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Evaluation of the Management of Cases with Pain Complaint and Practices in Emergency Departments

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ABSTRACT:

Background: Pain is one of the most common reasons for presentation to emergency departments (EDs) and is present in 80% of patients. In our country, no standardized procedure has yet been developed for the management of patients with pain complaints. We conducted a study to retrospectively examine the pain control approach and management practices of physicians of different seniority to patients with pain complaints in EDs and to establish a certain standard by determining the differences between pain treatment, drugs used, and users.

Materials and Methods: This study was carried out prospectively with a questionnaire administered to physicians working in hospitals in Hatay province and Emergency Medicine clinics in six major cities after permission from the Hatay Mustafa Kemal University Non-Interventional Research Ethics Committee. In the questionnaire, 32 questions were asked about the physicians' professional status, demographic data, approach, and management of patients with pain complaints, and the data were processed and evaluated using SPSS 22.

Results: A total of 273 Emergency Medicine Specialists (EMSs), Emergency Medicine Assistants (EMAs), and General Practitioners (GPs) participated in the study. Among the study participants, 37.4% (n=102) used pain scales, while 42.5% (n=116) routinely used pain scales at discharge. In the study, non-steroidal anti-inflammatory drugs were the first preferred analgesic agent for headache, low back pain, extremity pain, and dysmenorrhea. In burn patients, acetaminophen (n=122) and fentanyl (n=52) were the preferred drugs. EMS and EMA preferred fentanyl more frequently in abdominal pain and burn patients than the GP group (p=0.001). In patients with chest pain, morphine use by EMS and EMA was significantly higher than in the GP group (p<0.001).

Conclusion: It was observed that there was no standardized approach in the management of patients with pain complaints in ED and the use of pain scales was low. It was concluded that the level of education and experience of physicians are important in the choice of analgesia.

KEYWORDS:

Emergency department, Pain complaint, Case management, Analgesics.

1. Introduction

Pain is the most common symptom in patients presenting to the EDs and is the first complaint in approximately 80% of patients. EDs serve as units where acute conditions are taken under control for patients and ın this process, the cause of the patient's pain is determined, as well as supportive treatment for their symptoms. Pain is a condition that is seen as a bad experience for patients presenting to the ED and has physiologic consequences. Inadequate treatment of pain remains an important problem in pain management. [(1)] The degree of pain of the patient should play a role in deciding the urgency and treatment of the patient. Therefore, pain scoring should be used both to measure the degree of pain and to determine the response to treatment. [(2)]

Although it is known that there are no standardized approaches to pain management in the EDs of our country, there are inadequacies in the determination studies regarding the current situation when internationally accepted standards are taken into consideration. Moreover, it is observed that there are significant

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clinical practice differences in the EDs of our country, such as the use of pain scales is not very common, opioid use is low, and the intramuscular route is more commonly used. [3-5]

After pain is perceived by nociception in humans, it is transmitted to the central nervous system (CNS) via the sensory nervous system, and the pain sensation evaluated here is characterized as a bad experience for humans, and the body develops a response against the mechanism that causes pain. [6]

The impact of pain management practices in EDs is unknown. To improve pain management in EDs, it is important to understand the current state of clinicians' analgesic practice as well as patients' pain experiences. These issues have not been investigated with sufficient studies. The scientific aim of this study is to contribute to the literature by evaluating the pain control approach and management practices of physicians of different seniority to patients with pain complaints in emergency departments and to establish a certain standard by determining the differences in pain treatment, drugs used, and users.

2. MATERIALS AND METHODS

This study was conducted at Hatay Mustafa Kemal University Faculty of Medicine, Department of Emergency Medicine, with the ethics committee permission obtained from Hatay Mustafa Kemal University Non-Interventional Clinical Studies Ethics Committee, with the date and number 17.02.2022/14. Physicians working in emergency medicine clinics in six major cities of Turkey (Istanbul, Ankara, Izmir, Antalya, Adana, Gaziantep), emergency physicians working in EDs of state hospitals in Hatay province, and emergency medicine assistants (EMAs), emergency medicine specialists (EMSs) and general practitioners (GPs) working in the ED of Hatay Mustafa Kemal University were included in the study. The study included 273 doctors working in these hospitals in March and April 2023, the dates of the study.

In this prospectively planned study, the participants working in the included regions were administered the 'Approach to Pain and Pain Management Practices in Emergency Departments' questionnaire consisting of 32 questions describing demographic data, educational status, and approach and management of pain.

During the evaluation, a 5-point Likert scale was used in the questionnaire, and the answers given in this scale were evaluated by giving 0 points for "never", 1 point for "rarely", 2 points for "undecided", 3 points for "mostly" and 4 points for "always".

While evaluating the findings obtained in the study, SPSS (Statistical Package for Social Sciences) for Windows 22.0 program was used for statistical analysis. Descriptive findings are given as numbers and percentages, mean and standard error. Comparisons between groups were analyzed by chi-square test and significance test of the difference between two means (t-test and ANOVA). For the chi-square test and the significance test of the difference between two means, a p value less than 0.05 was considered significant.

3. RESULTS

A total of 273 participants, 110 (40.3%) female and 163 (59.7%) male, were included in our study. Of these participants, 90 (33%) were EMAs, 85 (31.1%) were EMS, and 98 (35.9%) were GPs. The majority of participants 94 (34.4%) had 1-5 years of experience. Demographic data of the participants are presented in Table 1.

Table 1: Combined demographic data of participants

Demographic Data	N	%
Title		
- EMAs	90	33.0
- EMSs	85	31.1
- GPs	98	35.9
Gender		
- Female	110	40.3
- Male	163	59.7
ED Work Experience		
- < 1 year	71	26.0
- 1-5 years	94	34.4
- 5-10 years	55	20.1
- 10-15 years	31	11.4
- > 15 years	22	8.1

EMAs: Emergency Medicine Assistants, *EMSs: Emergency Medicine Specialists, GP: General Practitioners*

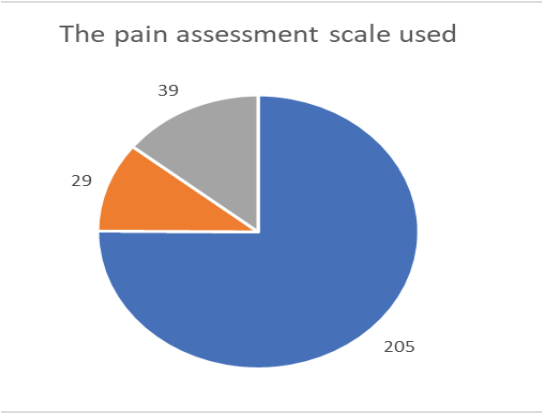
While 20 (7.3%) of the participants had less than 1,000 monthly visits to the ED where they worked, 122 (44.7%) of the participants had more than 20,000 monthly visits to the ED where they worked. The most common answer given by the participants on the rate of pain complaints was between 50-74% with 134 (49.1%) participants.

While 133 (49.7%) of the participants stated that the triage officer did not use any pain scale in the ED, 17 (9.9%) participants stated that they did not use a pain scale during the working period. 28 (10.3%) of the participants said that they did not use a routine pain scale at the patient's discharge from the ED. Table 2 shows the participants' information on pain scale use in the ED.

Table 2: Participants' use of the pain scale in the ED

Pain Scale Usage	N	%
Triage Nurse's Frequency of Using Pain Scale		
- Never	133	49.7
- Rarely	76	27.8
- Unsure	41	15.0
- Mostly	15	5.5
- Always	8	2.9
Frequency of Using Pain Scale While Working in the ED		
- Never	17	9.9
- Rarely	91	33.3
- Unsure	40	14.7
- Mostly	102	37.4
- Always	13	4.8
Routine Use of Pain Scale Before Discharge in the ED		
- Never	28	10.3
- Rarely	76	27.8
- Unsure	22	8.1
- Mostly	116	42.5
- Always	31	11.4

Among the participants, 205 (75.1%) used numeric pain scales, 29 (10.6%) used Visual Analog Scale (VAS), and 39 (39%) used other pain scale methods (Figure 1).



Numeric pain scale: 205 Visual Analog Scale:39 Other: 29
Figure 1: Types of pain scales

It was observed that 24 (8.8%) of the participants had a common pain management view in their organization. Among the participants who answered the questions in the study, 8 (29%) stated that they always follow the door-painkiller time tracking, 121 (44.3%) participants stated that they always question the history of analgesia in patients with pain complaints, 21 (7.7%) participants stated that they always delay analgesia due to the possibility of delayed diagnosis or cover-up of the clinical condition. Analgesia history and use behavior are presented in Table 3.

Table 3: Analgesia history and usage behavior (with time expressions)

Question	Never (N, %)	Rarely (N, %)	Unsure (N, %)	Mostly (N, %)	Always (N, %)
In your ED, how frequently do you track door-to-analgesic time?	59 (21.6%)	65 (23.8%)	61 (22.3%)	80 (29.3%)	8 (2.9%)
How frequently do you inquire about the analgesic usage history prior to admission for patients presenting with pain?	2 (0.7%)	12 (4.4%)	12 (4.4%)	126 (46.2%)	121 (44.3%)
In patients presenting with pain to your ED, how frequently do you delay administering analgesics due to concerns of diagnostic delay or obscuring the clinical condition?	21 (7.7%)	83 (30.4%)	52 (19.0%)	96 (35.2%)	21 (7.7%)

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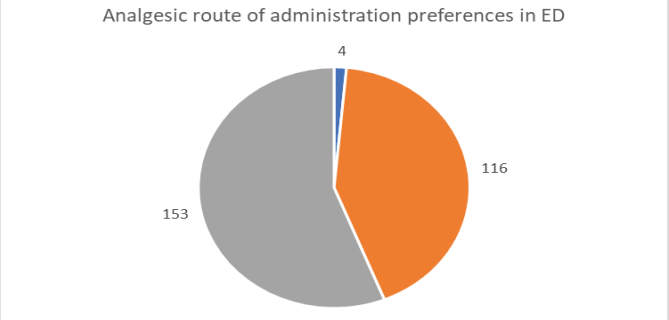
1 (0.4%) of the participants stated that they always administered oral analgesia. 34 (12.5%) participants said they repeated the pain scale after analgesia. 2 (0.7%) participants said they always preferred opioid analgesics, and 1 (0.4%) participant said they always had complications related to opioid use. 39 (14.3%) of the participants said they always looked at patients' old prescriptions, while 11 (4%) participants said they always saw a chronic patient presenting in the ED. While 80 (29.3%) of the participants thought that there was a weakness in outpatient care for those with chronic pain, 167 (61.2%) participants said that they always asked about the pain status of patients after analgesia.

Participants' reservations after opioid analgesic use were evaluated. 213 (78%) participants expressed concern about the risk of respiratory depression.

While 3 (1.1%) of the study participants thought that they might become addicted with a single dose, 103 (37.7%) participants stated that they had never used opioid antagonists. While 215 (78.8%) of the participants stated that they never prescribed opioids, 5 participants (1.8%) stated that they always encountered someone addicted to opioids in the ED.

The most common reason for avoiding opioid prescription at discharge from ED was the risk of addiction, stated by 241 (88.3%) participants.

When the preference for the route of administration of analgesia in ED was analyzed, 116 (42.5%) participants preferred intramuscular, 153 (56%) participants preferred intravenous, and 4 (1.5%) participants preferred oral administration (Figure 2).



oral: 4 intravenous:153: intramuskuler:116
Figure 2: Distribution of preferences for analgesic route of administration in the emergency department

Table 4 shows the participants' order of preference for analgesics for various pains and age groups when using them in the ED or prescribing them at discharge.

Table 4: Participants' preferred analgesic choices by scenarios

Scenario	Paracetamol	NSAIDs	Tramadol	Fentanyl	Morphine	Other	Meperidine
In ED Clinical Scenarios							
- Headache	87	182	0	1	0	1	2
- Back Pain	15	248	7	3	0	0	0
- Abdominal Pain	68	61	39	72	3	28	2
- Renal Colic	21	164	33	45	2	6	2
- Extremity Pain	28	221	10	12	1	1	0
- Burn	68	122	19	52	8	3	1
- Dysmenorrhea	38	225	2	3	0	5	0
- Chest Pain	98	39	3	23	85	24	1
- Elderly Patient	225	30	8	7	0	2	1
- Pregnant Patient	270	0	0	1	0	0	0
- Pediatric Patient	85	187	0	1	0	0	0

Scenario	Paracetamol	NSAIDs	Tramadol	Fentanyl	Morphine	Other	Meperidine
At Discharge Prescription Preferences							
- Headache	85	187	0	1	0	0	0
- Back Pain	26	244	0	1	0	2	0
- Abdominal Pain	97	100	0	2	0	73	1
- Renal Colic	26	231	0	5	0	11	0
- Extremity Pain	30	242	0	1	0	0	0
- Burn	68	196	2	2	0	5	0
- Dysmenorrhea	42	226	0	0	0	5	0
- Chest Pain	132	79	2	2	4	51	3
- Elderly Patient	236	31	1	0	1	1	0
- Pregnant Patient	269	3	0	1	0	0	0
- Pediatric Patient	264	8	0	1	0	0	0

After the participants were grouped according to their years of practice, their responses to the painful patient scenarios were evaluated. For the scenario of "a patient with chronic kidney disease presenting with abdominal pain who has not yet undergone dialysis", the rate of waiting for analgesia administration was found to be significantly higher in physicians with more than 15 years of practice ($p=0.002$). For patients who were followed up with the scenario of "48-year-old male patient presenting with typical chest pain, natural physical examination, and no Electrocardiogram findings", the rate of physicians who worked between 1-5 years was significantly higher than the other periods ($p=0.004$). In the case of "patient presenting with headache, Glasgow Coma Scale (GCS) <15 and lateralizing findings on physical examination", it was observed that physicians who had been working for less than 1 year or had experience between 1-5 years would administer analgesia more frequently than the other groups ($p=0.01$). In the scenario of "patient presenting with unilateral flank pain and deficiency on physical examination", 73.2% of physicians with less than 1 year of experience stated that they would not delay the administration of analgesia, which was significantly higher than the other groups ($p<0.001$). In the "78-year-old patient presenting with back pain" scenario, 59.2% of physicians with less than 1 year of experience stated that they would not delay the administration of analgesia, which was significantly higher than the other groups ($p=0.033$).

A comparison was made between the painkiller preferences of the participants who participated in the study and were grouped as EMS, EMA, and GP working in Emergency Medicine, according to the type of pain by using the chi-square test, and significant results were found in abdominal pain, chest pain, burns, and elderly patients. Fentanyl preference of EMS and EMA in patients with abdominal pain was significantly higher than that of the GP group ($p<0.001$).

Participants in the GP group preferred NSAIDs more frequently than the other groups in burn patients ($p=0.001$).

In the grouping according to occupation, participants in the GP group frequently preferred NSAIDs and paracetamol for chest pain, while the preference for morphine was significantly higher in the EMA and EMS groups ($p<0.001$).

Although acetaminophen was found to be the most preferred drug group in elderly patients, NSAID use was significantly higher in the GP group ($p=0.008$).

Table 5 shows the comparison of analgesics prescribed at discharge according to occupations and pain types. In the comparison between occupational groups for patients presenting with abdominal pain, it was observed that the preference for NSAIDs was significantly higher in the GP group participants compared to the other groups ($p<0.001$).

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Table 5: Discharge analgesic prescription preferences by professional groups

Scenario	Chi-square	p-Value	Relationship Ratio
Headache	5.889	0.208	6.175
Back Pain	9.58	0.143	10.69
Abdominal Pain	39.48	<0.001	41.279
Renal Colic	9.6	0.143	9.76
Extremity Pain	5.137	0.274	5.75
Burn	5.05	0.751	6.26
Dysmenorrhea	6.122	0.19	5.81
Chest Pain	19.5	0.077	22.35
Elderly Patient	10.14	0.255	10.26
Pregnant Patient	3.55	0.469	4.59
Pediatric Patient	3.44	0.486	3.87

No significant difference was found in the frequency of pain scale use by occupational groups while working in the ED (p=0.842). There was no significant difference in the frequency of routine pain scale application during discharge according to occupational groups (p=0.777).

According to occupational groups, a comparison was made between the waiting situations and patient scenarios. In the scenario "Hypotensive patient with back pain and nausea in the epigastric region", the frequency of analgesia administration was significantly higher in the GP group compared to the other groups (p=0.001). In the scenario of "Patient presenting with headache, GCS<15 and lateralizing findings on physical examination", the GP group participants administered analgesia significantly earlier than the other groups (p=0.005). In the scenario of "patient presenting with unilateral side pain and deficiency on physical examination", the frequency of administering analgesia without waiting was significantly higher in the GP group participants compared to the other groups (p<0.001). In the scenario of "78-year-old patient presenting with back pain", the frequency of EMS group participants to keep the patient waiting for analgesia was significantly higher than the other groups (p=0.001).

Concerns about the use of opioid analgesia were compared according to occupational groups. The EMS group was significantly less concerned about the risk of respiratory depression than the other groups (p<0.001). The change in the level of consciousness in the direction of deterioration of control was found to be a more frequent concern for participants in the GP group (p<0.001). Participants in the EMS group had fewer reservations about side effects compared to the other groups (p<0.001). Participants in the GP group had significantly more reservations about the difficulty in accessing the antidote than the other groups (p<0.001).

4. DISCUSSION

This study represents a broad-based, multicentre investigation of ED patients' experience of pain. Consistent with previous emergency medicine research involving predominantly single-centre studies, our results suggest that pain continues to be undertreated in the ED. Pain was the main complaint during patient visits. This high prevalence of pain has important implications for the allocation of resources in emergency medical care and for education and research efforts. [7,8]

The type of pain and the analgesic agent used in the management of patients with pain complaints in EDs may vary according to the experience and training of the physicians using the analgesic agent.

In a study conducted in Turkey, pain management was evaluated on EMSs and EMAs throughout Turkey. In this study, a total of 386 participants were reached, and 63.3% of these participants were men. [9]

When the demographic distribution of the participants was examined, it was found that the gender distribution across the country, the study sample in the literature, and the gender distribution in our study were similar.

EDs are acute treatment units for patients. In a study examining the quality standards, the number of ED admissions for the United States, which had a population of 316,497,500 in 2013, was found to be 130,035,300, and the ratio to the entire population was observed to be 0.41. While this ratio was 0.31 in Australia in the same year, it was found to be 1.31 in our country. [10] In a 5-year ED admission analysis performed in our country, it was observed that the annual number of patient admissions to a tertiary ED increased gradually. [11] In 44.7% of the EDs where the physicians who participated in our study worked, the average monthly number of admissions was 20,000 or

more. The target group of the study was planned as cities with high population density, and the distribution of the participants in the study according to the population rates in these cities and according to previous studies conducted in our country is similar to the distribution of patients in our country.

In ED working practice, pain complaints constitute approximately 70% of admissions. [1] In a study by Hong et al. examining the characteristics of patients presenting to the ED in South Korea between 2016 and 2018, it was observed that 33.6% of patients were discharged, and 27.9% of these patients were patients with pain. [12] In another study conducted in our country, it was reported that 57-75% of the population admitted to the ED due to pain, while door-painkiller time follow-up was performed in 27.6% of the participants in the study, it was observed that this follow-up was never performed in 27.1%. [9]

In our study, 49.1% of the participants stated that between 50-74% of the admitted patients presented with pain. While 2.9% of the participants always followed the door painkiller time, 21.6% stated that they never followed it. The rates of patients presenting to the emergency department due to pain were found to be similar to the literature. However, it was observed that door-painkiller follow-up was performed at a lower rate compared to the literature. This may be used as an indicator for a decrease in hospital service management.

In 49.7% of the participants' EDs, pain scales were not applied in triage. While 37.4% of the participants mostly used pain scales, 42.5% routinely used pain scales at discharge. The numeric pain scale was the most frequently used scale, with a rate of 75.1%.

It was found that 8.8% of the participants adopted a common authority in pain management.

In the study conducted by Yıldız et al., the behaviors of physicians in certain scenarios on analgesia administration in the ED were examined [9]. In this study, it was observed that pain relief was postponed in cases such as abdominal pain, chest pain, confusion, and if the patient had an increased comorbid factor. The fact that the patient was pregnant or of advanced age was seen as another reason for postponement. [12]

The individual characteristics of the patients, the training of the physician who will administer analgesia, and his/her knowledge of the patient's pathologic condition are important in analgesia management. [13]

In our study, when the scenarios administered to the participants and their waiting for analgesics were evaluated, it was found that the participants were willing to administer painkillers in the early period in chest pain and epigastric pain, whereas patients could be kept waiting in scenarios where conditions such as abdominal pain, headache, trauma, pregnancy and comorbidity were observed.

Comparison between participant groups showed that GPs preferred to administer analgesia earlier in different scenarios with symptoms such as chest pain, epigastric pain, headache, flank pain, back pain, and comorbid conditions. Significant differences were also observed between the early administration of analgesia and the professional experience of the participants. In the comparison made on abdominal pain, epigastric pain, traumatized pregnant women, headache, flank pain, and geriatric patient pain, those with more professional experience were more likely to delay the administration of analgesics.

The basis of analgesic administration is the individual experience of the physicians, their educational status, and the patient factor. As found in our study, there are significant differences in analgesia delaying behaviors for physicians with different educational backgrounds.

In our study, the most common route of analgesic use was the intravascular route, with a rate of 56%. In the study conducted by Yıldız et al., the intravenous route preference rate was 57.5%. [9]

The American Heart Association (AHA) recommends the use of opioids for pain control in patients presenting with chest pain. [14] In a review by Yan et al., it was emphasized that opioid analgesics were frequently used in EDs for patients with chest pain. [15] In a study on acute abdominal pain, Shabbir et al. found that analgesia was administered to patients in an average of 1.4 hours. It was observed that patients with lower pain levels waited longer for analgesia. NSAID was found to be the most commonly preferred analgesic method. [16]

In the study conducted by Yıldız et al., it was observed that NSAIDs were used as the first

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choice in cases such as headache, low back pain, side pain, limb pain, and dysmenorrhea. [9] In the study conducted by Çetin et al. in 2021, the physicians mainly ordered NSAIDs (67.9%), and opioid analgesics were the most frequently administered analgesic if the second application was required. Also, the most frequently prescribed analgesics were NSAIDs in 44% of cases. [17]

In our study, NSAIDs were the first preferred analgesic in cases such as headache, low back pain, renal colic, extremity pain, and dysmenorrhea in patients followed up in the ED, and NSAIDs constituted the first drug group prescribed at discharge. Acetaminophen was the most commonly used analgesic for elderly patients, pregnant patients, pediatric patients, and chest pain, and acetaminophen was the most commonly prescribed analgesic agent at discharge. Morphine use by EMSs and EMAs was significantly higher in patients with chest pain than in GPs.

Analgesic recommendations in the literature and previous studies were found to be similar to the practice and prescribing preferences in our study.

While the use of opioid analgesics was significantly higher in EMSs and EMAs compared to GPs in burn patients, it was observed that GPs were more hesitant in the use of opiate analgesics in emergency practice, and the most important hesitation was the risk of respiratory depression.

Common side effects of opioid administration include sedation, dizziness, nausea, vomiting, constipation, physical dependence, tolerance, and respiratory depression. Physical dependence and addiction are clinical concerns that may prevent appropriate prescribing and thus inadequate pain management. When using opioid group drugs, it is important to be controlled and to consider the risk of addiction. [18]

The use and control of opiate use and control was observed more prominently for EMSs, who have deeper knowledge about pain management in the ED by receiving specialized training on patient management, and EMAs, whose training in this field continues, compared to GPs. Possible side effects mentioned in the literature were also observed as the most important reservations in our study.

As in our study, it has been shown that correct analgesic use is more accurate as the level of physician education increases. In the study conducted by Jones et al., it was shown that pain management was more accurate when correct analgesic use was taught with pain management training programmes, which supports our study. [19]

This study by Ali et al. has demonstrated the importance and necessity of correct analgesic use in the ED. In addition, in the light of these scientific studies, we aimed to show how the use of analgesics in EDs by doctors of different seniority varies according to the patient and the type of pain. [20]

5. CONCLUSION

NSAIDs are the most preferred painkillers for headache, low back pain, extremity pain, and dysmenorrhea, while acetaminophen is the second most preferred painkiller for burn patients, followed by fentanyl. In discharge prescriptions, NSAIDs were the first choice for headache, low back pain, abdominal pain, renal colic, extremity pain, burns, and dysmenorrhea. EMSs and EMAs preferred fentanyl more frequently for abdominal pain and burns compared to the GP group. Morphine use by EMSs and EMAs was significantly higher in patients with chest pain compared to GPs. In discharge prescriptions, EMAs and EMSs frequently chose acetaminophen for abdominal pain, while GPs chose NSAIDs.

In the light of these findings, it is noteworthy that there is no standardization in terms of pain management in EDs, the use of pain scales is low, and due to the lack of standards in this process, both the experiences of the patient presenting with pain and the training and experience of the physician managing the pain appear as important parameters in treatment. It is seen that this situation affects both patient satisfaction and ED functioning.

Emergency clinicians have an important responsibility to relieve pain in a timely, effective, and safe manner using all available modalities. Increased knowledge and skills of emergency clinicians in pain management have resulted in the judicious use of opioids. Standardisation is required for emergency clinicians to have confidence in evidence-based pain management and to incorporate it into their daily practice.

It is necessary to identify the problems that cause differences in pain management, which has a very important place for emergency services, to carry out studies to solve the problems and to establish a standard in this regard.

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Giant Azygos Vein Aneurysm Incidentally Detected During Breast Cancer Workup: A Case Report

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ABSTRACT:

Azygos vein aneurysms are extremely rare vascular anomalies of the mediastinum. Most are asymptomatic and incidentally found during imaging. Their rarity, non-specific presentation, and radiologic resemblance to other mediastinal masses make accurate diagnosis challenging. This case adds to the limited literature and emphasizes the value of preoperative imaging in diagnosis and surgical planning.A 54-year-old woman was admitted for evaluation of a left breast mass. She had no cardiopulmonary symptoms. Preoperative contrast-enhanced CT revealed a well-defined, homogeneously enhancing posterior mediastinal mass (8.1 × 3.9 cm) in continuity with the azygos vein. Given the lesion's size and unclear nature, surgical resection was performed via median sternotomy, with careful intraoperative handling to prevent thromboembolism. Pathology confirmed a thrombosed azygos vein aneurysm. Immunohistochemistry was positive for CD31, CD34, ERG, D2-40, calretinin, and WT-1, with a Ki-67 index <1%, consistent with a benign vascular lesion. The patient recovered well without complications. Azygos vein aneurysms, though rare, should be considered in the differential diagnosis of mediastinal masses. Cross-sectional imaging is essential for identifying vascular origin and planning safe resection. Surgical removal is indicated for large or uncertain lesions, particularly when thrombosis is present. Early diagnosis and intervention can prevent complications and offer definitive histological confirmation, especially in patients undergoing oncologic workup.

KEYWORDS:

Azygos Vein Aneurysm, Breast Cancer, surgical resection.

1. Introduction

Mediastinal venous aneurysms (MVAs) are extremely rare vascular abnormalities characterized by focal dilatation of mediastinal veins, most commonly involving the azygos vein or its tributaries [1]. Unlike arterial aneurysms, MVAs are often asymptomatic and are usually discovered incidentally during imaging studies performed for unrelated conditions. When symptoms do occur, they may include chest discomfort, cough, or dyspnea due to mass effect. Although typically benign, MVAs pose a risk of thrombosis, rupture, or embolism, particularly in large or enlarging lesions. Owing to their rarity and nonspecific imaging features, MVAs can be mistaken for other mediastinal masses such as lymphadenopathy, neurogenic tumors, or cysts. Contrast-enhanced computed tomography (CT) or magnetic resonance imaging (MRI) plays a crucial role in diagnosis by demonstrating vascular continuity and enhancement patterns. Management strategies range from conservative monitoring to surgical resection, especially in symptomatic patients or those with uncertain diagnosis [2]. Here, we report a rare case of azygos vein aneurysm discovered incidentally in a patient undergoing evaluation for breast cancer, which was subsequently confirmed intraoperatively and successfully resected.

2. Case Presentation

A 54-year-old female was admitted to the hospital due to a palpable mass in her left breast. She reported no symptoms such as cough, sputum production, chest pain, dyspnea, or dysphagia upon admission. Her appetite, mental status, and exercise tolerance remained normal. She had no history of hypertension,

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diabetes, or chronic bronchitis. The patient was admitted to the Department of Breast Surgery and was diagnosed with invasive carcinoma of the left breast. Preoperative contrast-enhanced chest CT revealed a low-density mass adjacent to the mediastinum in the right thoracic cavity, measuring approximately 8.1 × 3.9 cm (Figure. 1). The lesion had a well-defined margin and smooth borders. Contrast enhancement showed significant and homogeneous enhancement, and the mass was found to communicate with the azygos vein, raising suspicion of a venous aneurysm. The patient subsequently underwent venous aneurysm resection and thrombectomy under extracorporeal circulation without cardioplegic arrest. Upon opening the aneurysmal sac, the inner wall exhibited typical venous endothelial characteristics, and the aneurysmal cavity displayed a honeycomb-like structure with localized thrombus formation at the base (Figure 2). The lesion had a complex anatomical relationship with the azygos arch and adjacent intercostal vessels and received mixed arterial and venous blood flow. Careful dissection and mobilization of the aneurysmal wall were performed intraoperatively. The base of the aneurysm was delivered intraluminally through its neck and subsequently ligated at the neck. After resecting the majority of the aneurysmal wall, the neck was meticulously reinforced to ensure complete closure. Postoperative histopathological examination confirmed the diagnosis of a venous aneurysm. Postoperative recovery was uneventful, and the patient was discharged in good condition.

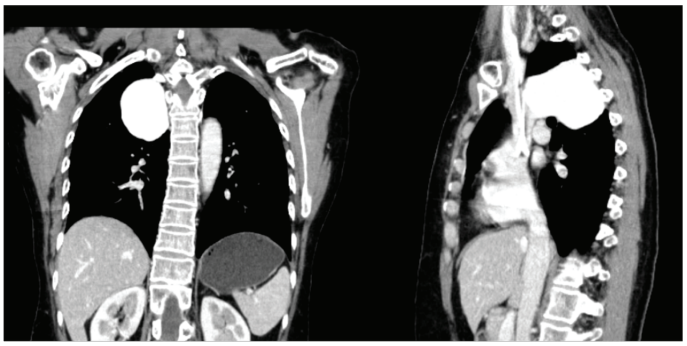

Figure 1: Contrast-enhanced chest CT showed a well-defined low-attenuation mass in the right thoracic cavity adjacent to the mediastinum, showing contrast enhancement and continuity with the azygos vein.

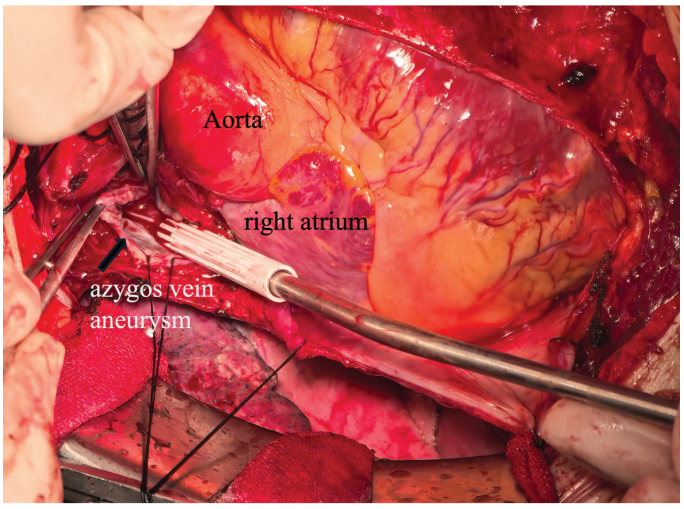

Figure 2: surgical resection of the venous aneurysm: The black arrow in the figure indicates the azygos vein aneurysm, whose inner wall exhibits typical venous endothelial characteristics. Localized thrombus is visible at the base, and the aneurysmal cavity displays a honeycomb-like structure with continuity to the azygos arch and adjacent intercostal vessels.

3. Discussion

Mediastinal venous aneurysms (MVAs), particularly those involving the azygos vein, are exceedingly rare vascular anomalies with unclear pathogenesis. Since their first description, fewer than 50 cases have been reported in the literature [3]. MVAs are typically asymptomatic and are often discovered incidentally during imaging for unrelated conditions, as in the present case. The underlying mechanism remains uncertain and may involve congenital weakness of the venous wall; however, acquired factors such as increased intrathoracic pressure or trauma have also been proposed [4]. Radiologically, MVAs can mimic various mediastinal masses, including lymphadenopathy, neurogenic tumors, pericardial cysts, or malignancies. Nevertheless, contrast-enhanced CT or MRI can help differentiate venous aneurysms by demonstrating vascular characteristics, such as smooth margins and enhancement patterns consistent with blood vessels [4]. In this case, the lesion showed homogeneous enhancement and a clear continuity with the azygos vein, leading to a preoperative suspicion of a venous aneurysm.

Currently, there are no established guidelines for the management of MVAs. However, several case reports and reviews have suggested general indications for surgical intervention [5-8]. These include: (1) diagnostic uncertainty requiring histological clarification to rule out malignancy or other mediastinal tumors; (2) the presence of symptoms such as chest pain, cough, dysphagia,

or superior vena cava compression; (3) large aneurysms (generally >4 cm in diameter) or those demonstrating progressive enlargement; (4) imaging evidence of intraluminal thrombosis, posing a risk of pulmonary embolism; and (5) concurrent oncologic surgery requiring exclusion of mediastinal pathology. In such cases, surgical resection provides not only a definitive diagnosis but also prevents potential complications.

Histopathological analysis of the resected specimen revealed positive immunohistochemical staining for CD31, CD34, and ERG, supporting a vascular endothelial origin. D2-40, calretinin (CR), and WT-1 were also positive, possibly indicating expression related to perivascular mesothelium or lymphatic endothelium, consistent with a complex vascular cystic lesion. The Ki-67 proliferation index was less than 1%, indicating extremely low proliferative activity. Based on morphologic and immunohistochemical features, the final diagnosis was a thrombosed azygos vein aneurysm, a benign vascular malformation (Figure. 3).

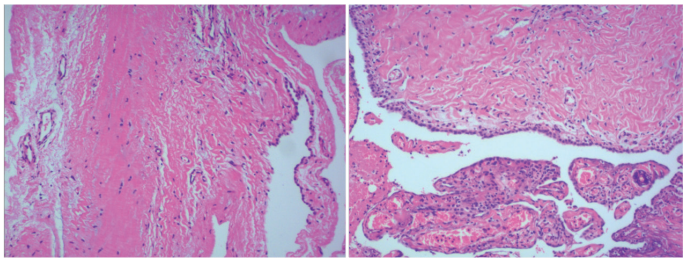

Figure 3: Histopathological features of the venous aneurysm: Hematoxylin and eosin (H&E) staining shows proliferation of fibrous tissue with surface mesothelial cell hyperplasia. Within the cyst wall, numerous proliferative vascular structures are present, some exhibiting thickened walls. These findings support the diagnosis of a venous aneurysm. (Original magnification: ×100)

In the present case, a large azygos vein aneurysm (AVA) was incidentally detected on preoperative imaging and subsequently confirmed intraoperatively, with associated thrombus formation. The lesion was successfully resected under extracorporeal circulation. A review of the literature reveals several comparable cases: Kreibich et al. (2017) reported a symptomatic large AVA treated surgically with favorable outcomes [1]; Kurihara et al. (2012) described an asymptomatic AVA that developed thrombosis during long-term follow-up and was also managed surgically [9]; and Ko et al. (2013) presented a series of 10 idiopathic AVA cases with variable clinical presentations, in which management strategies included surgical resection, endovascular intervention, or conservative observation [10]. Compared with these cases, the current patient exhibited representative features in terms of clinical presentation, radiological findings, and treatment strategy, and highlights the potential for AVAs to be misdiagnosed as mediastinal tumors, thereby prompting surgical exploration. Although standardized treatment guidelines are lacking, cumulative experience suggests that surgical resection remains the mainstay of management for symptomatic cases, those with thrombosis, or when the diagnosis is uncertain. This underscores the importance of accurate preoperative recognition of venous lesions to guide appropriate clinical decision-making.

4. Conclusions

In this case, the aneurysm measured approximately 8 × 4 cm and, although asymptomatic, exceeded the typical threshold for intervention and presented with significant diagnostic uncertainty. Contrast-enhanced CT clearly demonstrated continuity with the azygos vein and homogeneous enhancement, facilitating preoperative suspicion of a vascular anomaly and guiding surgical planning. Intraoperatively, the lesion's anatomical complexity—including its proximity to the azygos arch and intercostal vessels—necessitated careful dissection and vascular flow control to prevent thromboembolism, particularly given the presence of mural thrombosis. This case highlights the diagnostic challenges and operative considerations associated with mediastinal venous aneurysms (MVAs), a rare but clinically relevant entity that may mimic other mediastinal masses. As high-resolution imaging becomes increasingly integrated into oncologic workups, incidental detection of such lesions is expected to rise. Consequently, a growing body of case reports like this one may contribute to the development of consensus-based guidelines for the diagnosis and management of MVAs. Future directions should include the creation of risk stratification tools and refined surgical indications based on lesion size, symptomatology, thrombotic risk, and anatomical complexity. Accurate radiologic-pathologic correlation remains essential for guiding appropriate treatment strategies and avoiding unnecessary interventions.

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HMG-CoA reductase inhibitors (statins): Analgesic and Anti-Inflammatory Evaluation Using Various Animal Models

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ABSTRACT:

There are certain drugs that are known to decrease pain and inflammation. Among them are corticosteroids and non-steroidal anti-inflammatory drugs. But these provide only symptomatic relief. Many adverse effects are seen with chronic use of these drugs. Therefore, finding a new, reliable, and safe analgesic and anti-inflammatory drug is still the need of the hour. Statins are known to be one of the best agents for the treatment of cardiovascular diseases. Recently, the analgesic, anti-inflammatory, and antioxidant actions of statins have been demonstrated. The most probable mechanism behind their analgesic property includes decreased production of pro-inflammatory mediators such as tumor necrosis factor-alpha, bradykinin, interleukin-1 beta, IL-6, and IL-8.

Materials and Methods: *Analgesic anti-inflammatory activity of HMG-CoA reductase inhibitors (statins) was evaluated using Swiss albino mice. Analgesic activity was evaluated using the Hot Plate, Acetic acid-induced writhing, and Haffner's tail clip methods. Anti-inflammatory activity was evaluated using Carrageenan-induced left hind paw edema (Plethysmographic method).*

Results: *This study showed that the analgesic potential of rosuvastatin and atorvastatin was comparable to diclofenac; in the hot plate, the tail clip, and in the acetic acid-induced writhing method. The atorvastatin and rosuvastatin had equi-analgesic effects, which were significantly higher than that of the control ($p<0.01$) and ($p<0.001$), respectively; however, diclofenac was found superior in this regard ($p<0.01$). In the carrageenan-induced hind paw edema model (Plethysmographic method), both atorvastatin and rosuvastatin exhibited anti-inflammatory action ($p<0.01$), and the activity of atorvastatin was comparable to that of diclofenac. Conclusion: Both statins, rosuvastatin and atorvastatin, showed analgesic and anti-inflammatory action comparable to diclofenac.*

KEYWORDS:

Statins, Tail clip, Hot plate method, Plethysmometer.

1. Introduction

Drugs known for decreasing pain (analgesics) and inflammation, such as non-steroidal anti-inflammatory drugs (NSAIDs) and corticosteroids, give only symptomatic relief. Many adverse effects are observed with the chronic use of these drugs. Therefore, finding a new, reliable, and safe analgesic and anti-inflammatory drug is still in process.[1,2]

Statins are known to be one of the most powerful agents for the treatment of several cardiovascular diseases. They are becoming the first choice of drugs for conditions like hypertension, diabetes mellitus, and other known cardiovascular disease risk factors.[3] The statins belong to a class of agents which decreases lipid levels and tends to inhibit 3- hydroxy-3-methylglutaryl coenzyme A reductase enzyme.[4] Among Statins, the most commonly used drugs are Rosuvastatin and Atorvastatin. Recently, Statins have been demonstrated to possess anti-inflammatory, analgesic, and antioxidant properties. The most probable mechanism

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behind their analgesic action includes decreased production of pro-inflammatory mediators like bradykinin, TNF-alpha, interleukin-1b, IL-6, and IL-8.[2] Various experimental evidence and some clinical investigations have shown that statins can exert several cholesterol-independent, cardioprotective actions. Statins have the ability to increase nitric oxide (NO) generation and eNOS enzyme levels in endothelial cells. They are also powerful modulators of eNOS function. Therefore, Statins can be thought to have very powerful anti-inflammatory actions that are largely eNOS dependent.[2]

Diclofenac is a well-researched, widely used non-steroidal anti-inflammatory drug (NSAID) that possesses analgesic, anti-inflammatory, and antipyretic qualities. It is used to treat a variety of inflammatory conditions, which are acute as well as chronic in nature. Like all NSAIDs, the mechanism of action of diclofenac is to prevent prostaglandin synthesis by inhibiting the enzymes, cyclooxygenase-1 and cyclooxygenase-2.[5] Hence, this study was designed to evaluate and analyze the pain-reducing and anti-inflammatory actions of rosuvastatin, atorvastatin, and diclofenac in various animal models (Mice) of pain.

2. Materials & Methods

Swiss albino mice of either sex (25-30gms) were used for the study. The animals were housed under uniform standard laboratory conditions. They were provided with food and water ad libitum. The animals were acclimated for seven days before experiments were performed. The experimental protocol was approved by the Institutional Animal Ethics Committee (Ref. SKNMC/IAEC/App/2022/18). Mice were distributed randomly into four groups, with each group consisting of 6 animals (n = 24). Each animal was administered the drug by oral route to evaluate the single-dose analgesic and anti-inflammatory action in the following dosage.

Test Group I: Received Rosuvastatin 10mg/kg[2]

Test Group II: Received Atorvastatin 10mg/kg[6[

Standard Group: Received Diclofenac 2mg/kg and Control Group: Normal saline 0.5 ml

All the animals were screened, and marked into four different groups, and baseline readings were recorded for the following battery of tests:

Analgesic Activity:

Hot Plate Method:

Animals were placed on the hot plate, and the temperature was maintained at 55-65 degrees Celsius. The responses, such as jumping, licking of the paws, and withdrawal of the paws, were recorded. The response time of the animals was recorded with the help of a stopwatch. Test compounds were administered orally, and the duration of the latency period was recorded after 20, 60, and 90 minutes.[7]

Acetic Acid-induced writhing:

This method was used for the assessment of nociceptive responses to chemical stimuli. The experimental drugs were given with the help of oral gavage 30 minutes before the beginning of the experiment. By using a 27-gauge ½-inch needle, 0.1 ml of 1% acetic acid solution was injected via the intraperitoneal route. Each mouse was kept in the observation cages to assess their responses and behavior. Five minutes were allowed to pass during which the commencement of writhes was seen. Animals were monitored, and the number of writhes was observed for 10 minutes. A writhe is defined as the simultaneous stretching of at least one hindlimb along with the stretching of the abdomen.[7,8] This is required for scoring purposes.

Percentage Inhibition (W% %) in mice was

calculated as = {(Wc – Wt)/ Wc} x 100 where,

Wc - Total No. of writhes in the control group

Wt – Total No. of writhes in the test group

Haffner's Tail clip method:

In this tail clip method, an artery clip was used as a mechanical stimulus. It was placed at the root of the tail of each mouse, which acted as a painful stimulus. An immediate response from the animals was seen, such as biting the clip or tail, where the clip was placed. With the help of a stopwatch, the time elapsed between the application of an artery clip and the obtained response was noted. Test compounds were given orally to evaluate analgesic activity. After 15, 30, or 60 minutes, the same process was repeated, and the reaction time was calculated.[7]

Anti-inflammatory Activity:

Anti-inflammatory activity of the statins was studied using the carrageenan-induced left hind paw edema (Plethysmographic method). Animals were grouped into six groups, each with six animals in each, and received treatment as per their groups orally. Half an hour after

administration of the respective drug, 0.01mL of 1% carrageenan (Sigma Aldrich, Chemical) suspension was injected subcutaneously into the plantar surface of the left hind paw. The volumes of albino mouse hind paws in the test, control, and standard groups were measured using a Plethysmometer (Make: Ugobasile) at 0 and 3 hrs after the induction of inflammation, and Edema was expressed as an increase in paw volume due to carrageenan injection.[7,9] Edema was seen, and the % reduction in Edema in all groups was measured using the following standard formula:

% inhibition = 100 (1-Vt/Vc)

Where Vc = edema volume in control and Vt = edema volume in treated groups

Statistical Analysis

The data was analysed by using the SPSS software (subjected to relevant statistical tests). $P < 0.05$ was taken to be statistically significant.

3. Results

Analgesic Activity:

The results obtained are summarized in Table 1-3 and Figure 1 for different animal pain models.

Hot Plate Test:

Table 1: Effect of drugs on mean reaction time in hot plate method (cut off 10 secs.)

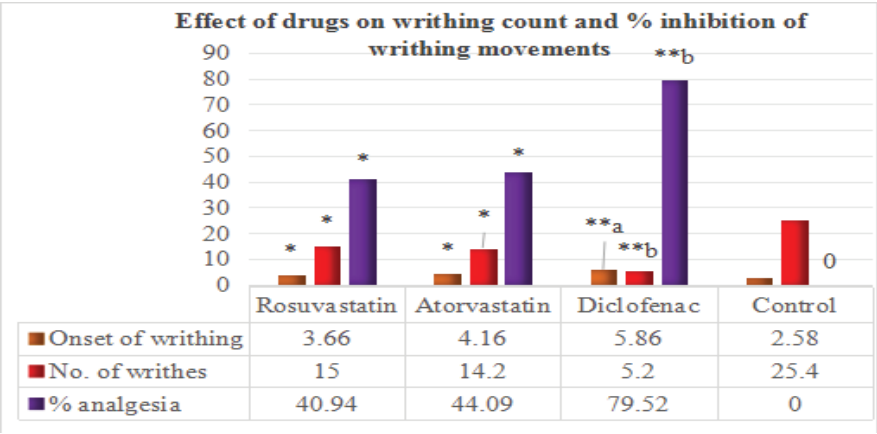
Treatment groups	Mean reaction time (in secs.) ±SD			
	After 0 min	After 20 min	After 60 min	After 90 min
Rosuvastatin	2.18 ± 0.18	3.13 ± 0.15*	6.3 ± 0.43**	8.61 ± 0.68**
Atorvastatin	2.12 ± 0.16	3.16 ± 0.19*	6.0 ± 0.33**	8.23 ± 0.45**
Diclofenac	2.7 ± 0.23	3.71± 0.14**	6.63± 0.18**	9.05 ± 0.68**a
Control	2.08 ± 0.15	2.45 ± 0.42	2.46 ± 0.36	2.35 ± 0.41

Data was analyzed using a one-way analysis of variance (ANOVA). Values expressed as mean ± SD; n=6; *p<0.01, **p<0.001; compared to control. ap<0.01; compared to standard

From Table 1, it is evident that at all the time intervals (i.e., 0, 20, 60, and 90 min), a significant rise in mean reaction time was seen in the rosuvastatin and atorvastatin group as compared to the control group ($p<0.01$, $p<0.001$), suggestive of equi-analgesic action. It was also seen that at all time intervals, their analgesic activity was comparable to diclofenac; however, diclofenac was found superior to statins in this regard ($p<0.01$).

Writhing Test:

Figure 1: Effect of drugs on writhing count and % inhibition of writhing movements



Data was analyzed using a one-way analysis of variance (ANOVA). Values expressed as mean ± SD; n=6; *p<0.01, **p<0.001; compared to control. ap<0.01, bp<0.001; compared to standard

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Both the test drugs significantly ($p<0.001$) showed inhibition of writhing counts and 40.94% and 44.09% inhibition of writhing movements, respectively, in the test groups. However, in the standard group, significant($P<0.001$) inhibition of writhing counts and 79.52% inhibition of writhing movements were observed in comparison to the control group.

Haffner's Tail–Clip Method:

Table 2: Analgesic effect of drugs by tail-clip method (cut off 15 secs.)

Treatment groups	Mean reaction time (in secs.) ±SD		
	After 0 min	After 15 min	After 30 min
Rosuvastatin	2.95 ± 0.24	5.41 ± 0.56*	8.43 ± 0.93**
Atorvastatin	2.81 ± 0.37	5.11 ± 0.83*	8.15 ± 0.61**
Diclofenac	3.01 ± 0.3	6.13 ± 0.43**[a]	9.45 ± 0.65**[a]
Control	2.81 ± 0.23	2.91 ± 0.38	3.18 ± 0.48

Data was analyzed using a one-way analysis of variance (ANOVA). Values expressed as mean ± SD; n=6; *p<0.01, **p<0.001; compared to control. ap<0.01; compared to standard

From Table 2, it is evident that, at 15 and 30 min intervals, there was a significant increase in mean reaction time ($p<0.01$, $p<0.001$) recorded in both test groups compared to the control group, suggestive of their equi-analgesic potential. This analgesic activity was comparable to diclofenac when compared to the control group; however, it was found that diclofenac was superior in this regard ($p<0.01$).

Anti-inflammatory Activity:

Table 3: Effect of drugs on carrageenan-induced mouse paw edema

Treatment groups	Left hind paw volume in ml (mean±SD)	
	0 min	180 mins
Rosuvastatin	0.143 ± 0.015 (18.75%)	0.316 ± 0.040 (28.67%)**
Atorvastatin	0.116 ± 0.032 (34%)	0.213 ± 0.045 (51.9%)**[a]
Diclofenac	0.106 ± 0.015 (39.7%)	0.183 ± 0.047 (58.6%)**[b]
Control	0.176 ± 0.015	0.443 ± 0.055

Figures in parentheses indicate the % anti-inflammatory activity. Data was analyzed using a one-way analysis of variance (ANOVA). Values expressed as mean ± SD; n=6; *p<0.01, **p<0.001; compared to control. ap<0.01, bp<0.001; compared to standard.

Carrageenan-Induced Paw Edema Model:

From Table 3, it is evident that the mean hind paw volume at 0 min was comparable in all four groups. It was observed that the mean paw volume was significantly lower in all three drug-treated groups when compared to the control group ($p<0.001$) at 180 minutes. The diclonefac group, however, showed a greater percentage of inhibition of acute inflammation than the rosuvastatin and atorvastatin groups. In a similar way, the percentage inhibition in the atorvastatin group was considerably higher than in the rosuvastatin group ($p<0.01$).

4. Discussion

A nociceptor is a type of sensory receptor that becomes active only when chemical, thermal, or mechanical stimuli surpass a high threshold, which is why it is designated as such [10]. Thermal nociceptors respond to harmful heat across different temperature levels. The first identified nociceptor of this kind was the Transient Receptor Potential cation channel, subfamily V, member 1 (TRPV1), also referred to as the capsaicin receptor [11, 12]. This receptor is activated when the temperature exceeds 42°C, the threshold for heat-induced pain [10]. In thermal testing, these receptors are stimulated at various temperature levels. Nonetheless, the precise mechanisms by which these channels interact and how the body determines that a temperature exceeds the pain threshold remain unclear.

In the present study, rosuvastatin and atorvastatin in the doses used showed a significant antinociceptive effect in the hot plate test, which is the most sensitive test to evaluate centrally acting analgesics.[13,14] This was clearly evident from the experiment results, where a

significant increase was observed in the mean reaction time with the gradual increase of time in both the test drugs. The antinociceptive effect was found to be greater in the diclofenac group. All these findings concur with the results of earlier studies in this regard, which mention that the pain-reducing action of statins begins a little earlier, and this finding was similar to our results.[6] Therefore, their usefulness can be seen in varied acute painful conditions. Results from Table 1 also specify that the mean reaction time in the rosuvastatin and atorvastatin groups at all the time intervals (i.e., 20, 60, and 90 min) significantly rose compared to the control, suggestive of their early and sustained analgesic potential, pointing out their antinociceptive effect through supraspinal mechanisms. [13,14]

The acetic acid-induced writhing method is a widely known experimental method to establish the action of peripherally acting analgesics. [1] Diclofenac was observed to have maximum analgesic potential in this model, confirming its potent peripheral analgesic action. However, both rosuvastatin and atorvastatin also showed significant analgesic action comparable to diclofenac. Most probable mechanism behind their analgesic action includes decreased production of pro-inflammatory mediators like bradykinin, TNF-alpha, interleukin-1 1b, IL-6, and IL-8.[2]

Haffner's tail clip method is used to study mechanical pain stimulation. This test procedure is based on the observation that morphine-like drugs selectively prolong the reaction time of the reflex to dislodge the tail artery clip in mice, showing central analgesic activity.[15] From the results, it is evident that the mean reaction time has increased in both the test groups at 15 and 30 minutes, suggesting the analgesic potential and involvement of central mechanisms for the analgesic activity.

It is a known fact that the standard and renowned experimental method to depict acute inflammation is the Carrageenan-induced hind paw edema model. Since carrageenan has no apparent systemic effects and is not known to be antigenic, it is the preferred irritant for testing the action of anti-inflammatory drugs.[6] This model helps find drugs that show anti-inflammatory potential and are clinically beneficial, as well as demonstrate good repeatability.[16] Carrageenan-induced paw edema is actually a biphasic response. In the first phase, several mediators are released, like serotonin, histamine, and kinins, while the second phase is mediated via the release of slow-reacting substances and prostaglandin.[17] Carrageenan-induced hind-paw edema inhibition by atorvastatin and rosuvastatin is the result of their ability to prevent the release of various inflammatory mediators. As can be seen from Table 3, the mean hind paw volume in all three drug-treated groups was found to be significantly lower when compared to the control group ($P < 0.001$) at 180 minutes. Percentage (%) inhibition of inflammation was greater in the standard (diclofenac) group when compared to atorvastatin and rosuvastatin, suggestive of predominant peripheral action of diclofenac and modest peripheral action exhibited by statins, especially by rosuvastatin, since the percentage inhibition in the atorvastatin group was greater than that of rosuvastatin. [Table 3].

In this study statins have shown analgesic and anti-inflammatory in all animal pain models, mechanism involved are central as well as peripheral probably by inhibiting production of pro-inflammatory mediators like bradykinin, TNF-alpha, interleukin- 1b, IL-6 and IL-8 for analgesic action and powerful modulation of eNOS function causing anti-inflammatory action.

5. Conclusion

From the results of this study, it can be concluded that HMG-CoA reductase inhibitors (statins), one of the best agents for the treatment of cardiovascular diseases, also possess analgesic and anti-inflammatory activity comparable to the established drug diclofenac, i.e., NSAID. All the above findings, which are corroborated by comparable studies that have been published, suggest that statins can be effective analgesics and anti-inflammatory drugs; however, further human studies are required to confirm the results of this study.

6. Limitations

It is single dose animal study with small sample size so we could not comment on ADRs of statins as analgesics after long term use; as well their efficacy in chronic pain was not studied. Further studies with multiple doses in larger sample size are needed.

7. Conflict of Interest

No conflict of interest was found in our study.

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Geriatric prescribing patterns: A Beers 2023 criteria pharmacoepidemiologic analysis, Alexandria, Egypt

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ABSTRACT:

Background: *In the geriatric population, the appropriateness of medication requires meeting multiple criteria, such as having a clear evidence-based indication, being well-tolerated by the majority, and being sufficiently cost-effective. In African countries, standardized Potentially Inappropriate Medication use criteria are rarely used.*

Aim: *We aimed in this study to estimate the prevalence of inappropriate medication prescribing patterns among geriatric patients aged 65 years or more in Alexandria, Egypt, and to determine the characteristics and risk factors for such patterns.*

Methods: *Cluster sampling was used as the sampling technique, and the sample size reached 1,000 prescriptions representing all districts of the Alexandria governorate. The updated 2023 Beers Criteria were utilized to identify potentially inappropriate prescribing. Inappropriate prescribing was considered if at least one inappropriate medication fell into at least one of the categories mentioned in the 2023 Beers Criteria.*

Results: *About 64.1% of prescriptions included three or more medications. Notably, 31.8% of prescriptions contained three medications, while 25.5% included four medications. A total of 463 (46.3%) prescriptions were potentially inappropriate. The full logistic regression model, which included all predictors, was statistically significant, χ^2 (20, N=1147) = 372.825, p < .001. Several independent variables made a unique statistically significant contribution to the model, including district, physician grade, and the number of medications prescribed.*

Conclusion: *It is advisable to standardize prescribing systems, particularly for the older population.*

KEYWORDS:

Geriatric, Prescribing patterns, Beers, Pharmacoepidemiologic, Egypt.

1. Background

In the geriatric population, the judgment of whether a medication is appropriate necessitates fulfilling a multitude of criteria such as having a clear evidence-based indication, being well-tolerated by the majority, and being sufficiently cost-effective. Appropriate prescribing in older people also encompasses considering an individual patient's life expectancy, avoiding preventive therapies in those with a poor survival prognosis, and promoting drugs with favorable risk-benefit ratios. Conversely, medicines that are potentially inappropriate in geriatrics either lack a clear evidence-based indication, carry a substantially higher probable risk of adverse events compared to younger people, or lack considerable cost-effectiveness. [1]

With the ongoing change in demographics and the increasing number of older patients, medication use has gradually turned into polypharmacy, which is the prescribing of multiple medications, most commonly defined as ≥ 5 medications for the older patients' therapeutic management. This, in turn, increases the risk of associated adverse events, causing morbidity and even leading to mortality. Consequently, polypharmacy has become a major concern worldwide, especially in Low- and Middle-Income Countries (LMICs). [2,3]

Prescribers for the geriatric population with multiple comorbidities have a heightened potential to commit various types of prescribing

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errors. Several complex and interrelated factors contribute to these errors, including a lack of knowledge about the unique physiology of older adults, principles of geriatric medicine, and their implications for pharmacotherapy in this population. Overprescribing often results in inappropriate prescribing, while underprescribing can lead to the omission of necessary medications. [4,5]

Several countries have developed their standards for identifying potentially inappropriate medication prescribing errors in the older population, providing a clinical reference. The American Geriatrics Society's (AGS) Beers Criteria (Beers Criteria) is one such standard, designed to assess potentially inappropriate medication use in the older population. It is extensively used by clinicians, researchers, medical administrative staff, educators, and regulators. The criteria primarily focus on the safety of medication use in the older population. An advantage of the Beers Criteria is that it is evidence-based and utilizes the Delphi validation approach to reach expert consensus, thereby creating a list of inappropriate drugs for older patients. [6]

The recently updated version of the Beers Criteria was launched in 2023. The updated Beers Criteria defines the drugs and drug lists that the AGS and its panel of experts have reached a consensus to consider as potentially inappropriate for use in older patients. The panel categorized these drugs into the same five main categories developed in the previous 2019 update: medications considered potentially inappropriate, medications considered potentially inappropriate for patients with specific diseases or syndromes, medications to be used with caution, medications with potentially inappropriate drug-drug interactions, and medications requiring dose adjustment based on renal function. [7,8]

A recent systematic review on the prevalence of Potentially Inappropriate Medication (PIM) use among the older population revealed that African countries rarely use standardized PIM use criteria. The findings suggest that this may be the highest contributor to the high prevalence of PIM use in this region. [9] These findings represent a milestone that highlights the emerging need for a thorough evaluation of PIM use among the older population in African countries. They have mobilized our enthusiasm to start with Egypt as a prototype for such an evaluation in our diligent pursuit to minimize PIM use in the older population.

In Egypt, to the best of our knowledge, there are no available studies that specifically address inappropriate medication prescribing in older adults, despite the increasing proportion of this population segment. According to World Bank/ United Nations (UN) data accessed via FRED, individuals aged 65 years and older constituted approximately 4.97% of the total population in 2023, reflecting a gradual but significant demographic shift toward an aging society. [10]

Defining inappropriate medications using explicit lists of criteria, such as the Beers Criteria, may miss some issues like therapeutic duplication within a drug class.

Therapeutic duplication in drug class prescriptions (also called therapeutic duplicates) increases the potential risk of adverse drug reactions without providing any additional therapeutic benefits. Therefore, it is crucial to pay close attention to each prescribed drug due to the evidence-supported increase in both the risk of adverse drug events and the associated costs, which consequently contribute to the economic burden. Identifying therapeutic duplication is essential for optimizing polypharmacy. [11]

In this study, we aim to estimate the prevalence of inappropriate medication prescribing patterns among geriatric patients aged 65 years or older in Alexandria, Egypt, and to determine the characteristics and risk factors for such patterns.

2. Methods

a. Study population:

The total number of medication prescriptions for geriatric patients aged 65 years and older resides in the nine districts of Alexandria governorate, Egypt. The geographical distribution of these districts was obtained from the official Alexandria governorate website. Our sample size calculations included 1000 prescriptions, representing the study population. Data collection and assessment of medication prescriptions were conducted at community pharmacies within each district.

b. Sample size calculation:

Sample size estimation depends on the statistical analysis type. We planned to use three different types, calculating the minimum required sample size for each to ensure adequate power and precision:

i. For prevalence estimation, we employed the formula [12]:

$$n = \frac{Z^2 P(1-P)}{d^2}$$

Where: n sample size, **Z**= Z statistic for level of confidence, **p**=expected prevalence or proportion, **d**= precision.

Accepting 5% error in our estimate and to achieve a study power of 80%, with a 95% confidence level, Z= 1.96, d= .05, and p= 0.5 (the safest choice for estimating population proportion is 0.5 as it presents the largest sample size to be estimated). [13]

The estimated sample size calculated was 384 but as we used a sampling method other than simple random sampling a larger sample size was to be needed because of the "design effect", for a cluster sampling strategy the design effect might be estimated as 2 [13], so the calculated sample size after adjustment for "design effect" was 768.To reduce sampling error, a round figure for sample size of 1000 had been taken.

For Chi-square analysis: we consulted sample size estimation tables [14], aiming for 80% power, 5% significance level, 8 degrees of freedom (based on up to 9 categories for a variable), and an expected effect size of 0.2 [14]. The calculated sample size was 376, confirming that our selected sample size of 1000 is sufficient for conducting Chi-square analyses between each pair of selected categorical variables.

For multiple logistic regression: In logistic regression, a common guideline suggests that you need approximately 10 events for each variable to achieve reasonably stable estimates of the regression coefficients. [15]

We expected that the proportion of inappropriate medication prescribing would be at least 24%, comparable to the proportion studied for community-dwelling patients in the United States. [16] Consequently, there would be at least 240 events in our sample size of 1000. Therefore, adhering to the rule of thumb, we can conduct a multiple logistic regression analysis with up to 24 independent variables. In our case, we have only five independent variables, which means we have approximately 48 events or outcomes per variable. Thus, a sample size of 1000 would be adequate to perform a multiple logistic regression analysis.

c. Sampling technique:

To select a sample of medication prescriptions depending on the principle of simple random sampling we should get a list or sampling frame for these medication prescriptions (population of N units) and a sample of n units is selected randomly from that population of N units without replacement so that each of the possible samples has the same probability of selection.

As there is no available sampling frame for the population of medication prescriptions in the visited community pharmacies in each defined district, so it is justified to use the principle of cluster sampling.

Cluster sampling is most useful when the homogeneity among units within clusters is not more than the homogeneity among units in the population as a whole and this was evident for the present study because the variability between the individual prescriptions within each district-specific community pharmacy is almost the same between the individual prescriptions within all the visited community pharmacies in all districts (whole population) and this is due to the presence of the same market drug list available for prescription. In such instances, cluster sampling can result in a considerable reduction in sampling frame construction without resulting in a significant increase in sampling errors. [17]

d. Data collection:

i. Basic data collection:

At each visit, the following data had been documented from each sampled prescription through prescription reviewing: ***1. Patient characteristics:*** patient name, patient age, patient sex, and diagnosis. ***2. Physician characteristics:*** Clinic specialty (Orthopedic, Internal medicine, etc.)**,** and physician grade [General Practitioner (GP), specialist, or consultant]**.** ***3. Prescription characteristics:*** Number of medications**,** name of medications**,** dose of medications, presence of at least one drug–drug interaction**,** presence of at least one drug class duplication.

ii. Assessment for drug class duplication or drug-drug interactions:

The American Hospital Formulary Services (AHFS) Pharmacologic-Therapeutic Classification serves as a reliable reference for assessing therapeutic duplication.

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The AHFS Pharmacologic-Therapeutic Classification was developed and is maintained by the American Society of Health-System Pharmacists, the national professional association representing pharmacists who practice in inpatient, outpatient, home-care, and long-term-care settings. The American Society of Health-System Pharmacists has a long history of fostering evidence-based medication use and patient medication safety. [18] The assessment for drug class duplication was conducted according to the AHFS classification.

Drug-drug interactions were determined through screening the entire list of medications in the prescription against a reliable checking tool, ***Lexicomp's online Comprehensive Interaction Analysis Program***, which is a complete drug and herbal interaction analysis program. One can enter a patient's entire regimen, identify potential interactions, and obtain appropriate patient management steps. The analysis includes a summary of drug interactions with an assigned risk rating to identify the action steps necessary. Each letter designation (A, B, C, D, X) represents the severity level of the identified interaction. A detailed interaction monograph is displayed by clicking on the interacting drug name. Lexicomp's online Comprehensive Interaction Analysis Program excelled as a personal digital assistant pharmacopoeia for assessing drug interactions, thoughtfully designed to provide quickly accessible, exceptionally reliable information. A drug-drug interaction is defined as having at least one either D or X risk rating interaction using Lexicomp's online Comprehensive Interaction Analysis Program. [19]

iii. Assessment of inappropriate medication prescribing:

The medications were reviewed by the investigator using the updated 2023 Beers Criteria to identify potentially inappropriate prescribing. Inappropriate prescribing was considered if at least one inappropriate medication was identified in the prescription, falling into at least one of the mentioned categories stated in the 2023 Beers Criteria: medications considered potentially inappropriate, medications deemed potentially inappropriate for patients with specific diseases or syndromes, medications that should be used with caution, and medications with potentially inappropriate drug-drug interactions. [8]

e. Statistical analysis:

Statistical analysis was conducted using IBM® SPSS® Statistics version 27. Data integrity was checked and verified via the frequency checks for the qualitative variables, as well as minimum and maximum checks for the quantitative variables, ensuring that no errors or missing values are present. Descriptive statistics were utilized to present qualitative variables in terms of frequencies and percentages. For the analytical statistics, a multivariate logistic regression analysis was conducted to examine the associations between the independent variables—' Patient age,' 'Patient gender,' 'Physician grade,' 'Clinical specialty,' and 'Number of prescription medications'—and the outcome variable, inappropriate medication prescribing according to the 'Beers Criteria 2023." The regression coefficients (βs) indicate the magnitude of increase or decrease in the log odds of the outcome variable for each unit change in the independent variable, while keeping all other variables constant. The Standard Error (SE) estimates the variability (precision) of the regression coefficient.

Selecting a model-building strategy is closely tied to the choice of independent variables. In logistic regression, two approaches are commonly employed, each having its own focus and purpose: direct (also known as full, standard, or simultaneous) and stepwise (known as Statistical). [20] These strategies are not necessarily interchangeable, as they can generate different model fit statistics and independent variable estimates from the same data. Thus, it is crucial to identify the appropriate model that aligns with one's research objectives. The direct approach is a default of sorts, as it enters all independent variables into the model at the same time and makes no assumptions about the order or relative worth of these variables. The direct approach is best if there is a priori knowledge of the independent variables and their relevance to the outcome variable. [21]

Stepwise regression identifies independent variables to keep or remove from the model based on predefined statistical criteria influenced by the unique characteristics of the sample being analyzed. Although stepwise regression is frequently used in clinical research, its use is somewhat controversial because it relies on automated variable selection that tends to take advantage of random chance factors in a given sample. Additionally, stepwise regression may produce models that do not seem entirely reasonable from a biological perspective. Given these concerns, some argue that stepwise regression is best reserved for preliminary screening or hypothesis testing only, such as with novel outcomes and limited understanding of

independent variable contributions. Accordingly, we preferred to use the direct (enter) method for model building in our study. [22]

6. Results

Our study included a total of 1,000 patient prescriptions, of which 580 patients were males (58%) and 420 were females (42%). The mean age of the patients was 71.85 ± 4.3 years, ranging from 63 to 86 years. The majority of the patients (76.3%) were between 66 and 80 years old. Patients were almost evenly distributed across the nine districts, each accounting for approximately 11% of the total population. **Table 1** shows the demographics of the study patients according to their assessed prescriptions.

The majority of assessed prescriptions (72.4%) were issued by specialist physicians, with internal medicine being the most common specialty (42.8%), as shown in **Table 2**. Additionally, 64.1% of prescriptions included three or more medications. Notably, 31.8% of prescriptions contained three medications, while 25.5% included four medications. These findings highlight the significant role of specialists in the prescribing process and the widespread occurrence of polypharmacy in older patients.

Table 1. Demographic characteristics for patients whose prescriptions were assessed

Patient demographics	(Mean ± SD)	(Min. - Max.)	Frequency (%)
Gender			
Males			580 (58)
Females			420 (42)
Age in years:	**(71.93 ± 4.24)**	**(65 - 86)**	
65 – 70			456 (45.6)
71 – 75			332 (33.2)
76 – 80			152 (15.2)
81 – 85			57 (5.7)
86 – 90			3 (0.3)
District			
Al Montazah Awal			111 (11.1)
Al Montazah Tany			111 (11.1)
Eastern District			111 (11.1)
Central District			111 (11.1)
Western District			111 (11.1)
Al Gomrok			111 (11.1)
Al Amreya			110 (11.0)
Al Agamy			112 (11.2)
Borg Al Arab			112 (11.2)

Table 2. Characteristics of prescriptions included in the study

Prescription characteristics	Frequency (%)
Prescribing Physician Grade	
General Practitioner (GP)	46 (4.6)
Specialist	724 (72.4)
Consultant	230 (23)
Prescription specialty	
Internal medicine	428 (42.8)
Neuro-psychiatry	88 (8.8)
Urology	125 (12.5)
Orthopedic	57 (5.7)
Chest	145 (14.5)
General Surgery	71 (7.1)
E.N.T.	40 (4.0)
Ophthalmology	46 (4.6)
Prescription's number of medications	
One	26 (2.6)
Two	244 (24.4)
Three	318 (31.8)
Four	255 (25.5)
Five	83 (8.3)
Six	49 (4.9)
Seven	24 (2.4)
Eight	1 (0.1)

The full model containing all predictors was statistically significant, χ^2 (20, N=1147) = 372.825,

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$p < .001$, indicating that the model was able to distinguish between respondents who were compliant versus non-compliant with Beers criteria 2023. The model explained between 31.1% (Cox & Snell R^2) and 41.6% (Nagelkerke R^2) of the variance in compliance status and correctly classified 77.5% of cases. The Hosmer-Lemeshow test was non-significant, χ^2 (8) = 14.954, $p = .060$, indicating good model fit. [21,22]

As shown in **table 3**, several independent variables made a unique statistically significant contribution to the model (district, physician grade, and the number of medications prescribed).

The findings show that the strongest predictor of non-compliance with Beers criteria 2023 among assessed prescriptions was the number of medications prescribed, recording an odds ratio of 3.234. This indicates that the odds of non-compliance are 3.234 times higher for each additional medication prescribed, while controlling for all other factors included in the model.

The findings show that the strongest predictor of non-compliance with Beers Criteria 2023 among assessed prescriptions is the number of medications prescribed, with an odds ratio of 3.234. This indicates that the odds of non-compliance are 3.234 times higher for each additional medication prescribed, while controlling for all other factors included in the model. Although the odds ratio for physician grade General Practitioner (GP) is higher in value, at 10.105, indicating that physicians with a grade of 1 have significantly higher odds of having non-compliant prescriptions, this higher odds ratio reflects a specific subgroup effect rather than a general trend applicable to all cases. The Beers criteria 2023 appropriateness status for the significant contributors is shown in **Table 4**, and a general Beers criteria 2023 appropriateness distribution according to the physician grade is shown in **Figure 1**.

Table 3: Logistic Regression Predicting Likelihood of Compliance with Beers Criteria

Predictor	β	SE	P-value	Odds Ratio	95% CI for Odds Ratio
District (Al Montazah Tany)	0.879	0.340	0.010	2.409	1.236 – 4.695
District (Eastern District)	1.177	0.339	0.001	3.245	1.678 – 6.277
District (Central District)	1.109	0.341	0.001	3.032	1.568 – 5.862
District (Western District)	0.973	0.332	0.003	2.646	1.400 – 5.000
Physician Grade (GP)	2.313	0.438	<0.001	10.105	4.288 – 23.799
Physician Grade (Specialist)	0.806	0.209	<0.001	2.240	1.496 – 3.355
Number of Medications Prescribed	1.174	0.085	<0.001	3.234	2.746 – 3.812
Constant	-6.939	1.419	<0.001	0.001	

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Table 4: Beers criteria 2023 categories appropriateness status for significant contributors

Predictor	Potentially Inappropriate medication n (%)		Potentially Inappropriate medication for a certain disease n (%)		Medication should be used with caution n (%)		The medication dose should be adjusted n (%)		Potential drug-drug interaction n (%)		Duplication n (%)	
	Appropriate	Inappropriate	Appropriate	Inappropriate	Appropriate	Inappropriate	Appropriate	Inappropriate	Appropriate	Inappropriate	Appropriate	Inappropriate
District												
Al Montazah Awal	99 (89.2)	12 (10.8)	98 (88.3)	13 (11.7)	89 (80.2)	22 (19.8)	98 (88.3)	13 (11.7)	95 (85.6)	16 (14.4)	99 (89.2)	12 (10.8)
Al Montazah Tany	79 (71.2)	32 (28.8)	91 (82)	20 (18)	88 (79.3)	23 (20.7)	94 (84.7)	17 (15.3)	93 (83.8)	18 (16.2)	91 (82)	20 (18)
Eastern District	86 (77.5)	25 (22.5)	86 (77.5)	25 (22.5)	98 (88.3)	13 (11.7)	97 (87.4)	14 (12.6)	93 (83.8)	18 (16.2)	99 (89.2)	12 (10.8)
Central District	83 (74.8)	28 (25.2)	86 (77.5)	25 (22.5)	91 (82)	20 (18)	95 (85.6)	16 (14.4)	89 (80.2)	22 (19.8)	94 (84.7)	17 (15.3)
Western District	87 (78.4)	24 (21.6)	90 (81.1)	21 (18.9)	96 (86.5)	15 (13.5)	100 (90.1)	11 (9.9)	89 (80.2)	22 (19.8)	104 (93.7)	7 (6.3)
Al Gomrok	93 (83.8)	18 (16.2)	89 (80.2)	22 (19.8)	99 (89.2)	12 (10.8)	100 (90.1)	11 (9.9)	94 (84.7)	17 (15.3)	104 (93.7)	7 (6.3)
Al Amreya	90 (81.8)	20 (18.2)	87 (79.1)	23 (20.9)	94 (85.5)	16 (14.5)	99 (90)	11 (10)	93 (84.5)	17 (15.5)	103 (93.6)	7 (6.4)
Al Agamy	97 (86.6)	15 (13.4)	91 (81.3)	21 (18.8)	99 (88.4)	13 (11.6)	103 (92)	9 (8)	97 (86.6)	15 (13.4)	106 (94.6)	6 (5.4)
Borg Al Arab	92 (82.1)	20 (17.9)	93 (83)	19 (17)	89 (79.5)	23 (20.5)	98 (87.5)	14 (12.5)	92 (82.1)	20 (17.9)	107 (95.5)	5 (4.5)
Physician Grade												
GP	33 (71.7)	13 (28.3)	33 (71.7)	13 (28.3)	31 (67.4)	15 (32.6)	43 (93.5)	3 (6.5)	33 (71.7)	13 (28.3)	40 (87)	6 (13)
Specialist	583 (80.5)	141 (19.5)	575 (79.4)	149 (20.6)	598 (82.6)	126 (17.4)	633 (87.4)	91 (12.6)	602 (83.1)	122 (16.9)	654 (90.3)	70 (9.7)
Consultant	190 (82.6)	40 (17.4)	203 (88.3)	27 (11.7)	214 (93)	16 (7)	208 (90.4)	22 (9.6)	200 (87)	30 (13)	213 (92.6)	17 (7.4)
Number of prescription medications												
1 - 3	533 (90.6)	55 (9.4)	523 (88.9)	65 (11.1)	560 (95.2)	28 (4.8)	558 (94.9)	30 (5.1)	574 (97.6)	14 (2.4)	581 (98.8)	7 (1.2)
4 - 6	265 (68.5)	122 (31.5)	269 (69.5)	118 (30.5)	277 (71.6)	110 (28.4)	306 (79.1)	81 (20.9)	257 (66.4)	130 (33.6)	320 (82.7)	67 (17.3)
7 - 8	8 (32)	17 (68)	19 (76)	6 (24)	6 (24)	19 (76)	20 (80)	5 (20)	4 (16)	21 (84)	6 (24)	19 (76)

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Table 5. Frequency distribution of the inappropriate medications identified based on the Beers criteria 2023

Drug name	Frequency	%
Indomethacin	281	28.13
Nitrofurantoin	130	12.96
Hyoscyamine	78	7.83
Amitriptyline	69	6.91
Ketorolac	22	2.18
Nifedipine (immediate release)	26	2.55
Chlorpheniramine	57	5.65
Thioridazine	18	1.82
Diphenhydramine (oral)	15	1.46
Methyldopa	30	3
Amiodarone	34	3.37
Metoclopramide	91	9.11
Digoxin	77	7.75
Spironolactone > 25 mg/day	32	3.18
Benztropine	16	1.63
Haloperidol	12	1.19
Chlorzoxazone	5	0.46
Orphenadrine	8	0.82

Table 6. List of the most frequent drug–drug interactions detected according to Beers criteria 2023

Drug – drug interaction	Frequency	%	Risk category
Amiodarone + Digoxin	25	14.5	D
Diclofenac + Furosemide	19	11	D
Carbamazepine + Phenytoin	13	7.5	D
Amiodarone + Warfarin	9	5.2	D
Captopril + Allopurinol	8	4.6	D
Others	99	57.5	

Table 7. List of the most frequent five drug class duplications detected according to the Beers criteria 2023

Drug class duplication	Frequency	%	AHFS* Code
Diclofenac + Ibuprofen	41	16.9	28:08.04.92
Allopurinol + Colchicine	35	14.4	92:16
Nitrofurantoin + Trimethoprim	25	10.3	8:36
Diclofenac + Indomethacin	24	9.9	28:08.04.92
Amitriptyline + Clomipramine	12	4.9	28:16.04.28
Others	106	43.6	

*American Hospital Formulary Services

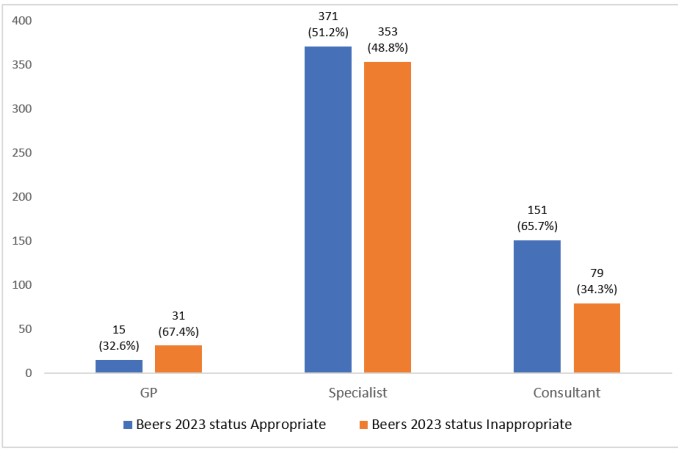

Figure 1. Distribution of appropriateness according to Beers criteria 2023 by physician grade

7. Discussion

Inappropriate prescribing can be attributed to various factors that must be addressed to optimize current prescribing approaches. Conceptually, prescribing can be seen as a function influenced by the patient, the prescriber, and the environment.

The primary factor in prescribing decisions should be the clinical needs of the patient. Prescribing should prioritize evidence-based therapies and minimize the use of medications that lack

clinical necessity or proven efficacy. Additionally, patients' expectations can considerably influence these decisions. [23]

Prescribing is primarily the responsibility of physicians who rely on their clinical experience and personal attitudes to make final decisions. Inadequate training in geriatric pharmacotherapy is a significant factor that can heavily contribute to inappropriate prescribing. For instance, some prescribers may avoid prescribing a medication or increasing its dose simply because the patient is an older patient, a practice known as ageism.

Additionally, inappropriate prescribing can arise from a lack of communication between physicians practicing in different settings or even between specialists practicing in the same setting. [23,24]

The environment in which the prescriber operates can significantly influence prescribing decisions. For instance, acute care settings often do not prioritize the review of patients' chronic and preventive medications. Additionally, a lack of systems or structures for sharing drug-related information during transitions between care settings can negatively impact the quality of care provided. [25]

It should be noted that several medications were not evaluated for various reasons. Some medications, such as Trimethobenzamide, Mesoridazine, and Desiccated Thyroid extract, were not available in Egypt. Additionally, some medications, such as Pentazocine and Amphetamines, are classified as controlled substances by the Egyptian Ministry of Health (MOH) and, consequently, cannot be dispensed at ordinary community pharmacies. These factors may contribute to the underestimation of inappropriate prescriptions. In our study, we identified the most prevalent potentially inappropriate medications according to the Beers Criteria 2023. This included indomethacin, a non-steroidal anti-inflammatory drug (NSAID); nitrofurantoin (antimicrobial); hyoscyamine (antispasmodic); amitriptyline (antidepressant); amiodarone (antiarrhythmic); methyldopa (antihypertensive); and chlorpheniramine (antihistamine). Together, these drugs accounted for approximately 87% of all potentially inappropriate medications. **(Table 5)**

The study identified that the most frequent drug–drug interaction (DDI) was between amiodarone and digoxin, accounting for 14.5% of all interactions. This combination significantly increases the risk of digoxin toxicity due to amiodarone's inhibition of P-glycoprotein, which reduces digoxin clearance. Clinical consequences may include bradycardia, arrhythmias, and gastrointestinal disturbances, necessitating close monitoring of serum digoxin levels and dose adjustments [26,27].

Other notable DDIs included diclofenac with furosemide (11%) and carbamazepine with phenytoin (7.5%). Diclofenac may blunt the diuretic and antihypertensive effects of furosemide, posing risks of fluid retention, hypertension, and renal impairment, particularly in elderly or renally compromised individuals [28]. The carbamazepine-phenytoin combination presents risks of central nervous system toxicity, sedation, and potential hepatic enzyme induction, leading to altered plasma levels and reduced seizure control [29]. All of these interactions fall under risk category D, as per standard drug interaction classifications, indicating the need for careful monitoring and potential therapy modification (Table 6).

In terms of drug class duplications, the most frequent combination was diclofenac and ibuprofen, observed in 16.9% of duplication events. Both agents belong to the NSAID class, and their concurrent use substantially increases the risk of gastrointestinal bleeding, cardiovascular complications, and renal dysfunction, concerns explicitly highlighted in the American Geriatrics Society (AGS) 2023 Beers Criteria [8].

Other frequently observed duplications included allopurinol with colchicine (14.4%), which may enhance the risk of myopathy, bone marrow suppression, and gastrointestinal intolerance, especially in patients with impaired renal function [30]. The combination of nitrofurantoin with trimethoprim (10.3%) may lead to additive nephrotoxicity and hematological adverse effects, and nitrofurantoin in particular is contraindicated in older adults with poor renal function [8,32].

Of special concern is the duplication of tricyclic antidepressants, such as amitriptyline with clomipramine (4.9%), which amplifies anticholinergic burden, increasing the risk of delirium, falls, and cognitive decline in older patients [8,33].

These findings underscore the critical importance of integrating comprehensive medication reviews, clinical decision support tools, and evidence-based resources such as the Beers Criteria into routine practice. Doing so can significantly reduce the risk of preventable

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adverse drug events, particularly among older adults and those with complex medication regimens.

Comparing our findings with existing literature, we observed that indomethacin, amitriptyline, methyldopa, and antihistamines are consistently identified as potentially inappropriate medications, aligning with previous studies [33-35]. However, there were differences in other medications, highlighting the variability in the application of the Beers Criteria across different countries.

The odds ratio of 10.105 for physician grade (GP) indicates significantly higher odds of non-compliant prescriptions. However, this effect is specific to a particular subgroup and should not be generalized across all study cases. This distinction underscores the difference between "general applicability" and "subgroup effect".

"General Applicability" means that the number of medications prescribed, as a continuous variable, is a universal predictor of non-compliance with the 2023 Beers criteria. This implies that as the number of medications increases, the likelihood of non-compliance rises for all patients, regardless of other factors. Conversely, "Subgroup Effect" refers to the physician grade (GP) being a categorical variable with a higher odds ratio, but its influence is specific to a subset of the data (patients treated by grade GP physicians). While the impact of physician grade is significant, it is limited to a particular group of patients, rather than being a universal factor across all patient prescriptions. [20]

The number of medications prescribed has a cumulative effect, with each additional medication increasing the odds of non-compliance by a factor of 3.234. This incremental increase quickly compounds, making it a strong predictor of non-compliance. On the other hand, the odds ratio of 10.105 for physician grade (GP) reflects a single comparative effect rather than an accumulating risk. The number of medications prescribed likely varies widely and frequently among patients, exerting a more consistent influence on compliance compared to the relatively stable category of physician grades.

In summary, the number of medications prescribed is considered the strongest predictor of non-compliance with the Beers criteria 2023 due to its broad applicability, cumulative impact, and consistent influence across the entire patient population, despite the higher odds ratio associated with physician grade (GP).

Managing the complexity of medication regimens is hence crucial for improving compliance with the 2023 Beers criteria. Roux et al. (2020) conducted a retrospective population-based cohort study using the Quebec Integrated Chronic Disease Surveillance System (QICDSS). This system monitors drug claims for older adults aged 65 and above, living in the community, who have chronic diseases or are at risk of developing them, and are covered by public drug insurance plans. The study found that having more medications and multiple chronic diseases, especially mental disorders (RR: 1.50; 95% CI: 1.49–1.51), significantly affected medication management. [36]

The overall findings in assessing compliance with the Beers Criteria 2023 clearly emphasize the significance of regional variations, physician qualification levels, and the complexity of prescribed medication regimens in determining the prescription status as either compliant or non-compliant with the Beers Criteria 2023. By focusing on these areas, interventions may increase adherence and lead to better patient outcomes.

A systematic review by Garcia (2006) identified five strategies to minimize inappropriate prescribing in older adults: seeking pharmacist recommendations, using computerized alerts, reviewing patient medications, applying Beers' criteria, and educating patients to enhance compliance. [37] Other research has shown that methods like geriatric medicine services, pharmacist participation in patient care, and computerized decision support can improve prescribing appropriateness for older patients in various settings. [38, 39].

8. Conclusions

Building on these findings and recommendations, we propose standardizing prescribing systems, particularly for the older population, to minimize inappropriate prescribing practices. Integrating updated Beers criteria into the prescribing systems for older patients to serve as reliable clinical decision support tools could be very beneficial, especially given the implementation of Egypt's universal health insurance system. This approach aims to achieve the ultimate objective of reducing the economic burden and improving patient well-being by avoiding inappropriate prescribing in the older population.

List of abbreviations:

LMICs	Low- and Middle-Income Countries
AGS	American Geriatrics Society
PIM	Potentially Inappropriate Medication
GP	General Practitioner
AHFS	American Hospital Formulary Services
SE	Standard Error
MOH	Ministry of Health
NSAID	Non-steroidal anti-inflammatory drug
QICDSS	Quebec Integrated Chronic Disease Surveillance System
(UN)	United Nations

Declarations:

Ethics approval and consent to participate:

"An ethical approval was obtained and uploaded as a supplementary file."

Consent for publication:

'Not applicable'

Consent:

'Not applicable'

Availability of data and materials:

All data generated or analysed during this study are included in this published article [and its supplementary information files].

Competing interests:

'Not applicable'

Funding:

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Authors contributions:

Both authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by both authors. The first draft of the manuscript was written by **Hesham Metwalli Abd El Moneim Mousli** and both authors commented on previous versions of the manuscript. both authors read and approved the final manuscript.

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'Not applicable'

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Medicinal Mushrooms: A Review of Bioactive Compounds, Pharmacological Mechanisms and the Translational Roadmap to Clinical Therapeutics

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ABSTRACT

Mushrooms, which have gained intriguing recognition in ethnobotany due to their affordability and eco-friendliness, remain underutilized despite their potential as therapeutic agents. Abundant evidence supports their long-term effectiveness against various health disorders, including immunodeficiency, cancer, and metabolic diseases. However, their clinical applications have faced substantial limitations. We conducted a critical analysis of the existing key findings on mushroom bioactive compounds, such as polysaccharides, terpenoids, and phenolics, and explore the molecular mechanisms underlying their effective immunomodulatory, anticancer, antiviral, and metabolic-modulating activities. While highlighting the significant therapeutic prowess of mushrooms, we also examined the development pathway, identifying the key challenges in standardization, quality control, pharmacokinetics, toxicology, and regulatory frameworks. Our findings highlighted the convincing necessity for tailored research and effective development approaches to reconcile disparities between conventional applications, laboratory studies, and human trials, thus uncovering the rich pharmaceutical prospects of mushrooms for novel, holistic therapeutic models.

KEYWORDS

Mushroom-derived compounds, Immunomodulation, Pharmacological properties, Therapeutic applications, Clinical translation, Regulatory frameworks

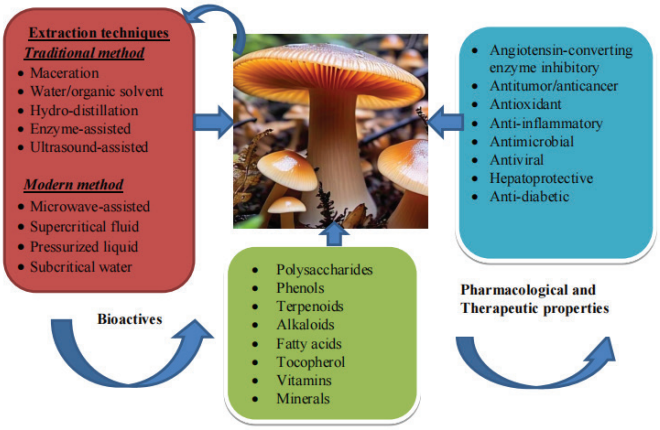

Fig. 1 Graphical abstract of the review

1. Introduction

The global spike in the quest for affordable and eco-friendly panacea for skyrocketing health-threatening diseases has stimulated research interest in novel sources with inherent prospective nutritional, industrial, and pharmaceutical benefits. Mushrooms have gained recognition as a potential source of nutrients and therapeutics due to their unique characteristics and diverse bioactive compounds. Abundant evidence has supported the utilization of mushrooms in traditional medicine for centuries for the treatment of certain human disorders. The recognition and utilization of mushrooms and their products vary globally, with Asian Asian cuisine notably valuing their nutritional and medicinal benefits. Their nutritional richness, including minerals, vitamins, and essential amino acids, accounts for classifying them as "superfoods" [1]. The increasing demand for bioactive foods and nutraceuticals, coupled with the predicted rise in global protein demand, has further spotlighted the prospects of mushrooms

as a beneficial source for enhancing public health and global wellness. Research has found mushrooms to contain a plethora of bioactive compounds, encompassing diverse polysaccharides, proteins, phenols, terpenoids, and alkaloids, which have been established to show various pharmacological effects, including antioxidant, antidiabetic, antiviral, anti-inflammatory, antimicrobial, antitumor, and anticancer activities [2-4]. The use of mushrooms for medication has grown beyond the traditional aspects, given their notable impacts in the management of various global health maladies, including cardiovascular diseases, viral conditions, and cancer [5-7]. Mushroom-based compounds have been found to exhibit these properties by modulating various biological pathways [8-10]. The significant advantages associated with mushroom production, such as sustainability, simplicity, efficiency, cost-effectiveness, and environmental friendliness, have also strengthened its position as a promising source of nutrition and bioactive ingredients. Mushroom production employs fruiting bodies and fungal biomass cultivation, solvent-assisted methods, precipitation, and novel technologies [1].

Plate 1 Oyster mushroom at Enugu-Ngwo, Enugu North in Enugu State, Nigeria

(A & B = matured mushroom that have been harvested; C = media containing mushroom spores being stacked, D= well-stacked spores inoculated on its media)

2. Methods

A comprehensive literature search was conducted using PubMed, Springer, ScienceDirect, Scopus, and Google Scholar. Peer-reviewed articles published in English between 2018 and 2025 were exclusively searched. Relevant studies were identified by using strategic keyword combinations and phrases, such as "mushroom bioactive compounds," "ethnopharmacology of mushrooms," and "mushroom pharmacology." The search yielded various studies that examined the phytochemical, ethnobotanical, and pharmacological properties of mushrooms. The retrieved articles were meticulously reviewed.

Bioactive chemical composition and nutritional value of mushrooms

Edible mushrooms are promising alternative protein sources, offering high protein levels ranging from 6.60 to 36.87 g/100 g dry weight [4, 11]. However, mushroom protein content depends on several factors like species, strain, maturation stage, substrate, and environmental factors. Compared to other protein sources (animals and plants), some mushrooms have protein values similar to milk, eggs, meat, and fish, while many mushrooms exceed legumes, cereals, nuts, and seeds in protein content. For nutritional value, 100 g of mushrooms can provide 29.41-66.0 % of the recommended dietary allowance (RDA for men and 35.80-80.35 % for women [4]. With respect to protein efficiency ratio (PER), several mushrooms like *Pleurotus ostreatus*, *Agaricus bisporus,* etc., have PER comparable to or exceeding beef jerky and legumes [4, 12]. The nutritional content of mushrooms, which are biologically important, includes: carbohydrate, protein, fat, fatty acid, mineral, vitamins, fiber, etc. [7]. In addition to the nutritional usefulness of mushrooms, many studies have indicated their rich bioactive chemical compositions (Table 1), which are of immense pharmaceutical significance [10]. These bioactive metabolites are polysaccharides, polyketides, terpenes, beta-glucans, polysaccharide-protein complexes, lectins, fungal immunomodulatory proteins (FIP), ribosome-inactivating proteins (RIP), antimicrobial/antifungal proteins, ribonucleases, laccases, and phenolic compounds [4, 13-14]. Wang et al [15] found a new compound (A homogeneous α-glucan (AM-1)) from *Agaricus blazei* coupled with the bioactive compounds previously reported. The advantages of mushrooms as a source of protein and bioactive compounds are worth noting (Figure 2); they can be produced more easily, cheaply, and with less environmental impact (sustainable production).

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It can also offer a promising option for those seeking plant-based or sustainable protein sources and bioactive compounds.

Table 1: Some chemical compositions of mushrooms and their potential pharmacological activities

Compounds	Type	Biological activities	References
Polysaccharides	β-glucans, chitin, mannose, mannoglucan, galactose, xylose, lentinan, hetero-galactomannan	Antioxidant, anti-inflammatory, anti-acetylcholinesterase, antitumor, antidiabetic,immune-enhancing, antimicrobial, hepatoprotective, and hypoglycemic effects	[4, 6]
	N/A	Anticancer, anti-inflammatory, antiviral, antioxidant, and hypercholesterolemic effects	[4, 6]
Phenols	Phenolic acids, flavonoids, tannins, lignans, hydroxycinnamic acid, polyphenols, and hydroxybenzoic acid	Antimicrobial, antioxidant, anti-inflammatory, and inhibitory activities against tumor effects	[6, 10]
Terpenoids	Ganoderic acids, ergosterol, fungal sterols	Anti-inflammatory, inhibitors of inflammatory mediators, and anticancer activities	[6, 16]
Flavonoids	Isorhamnetin, genkwanin, icariin, acacetin, kaempferol, eriocitrin, silymarin, silibinin, and apigenin	Anti-mutagenic, anti-inflammatory, anti-allergic, antiviral, and antitumor effects	[6, 10]
Alkaloids	Indoles (psilocybin), isoxazoles, tropanes, piperidines, quinolones	Anticancer activities	[17]
Steroids	N/A	Inhibit cancer cells, antiviral, antibacterial, immunomodulatory, and antidiabetic properties.	[6, 16]
Fatty acids	Linoleic acid, oleic acid, linolenic acid	Anti-inflammatory, pain-regulatory, antimicrobial, cardiovascular, and antioxidant activities	[18]
Tocopherol	N/A	Vitamin E activity, antioxidant, antimicrobial, and anti-quorum sensing effects	[6]
Vitamins	Vitamin D, B, C	Antioxidant, immunomodulatory, and anti-inflammatory effects	[10]
Minerals	Macro and micro elements	Antioxidant, anti-metabolic disorder, and wound-healing effects	[15-16]

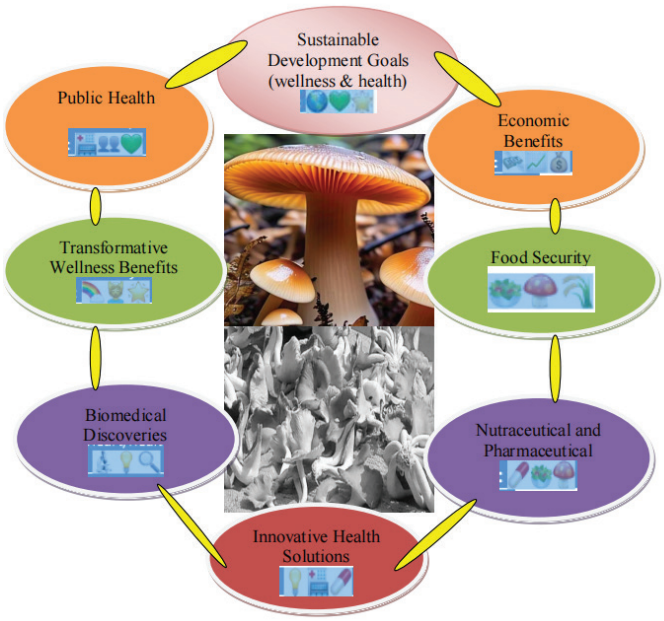

Fig. 2 Transformative potential of medicinal mushrooms: a summary framework

Isolation and identification of bioactive secondary metabolites from mushrooms

Conventionally, the main solvents used for the extraction of bioactive compounds from mushrooms are water and organic solvents. Water extraction offers moderate efficiency but stands the risk of degrading heat-sensitive components. A mixture of water-alcoholic extraction provides better extraction but involves a higher cost due to the solvent used. Maceration as a traditional technique is simple, reliable, but potentially time-consuming. The hydro-distillation method is a commonly employed technique, but it may require optimization for quality results. Enzyme-assisted extraction offers enhanced yield, reduced temperature, and extraction time, but is technically challenging. Furthermore, since it leverages the enzymatic degradation of cell walls, it offers quick release of bioactive substances. It has been employed for the effective extraction of certain compounds from mushrooms, like polysaccharides and enzymes. Nevertheless, the maintenance of enzyme stability and activity is a critical challenge for this method [6]. To mitigate the shortcomings associated with the conventional methods of extracting mushroom bioactive compounds, modern techniques have been developed; these include: (a) Ultrasound-assisted extraction (UAE), offering enhanced efficiency with reduced solvent use (b) Microwave-assisted extraction (MAE) extraction ensures efficiency and reduces thermal degradation (c) Supercritical fluid extraction (SFE) employs CO_2, yields higher extraction rates, especially for non-polar compounds (d) Pressurized liquid extraction (PLE) works based on optimized conditions, offering high extraction efficiencies (e) Subcritical water extraction boosts extraction yields without requiring high solvent volumes [6, 19]. In summary, modern extraction approaches generally offer higher efficiency and reduced solvent use, help prevent thermal degradation of bioactive compounds; however, some modern techniques like SFE require specialized equipment.

Factors affecting the bioactive disposition of mushrooms

Mushrooms contain many bioactive compounds, including polysaccharides, polyketides, phenolic compounds, and so on. The disparity in bioactive chemical composition of mushrooms is determined by certain factors, including species, cultivation conditions, and method of extraction [20-21]. According to Kumari et al [22], mushrooms' genetic disposition and their developmental history influence the chemical composition. Different mushroom species display distinctive genetic content, expressing unique bioactive compounds; of the many species of mushroom studied, *Ganoderma lucidum*, *Hericium erinaceus*, *Cordyceps militaris*, *Lentinus edodes*, and *Trametes versicolor* are thought to possess more pharmaceutical values. According to Parise *et al* [9], mushroom species suspected to possess activities against an array of COVID-19 infections include: *Agaricus blazei Murill, Ganoderma lucidum, Hericium erinaceus, Grifola frondosa, Inonotus obliquus, Lentinus edodes,* etc. The relationship between mushroom species and bioactive compound production is validated by *Inonotus obliquus* (Chaga), possessing a high proportion of polyketides, contrary to *Ganoderma lucidum* (Reishi), which contains a high profile of polysaccharides [15]. Furthermore, according to studies by Kumari et al [22] and Li et al [23], conditions including humidity, soil quality, temperature, and light, constituting environmental factors or cultivation conditions, have a tremendous impact on the chemical composition of mushrooms. For example, it has been observed that an increase in temperature enhances the synthesis of some bioactive substances in mushrooms, e.g., polysaccharides. Also, the yield of bioactive compounds like phenolic compounds has been improved by drought stress. Additionally, different extraction techniques, including supercritical fluid extraction, solvent extraction, and steam distillation, have been demonstrated to affect both the production and chemical constituents of mushrooms' bioactive compounds. Typically, more polysaccharides were obtained using

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ethanol than by distillation. Regarding phenolic compounds, a higher yield can be recorded by supercritical fluid extraction compared to extraction by the solvent method [24].

Pharmacological and therapeutic properties of mushrooms

Many studies have reported that the pharmacological potentials of mushrooms hold great promise. Interestingly, research has indicated immune improvement, lipid depletion, anticancer, anti-inflammatory, antioxidant, hepatoprotective, antitumor, antiviral, and several other biological activities as the pharmacological effects of mushroom extracts and their derived compounds (Table 2) [4, 6]. These pharmacological dispositions and the determinants of therapeutic potentials are thought to be due to the bioactive contents of the mushroom [15]. Edible mushroom proteins exhibit various therapeutic benefits, including: improved digestion, boosting nutrient absorption and immune function modification; anticancer properties, inhibiting cancer cell growth, inducing apoptosis, and arresting cell cycle progression; antimicrobial activity against bacteria, fungi, and other pathogens. Additionally, the bioactive protein portions of edible mushrooms like protein concentrates, hydrolysates, and peptides, Lectins, fungal immunomodulatory proteins, ribosome-inactivating proteins, and laccases boosted digestibility and potential health benefits, linked to ACE inhibitory, antioxidant, and anticancer immunomodulatory, antimicrobial, and antifungal activities [4,6].

Table 2: Therapeutic properties of bioactive proteins isolated from mushrooms

Bioactive protein	Biological activities	Mushroom sp	Nature	Reference
Protein hydrolysates	Neuroprotective, antioxidant, cytoprotective	*Pleurotus geesteranus*	hydrolysates	[4, 13]
Protein hydrolysates	Antioxidant, hepatoprotective, and antiproliferative, ACE inhibitory,	*Pleurotus ostreatus*	hydrolysates	[25-26]
Protein hydrolysates	Antioxidant, Lowers peroxidation (lipid)	*Ganoderma lucidum*	hydrolysates	[4]
Ribonuclease	Antiviral (HIV)	*Lepista personata*	ribonuclease (27.8 kDa)	[4, 27]
Ribotoxin	NA	*Cyclocybe aegerita*	ribotoxin-like enzymes (15 kDa)	[4]
Ostreatin	Novel biotechnological tool	*Pleurotus ostreatus*	ribotoxin-like proteins (131 amino acids and 14,263.51 Da)	[4]
Extract	Antitumor	*Pleurotus tuber-regium* *Pholiota nameko* *Boletus edulis*	Protein extract Protein extract Protein extract (16.7KD)	[28] [29] [30]
Extract	Antimicrobial	*Auricularia auricula-judae* *Mushroom (Oyster and button)* *Inonotus hispidus*	Aqueous protein extracts Protein extracts Proteins, peptides, and other compounds	[4] [4] [4]
Peptides	Antioxidant	*Agaricus bisporus*	Peptides, 1–3 kDa portion	[4]
Peptides	Antioxidant, ACE inhibitory	*Boletus mushroom*	KBMPHF1 (> 10 kDa), KBMPHF2 (3–10kDa) KBMPHF3 (1–3 kDa), and KBMPHF4(1 kDa)	[4]

The angiotensin-converting enzyme inhibitory activities

The bioactive peptides from mushrooms have been found to demonstrate angiotensin-converting enzyme (ACE) inhibitory effects, potentially treating hypertension. Peptides showing ACE inhibitory activities have been isolated from *Lentinula edodes* as 1265.43 Da and N-terminal (KIGSRSRFDVT [31], *S. rugosoannulata, Agaricus bisporus, Ganoderma sinense,* and *Grifola frondosa* in the form of peptide mixtures [4, 15, 23]. The ability of mushrooms to reduce blood pressure and cholesterol volume, prevent platelet accumulation and thrombosis, and shield from cardiovascular disease demonstrates their potential in ensuring cardiovascular health [10, 15]. Studies have highlighted that several mushroom species, like *Lentinula edodes*, *Stropharia rugosoannulata, and Grifola frondosa*, have produced peptides possessing ACE inhibitory effects. Additionally, *novel* peptides obtained from the hydrolysis of *Agaricus bisporus* scraps produced three ACE inhibitory peptides (LVYP, VYPW, and YPWT) displaying temperature, pH, and tolerance to digestive enzymes. These peptides work by binding to ACE active sites, zinc ions, or critical amino acids, inhibiting ACE activity [4]. Mushroom peptides may offer a promising alternative to synthetic ACE inhibitors, with potential benefits including fewer side effects and blood pressure reduction. Notwithstanding, clinical studies are crucial to confirm the efficacy and safety of mushroom peptides as ACE inhibitors.

Antioxidant properties

Mushroom-derivative compounds have been found to possess antioxidant activities, employing various mechanisms of action, including neutralization of free radicals, binding to metal ions to prevent oxidative reactions, hampering lipid damage, influencing cellular signaling pathways, and natural antioxidant defense architecture of the body [6]. Studies have uncovered that these mechanisms of action utilized by mushroom bioactive metabolites may be direct or indirect; it is direct if protons or electrons are mobilized to eradicate the free radicals, but when chelating metal ions and endogenous oxidases (enzymes) are impeded, it is indirect [4]. These bioactive compounds exhibit antioxidant properties, potentially mitigating oxidative stress and the diseases associated with it, such as aging, cancer, and atherosclerosis. Research has identified polysaccharides, phenolic compounds, triterpenoids, erinacines (unique diterpenoids), and ergothioneine as bioactive compounds from mushrooms demonstrating antioxidant activities. The bioactive compounds, particularly peptides involved in antioxidant activities, have been found to possess distinct characteristics such as amino acid composition (5-16 amino acids), molecular weight (0.65-3 kDa), and hydrophobic moiety, which enhance their antioxidant capabilities [4]. The mushroom species harbouring antioxidant bioactive compounds include *Schizophyllum* commune, *Hericium erinaceus, Agrocybe aegerita, and Ganoderma lucidum. Inonotus obliquus*, *Pleurotus ostreatus, Lentinula edodes*, *and Agaricus bisporus* [6, 10]. This review predicts the integration of mushroom-derived antioxidant compounds into functional foods, as well as the development of nutraceuticals and therapeutic agents.

Antitumor / anticancer effects

Research has found that the bioactive peptides from mushrooms exhibit antitumor activities, with potential therapeutic potency. These peptides employ two mechanisms of action, including mitochondrial-dependent pathway (obstructing cell proliferation and inducing apoptosis), and antioxidant and ACE inhibitory activity (enhancing antitumor properties). The bioactive compounds from mushrooms have demonstrated various anticancer activities by triggering programmed cell death, stimulating immune cells, suppressing vascular endothelial growth factor (VEGF) expression, mitigating oxidative stress, and cell cycle arrest, as mechanisms of action [6]. The bioactive compounds associated with anticancer properties include β-glucans (activate apoptotic pathways and stimulate immune cells), triterpenoids (suppress VEGF expression, blocking angiogenesis), and polysaccharides (stimulate immune cells and induce cell cycle arrest). β-glucans have displayed potential in treating numerous cancers, including gall bladder, liver, and breast cancer. Clinical trials have been conducted on mushroom-derived compounds, showing therapeutic potential.

Mushrooms, such as *Grifola frondosa* (Maitake) and *Agaricus blazei* (AndosanTM, made from its mycelium), have demonstrated anticancer activities. Again, studies highlighted numerous mushroom *novel* peptides: *Morchella importuna* peptide (MIPP) showed antitumor activity against human cervical cancer HeLa cells; Boletus mushroom peptides demonstrated antioxidant, ACE inhibitory, and anticancer activities; King Boletus mushroom protein hydrolysate (eb-KBM) displayed significant antioxidant, ACE inhibitory and anticancer activities against

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lung carcinoma and hepatocarcinoma cells [26]. The biological activities of eb-KBM have been linked to its rich hydrophobic and amino acid constituents. Again, *Cyclocybe aegerita*–derived ageritin has displayed antiproliferative properties, exhibiting selective toxicity against cancer cells. Also, the bioactive protein/protein extracts from edible mushrooms exhibited anticancer activities against various cancer cells, such as human non-small-cell lung cancer cells (*Boletus edulis* antitumor protein, BEAP), breast cancer (*Pleurotus tuber-regium* protein extract, PS60), and human breast cancer cells (*Pholiota nameko* protein, PNAP) [4]. Peptides reveal promise as potential antitumor agents, with further research required to explore their therapeutic potential.

Antimicrobial and antiviral activities

The bioactive compounds from mushrooms have demonstrated antibacterial, antifungal, and antiviral properties by disrupting microbial colony formation and causing the leakage of nuclear materials (DNA and protein). The antiviral activities displayed by bioactive compounds isolated from mushrooms involved diverse mechanisms of action, such as inhibiting viral replication, inducing overexpression of antiviral genes, enhancing immune cell activity and production, and preventing viral attachment to host cells [6]. Numerous studies have confirmed the antibacterial, antifungal, and antiviral effects of mushrooms [6, 15]. Several bioactive compounds isolated from different species of mushrooms, such as *Agaricus bisporus, Pleurotus ostreatus, Sanghuangporus sanghuang, Thelephora palmata, Hygrophorus crispus, Gyroporus castaneus, Neoboletus luridiformis, Gyromitra esculenta, and Lentinula edodes,* exhibited inhibitory activities against pathogens: *Staphylococcus aureus, Clavibacter michiganensis*, *Burkholderia aglumae*, *and Peptobacterium carotovorum*. Furthermore, research has found that bioactive compounds like triterpenoids (isolated from *S. sanghuang*), panisaldehyde (found in *P. ostreatus*), polyacetylenes and sulphur compounds (present in *L. edodes*), and terpenes (extracted from *F. velutipes* mycelium culture filtrate) exhibited antimicrobial properties [6]. The beta-glucans, flavonoids, ergosterol, and ganoderic acid contents play vital roles in the antimicrobial and antitumor properties of mushrooms [4]. Additionally, Liu et al [1] indicated the antiviral and antifungal effects of mushrooms. It is worth noting that peptides (mixtures of peptides) isolated from *Pseudoplectania nigrella*, *Russula paludosa*, and *Clitocybe sinopica* displayed significant effects against COVID-19 [32]. This property is possible due to its ability to bind and compromise the integrity of certain coronavirus proteins, such as the ACE-associated carboxypeptidase, the SARS-CoV HR2 Domain, and the COVID-19 major protease enzyme. Furthermore, a ribonuclease (ageritin) obtained from *Cyclocybe aegerita*, an edible fungus, displays antifungal, antibacterial, entomotoxic, antiviral, and nematotoxic activities. Its inhibitory effects against HIV-1 reverse transcriptase have also been indicated by research [4, 6]. Interestingly, it demonstrates these biological functions by exhibiting ribonucleolytic (cleaving phosphodiester linkages in 23-28S rRNAs, hampering protein synthesis, and causing apoptosis) and endonuclease activities (acting on plasmids and genomic DNAs) [4]. Rijia et al [6] indicated that the mushroom-derived compounds found to possess antiviral properties are: (a) Polysaccharide peptide (PSP), which shows antiviral effects by hindering viral replication and triggering antiviral genes; (b) Lentinan (LTN1) that boosts immune cell activity and production, enhancing defenses against infections; (c) Heliantriol F, semicohlidinol A, and semicohilidinol B, thought to possess antiviral activity against chikungunya virus; and (d) Polysaccharide Krestin (PSK), which exhibits antiviral effects on HIV and cytomegalovirus. In summary, the mushroom bioactive compounds exhibit antibacterial activities, adopting several mechanisms of action, including inhibition of metabolic pathways, disruption of polymerases, obstruction of protein synthesis, destruction of cell membranes, destruction of cell walls, and nucleic acid damage.

Anti-inflammatory properties

The bioactive compounds isolated from mushrooms have exhibited significant anti-inflammatory effects, engaging various mechanisms of action, including regulation of cytokine production, reducing inflammation, influencing immune cell activity, mitigating excessive inflammation, reducing oxidative stress, contributing to anti-inflammatory effects, influencing enzymatic activity, further reducing inflammation [6 Rijia et al. 2025]. Studies have identified mushrooms with anti-inflammatory activities: *Pleurotus florida*, *Sanghuangporous sanghuang*, *Trametes versicolor*, *Dictyophora indusiata*, *Grifola frondosa*, and *Pleurotus eryngii*. Bioactive compounds from mushrooms linked to anti-inflammatory activity are flavonoids, β-linked polysaccharides, ascorbic acid, beta-carotene, and lycopene [6, 33]. *Cordyceps militaris novel* selenium peptides (Se-Ps),

VPRKL(Se)M (Se-P1) and RYNA(Se) MNDYT (Se-P2) exhibited anti-inflammatory activities against lipopolysaccharide-stimulated inflammatory and oxidative stress around the colon-brain axis [4]. This activity is demonstrated by impeding the generation of inflammatory cytokines. Additionally, studies indicated that Se-Ps enhanced the functions of the intestinal mucosa and gut microbiota dysbiosis. *Tricholoma matsutake* peptides, SDIKHFPF and SDLKHFPF, suppressed ethanol-dependent cytokine-mediated responses by NF-κB inhibition and programmed cell death [23]. *H. erinaceus* metabolites have been found to possess the ability to combat inflammation and oxidative stress, critical determinants in neurodegenerative disorders. This metabolite may present a natural source for therapeutic agents targeting neuroinflammation and oxidative damage, which is substantiated by the suggestion that the metabolites may be developed into therapeutic agents for the treatment of Alzheimer's and Parkinson's diseases [6]. Again, it is worth noting that the ethanol extract of *P. eryngii* shows potential for the enhancement of neurological health. Edible mushroom bioactive compounds, like proteins, demonstrate potential therapeutic applications in disease prevention and cure, necessitating further research and exploitation. Continued research into mushroom bioactive compounds may lead to new natural anti-inflammatory agents.

Hepatoprotective properties

Studies have uncovered the liver-shielding properties of mushroom bioactive constituents. The bioactive compounds, polysaccharide-peptides (PSI and PSII) from *Pleurotus citrinopileatus* demonstrated potency in the treatment of non-alcoholic fatty liver disease (NAFLD) [34]. It also regulates gut microbiota and improves liver function [4]. A paracetamol-induced hepatitis in a rat model treated with *Volvariella volvacea*, *Lentinula edodes*, *Flammulina velutipes*, *Auricularia auricular*, *Tremella fuciformis*, *Grifola frondosa*, and *Tricholoma lobayense* water extracts exhibited hepatoprotective activities after incubation [10].

Antidiabetic effects

The number of diabetic patients is projected to hit about 380 million, from the reported 190 million, in the next 5 years (2030), stimulating research for natural sources of antidiabetic bioactivity [6]. Mushrooms and their derivatives have been proven to adopt a multifaceted approach while displaying antidiabetic effects, including: enhancement of insulin sensitivity, blocking the activities of enzymes participating in glucose metabolic processes, affecting glucose production in the hepatic region, reducing oxidative stress linked to free radicals, and exhibiting anti-inflammatory activity [6, 35]. This compelling mechanistic fact positions mushrooms for therapeutic exploitation in antidiabetic therapy. Diverse mushroom species indicated to possess antidiabetic properties include: *Pleurotus abalonus, Pleurotus florida, Pleurotus pulmonarius, Agaricus blazei Murill, Pleurotus eryngii, Cordyceps sinensis, Agaricus subrufescens, Hericium erinaceus,* and *Cordyceps militaris* [6, 36]. Findings revealed that mushroom bioactive compounds demonstrate antidiabetic effects, like polysaccharide-protein complexes present in *P. abalonus*, displaying hypoglycemic effects, phenolic compounds found in *P. florida*, playing a crucial role in antidiabetic activity, β-glucans and oligosaccharides present in *A. subrufescens*, boosting insulin resistance, and fractions of polysaccharide (CSP-1 found in *C. sinensis* trigger insulin production. Potentially, mushrooms may offer a valuable dietary component for managing diabetes, insulin sensitizers, and anti-hyperlipidemic agents. For clinical applications of mushroom-derived compounds in diabetes management, further research is required for the specific identification of compounds involved in antidiabetic activities [6].

Safety, toxicity, and quality control of mushroom-based products: Current status, challenges, limitations, and prospects

The pharmacological effects exhibited by a wide range of mushroom species, including antimicrobial, antiviral, anti-inflammatory, antidiabetic, hepatoprotective, angiotensin-converting enzyme inhibitory, antioxidant, and anticancer activities, have earned them an enviable position in the pharmaceutical landscape [6]. These pharmaceutical properties of mushrooms have stimulated well-known research interest currently. The clinical setbacks elicited by mushroom-related safety and toxicity concerns require attention to strengthen their effective use as therapeutic agents. Various plans can be employed to mitigate these issues, which include identifying mushroom species accurately by experts, standardizing extraction and testing procedures, thorough examination for safety and efficacy profile, and adequate adherence to safety regulations. Additionally, with research confirming the presence of certain toxic compounds in mushrooms, evaluation of mushrooms and their derived products to

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ascertain their toxicity and side effects on public health becomes very imperative.

Furthermore, pharmacokinetic studies are essential for determining the bioavailability, absorption, distribution, and elimination of mushrooms and their derivative products in the human system. Clinical trials are also crucial to evaluate the safety and efficacy of these products [37]. These trials, encompassing Phase I, Phase II, and Phase III, help to establish the efficacy, safety, and optimal dosing regimens for various applications. Clinical trials of mushrooms and their based products have faced significant limitations, including variable mushroom constituents and quality, poor understanding of bioavailability and pharmacokinetics, and interaction with several other medications. Abundant evidence has confirmed that clinical trials conducted on the Reishi (*Ganoderma lucidum*) extract for anticancer effects, Shiitake (*Lentinula edodes*) extract for immunomodulatory activities, and Chaga (*Inonotus obliquus*) extract for antimicrobial and antioxidant properties have recorded huge success [15]. Processes, such as validation and standardization, policy adherence verification, and regulatory framework, must be thoroughly confirmed before any products of plant origin can be accepted by the global community [38]. To ameliorate the molecular weight-associated limitation polysaccharides face from production to extraction, particularly from fruit bodies, culture broth, and cultured mycelium, extraction and analytical technologies are standardized, which ensures sufficient uniformity and product quality [6]. The extraction procedures require various standardization strategies, including solvent-based extraction, enzyme-assisted extraction, and ultrasonic-assisted extraction, coupled with analysis techniques, such as HPLC, GC-MS, and NMR spectroscopy [15]. The involvement of high-throughput biotechnological techniques, such as X-ray crystallography, nuclear magnetic resonance spectroscopy, and mass spectrometry, to delineate the link between the structural conformation of mushroom active ingredients and their pharmacological properties is also very crucial, ensuring the standardization of mushroom-based products [36].

With the spike in the pharmaceutical prospects of mushrooms, numerous future directions ought to be sought for their full utilization as therapeutic agents. It becomes very essential to streamline the various regulatory frameworks. For robust development, production, and marketing of mushroom-derived medicinal products, international collaboration is pivotal in setting up strict regulations, leading to enhanced global recognition and use. Standardized extraction, authentication, and testing protocols are necessary for sufficient product quality and consistency of mushroom-based bioactive ingredients [36]. This standardization can offer a favorable environment for data assessment emanating from diverse research, thereby boosting high-quality product development. Furthermore, a partnership must exist between industry and academia for enhanced development of high-quality pharmaceutical products. As researchers from academia contribute expertise towards the development and examination of the mushroom-based products (Figure 3), industry collaborators play a vital role in commercializing and marketing the products. Also, evaluating the interaction between mushroom-based active ingredients and other therapeutics like conventional drugs and plant-based products may result in the development of *novel* therapeutic innovations. To arrest regulatory issues, robust strategies must be set up, including (1) Classification of mushroom-derived products as either dietary supplements or medicinal products, (2) Provision of sustainable cultivation practices, (3) Continuous supply of high-quality mushroom biomass for the production of medicinal products, and (4) Implementation of public education and awareness campaigns.

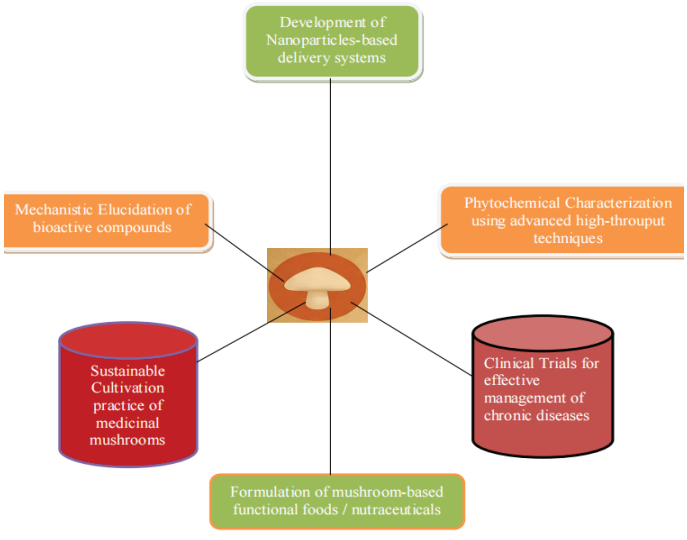

Fig. 3 Groundbreaking research opportunities in medicinal mushrooms

Regulatory framework of mushroom-based products

There is a need for standardization of mushroom-based pharmaceutical or neutraceutical products since the regulatory framework varies

from country to country. For example, mushroom-based products classified as dietary supplements are regulated by the FDA under the jurisdiction of the Dietary Supplement Health and Education Act (DSHEA) in the United States. Additionally, in Europe, mushroom-based products are categorized under food supplements or traditional herbal medicinal formulations, regulated by the European Medicines Agency (EMA). Similarly, in China, mushroom-based products are grouped as traditional Chinese medicine, supervised by the China Food and Drug Administration (CFDA). Furthermore, in India, mushroom-based products classified as Ayurvedic, Siddha, or Unani medicines are regulated by the Ministry of AYUSH. In Nigeria, the agency regulating mushroom-based products is not well-known; the National Agency for Food and Drug Administration and Control (NAFDAC) or the Federal Ministry of Agriculture and Food Security might play a crucial role, since these bodies regulate similar products.

To standardize mushroom-based pharmaceutical products in Nigeria, this review suggests implementing integrated processes, including (1) Establishing National Standards for industry involved in the productions of plant-based products, encompassing setting up examination procedures and prerequisites for labeling, (2) Consolidating Regulatory Framework, aimed to bring strict compliance with national standards and guidelines, (3) Translating Quality Control and Assurance policy to practical assessment and certification plans, (4) Supporting robust Research and Development towards the potency, safety, and potential interactions of plant-based products, (5) Carrying out Public Education, acquainting the end users with advantages and disadvantages of consuming mushroom-based products for enhanced decision-making, and (6) Strengthening co-operation among industries, stakeholders, regulatory bodies, and research institutions to contribute towards achieving standardization of the mushroom-based products.

To ensure standardization is attained and sustained, it is important to collaborate with agencies like NAFDAC, Federal Ministry of Agriculture and Food Security, National Agricultural Seeds Council of Nigeria, CropLife Nigeria, Standards Organization of Nigeria (SON), and National Biosafety Management Agency (NBMA).

3. Conclusion

Mushrooms provide intriguing opportunities for researchers to develop *novel* drugs, given the wide range of pharmacological effects demonstrated by their bioactive ingredients. The prospective pharmaceutical applications of these mushroom-derived active molecules are enormous, encompassing the treatment of life-threatening diseases, including cancer, infections, diabetes, and several other chronic diseases. However, significant challenges, including poor bioavailability, variable bioactive composition, and dose-dependent adverse effects, must be addressed to advance the clinical exploration of mushroom-based products. Additionally, the isolation of each mushroom bioactive constituent for medication is cumbersome due to the complex nature of mushroom components, which are found to work synergistically. Furthermore, regulatory authorization calls for top-notch clinical research and standardized pharmaceutical formulations. Leveraging improved extraction methodologies, nanoparticle-based delivery systems, and rigorous clinical trials, the therapeutic usefulness of mushroom-based products can be harnessed fully. Research focused on bioavailability, pharmacokinetics, and clinical effectiveness can drive the findings into effective medications for enhanced public health.

Ethics approval and consent to participate
It is not applicable.

Availability of data and materials
Not applicable

Consent for publication
The authors have given their consent for this article to be published.

Competing interests
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The Gut–Brain Axis in Neurodegeneration and Neural Repair: Microbiome-Driven Modulation of CNS Inflammation, Neurogenesis, and Recovery

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ABSTRACT:

Background: *The gut–brain axis represents a dynamic, bidirectional communication network linking the gastrointestinal microbiota with central nervous system (CNS) function. Emerging research implicates gut dysbiosis in the progression of neurodegenerative disorders such as Alzheimer's disease (AD) and Parkinson's disease (PD), as well as in the regulation of neuroinflammatory tone and adult neurogenesis. Understanding the microbial regulation of CNS integrity offers a novel vantage point for both neuropathology and recovery.*

Methodology: *This review synthesizes findings from recent preclinical and clinical studies that explore the mechanistic underpinnings of the gut–brain axis in neurodegeneration and neural repair. Emphasis is placed on germ-free models, microbiome sequencing in human cohorts, and interventional trials involving probiotics, dietary strategies, and fecal microbiota transplantation (FMT).*

Results: *Microbiota-derived metabolites such as short-chain fatty acids (SCFAs) and tryptophan catabolites influence neuroimmune responses, blood–brain barrier (BBB) integrity, and neurogenesis in the hippocampus and subventricular zone. Dysbiosis is associated with heightened microglial activation, impaired Aβ clearance, and α-synuclein aggregation. Conversely, modulation of the microbiota through probiotics or dietary interventions can attenuate neuroinflammation and improve cognitive and motor outcomes in both animal models and early-stage clinical trials.*

Conclusion: *The gut–brain axis is a critical modulator of CNS health, with disruptions contributing to neurodegeneration and offering a therapeutic window for repair. Future work should address causality, interindividual variability, and ethical considerations of microbiome manipulation to enable precision interventions targeting brain resilience and regeneration.*

KEYWORDS:

Gut–brain axis, microbiota, neurogenesis, neurodegeneration, short-chain fatty acids.

1. Background

The gut–brain axis represents a complex, bidirectional communication network linking the central nervous system (CNS) with the gastrointestinal tract, primarily mediated through neural, endocrine, immune, and microbial pathways. Recent advances in neurogastroenterology have identified the gut microbiota—comprising trillions of microorganisms—as a key modulator of brain development, function, and disease[1]. Microbiota-derived metabolites such as short-chain fatty acids (SCFAs), tryptophan catabolites, and neuroactive peptides influence neuronal excitability, glial function, and synaptic plasticity through blood-brain barrier (BBB) signaling, immune pathways, and vagal modulation[2,3].

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Disruption of the gut microbial ecosystem, or dysbiosis, has emerged as a contributing factor in a range of neurodegenerative diseases, including Alzheimer's disease (AD) and Parkinson's disease (PD). Mounting clinical and experimental evidence suggests that microbial imbalance not only precedes neurodegeneration but also amplifies neuroinflammation, BBB breakdown, and synaptic dysfunction, thereby exacerbating disease progression[4,5].

The gut microbiome functions as a systemic regulator of immune and neuroendocrine homeostasis, shaping host physiology far beyond the confines of the intestine. Mechanistic insights have shown that microbial signals can influence microglial maturation, astrocyte reactivity, and neurotrophic factor expression—all critical components of CNS health and repair[6,7]. Furthermore, gut microbiota regulates the integrity of the BBB, modulating tight junction expression and endothelial permeability, thus affecting CNS susceptibility to circulating immune signals and toxins[8].

This microbial influence extends into the degeneration-repair continuum of CNS disorders. While dysbiosis exacerbates neurodegeneration through immune priming and metabolic dysregulation, restoration of microbial balance has shown promise in enhancing neurogenesis, resolving inflammation, and facilitating recovery after injury (9, 10).

While previous reviews have broadly described gut–brain interactions, this review's unique contribution is a focused synthesis of how microbiota-derived signals regulate neuroinflammation, neurogenesis, and recovery, bridging mechanistic insights with translational strategies.

2. Gut Microbiota and CNS Homeostasis

Gut-Brain Axis: Microbiome-Derived Pathways Influencing CNS Neuroimmune Crosstalk

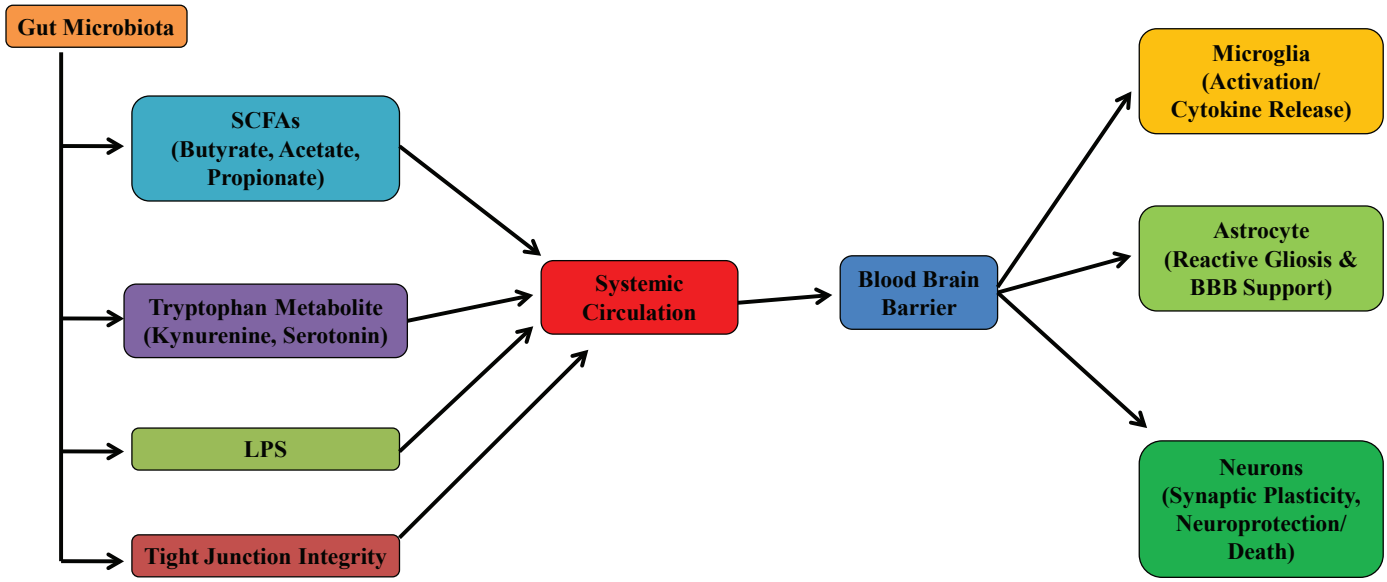

Figure 1: Schematic illustration of microbiome-derived signals influencing CNS function.

Short-chain fatty acids (SCFAs), tryptophan metabolites, and lipopolysaccharide (LPS), along with changes in gut barrier integrity, enter systemic circulation and interact with the blood–brain barrier (BBB). These signals modulate microglial activation, astrocytic reactivity, and neuronal function, leading to outcomes ranging from cytokine release and gliosis to altered synaptic plasticity, neuroprotection, or neurodegeneration.

2.1 Microbial Metabolites and Neuroactive Compounds

The gut microbiota produces a wide range of metabolites and neuroactive compounds that profoundly influence CNS physiology. Short-chain fatty acids (SCFAs)—notably butyrate, acetate, and propionate—are produced by bacterial fermentation of dietary fibers and have been shown to modulate microglial maturation, inflammatory responses, and even blood-brain barrier (BBB) function[11]. Butyrate, in particular, functions as a histone deacetylase (HDAC) inhibitor, enhancing gene expression linked to neuroprotection and synaptic plasticity.

Another essential pathway is the tryptophan–kynurenine axis, which connects gut microbiota activity with serotonergic and glutamatergic signaling in the brain. Commensal bacteria such as Bifidobacterium and Lactobacillus can shift tryptophan metabolism away from kynurenine production—associated with neurotoxicity—and toward serotonin biosynthesis[3]. Dysregulation of this pathway has been implicated in mood disorders, neuroinflammation, and cognitive decline.

Moreover, microbial species can synthesize or modulate levels of γ-aminobutyric acid (GABA), serotonin (5-HT), dopamine, and acetylcholine, influencing neural excitability and emotional behavior through vagal pathways or direct circulation of these metabolites[12].

2.2 Microbiota–Immune Interactions

The gut microbiota exerts profound effects on both systemic and CNS-resident immune cells, playing a pivotal role in shaping neuroimmune tone. Microbial-associated molecular patterns (MAMPs), including lipopolysaccharides (LPS) and peptidoglycans, are recognized by Toll-like receptors (TLRs) on immune and glial cells. Activation of TLR2 and TLR4 on microglia, for instance, can shift them toward a proinflammatory phenotype, promoting synaptic loss and neuronal dysfunction [13].

SCFAs also influence immune homeostasis by inducing regulatory T cell (Treg) differentiation, which can modulate neuroinflammation through peripheral cytokine profiles[14]. In addition, microbial signals have been shown to prime astrocytic IL-10 production, creating a neuroprotective milieu under homeostatic conditions.

However, dysbiosis—especially involving overgrowth of pathobionts—can tilt this balance toward a systemic inflammatory state, increasing levels of proinflammatory cytokines such as IL-1β, IL-6, and TNF-α, which can breach the BBB and disrupt neuronal signaling[15].

2.3 Maintenance of BBB Integrity

The integrity of the blood–brain barrier (BBB) is critical for maintaining CNS immune privilege. Gut microbiota modulates BBB function by regulating the expression of tight junction proteins—such as occludin, claudin-5, and ZO-1—via SCFA signaling and anti-inflammatory cytokine induction[16,17]. In germ-free mice, BBB permeability is significantly increased, a phenotype reversible by colonization with SCFA-producing bacteria.

Beyond SCFAs, other microbial signals directly or indirectly regulate BBB integrity. Tryptophan-derived indoles, produced by commensal bacteria such as *Lactobacillus* and *Clostridium* species, activate the aryl hydrocarbon receptor (AhR) on astrocytes and endothelial cells, upregulating tight junction proteins and exerting anti-inflammatory effects[18]. Secondary bile acids, generated by microbial metabolism of primary bile acids, influence endothelial signaling through farnesoid X receptor (FXR) and Takeda G protein receptor 5 (TGR5), modulating barrier permeability and vascular inflammation[19]. Additionally, polyphenol metabolites produced via microbial fermentation (e.g., urolithins, phenolic acids) have been shown to reduce oxidative stress and preserve endothelial tight junction integrity, thereby protecting BBB function[20].

Conversely, gut dysbiosis—through overproduction of endotoxins like LPS—disrupts BBB architecture and enhances

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leukocyte infiltration, thereby exacerbating CNS inflammation[21]. This breach in BBB integrity has been implicated in the pathophysiology of Alzheimer's disease, Parkinson's disease, and multiple sclerosis, providing a mechanistic link between microbial imbalance and neural vulnerability.

2.4 Role of the gut virome and mycobiome in gut-brain axis modulation

While most gut–brain axis studies emphasize bacterial communities, emerging evidence highlights important contributions from the gut virome and mycobiome. The gut virome, dominated by bacteriophages, can indirectly modulate host–microbiota interactions by altering bacterial population dynamics and metabolite production, thereby influencing systemic immunity and CNS function[22]. Similarly, fungal communities, though less abundant, play a role in gut–brain communication. Altered fungal diversity and overgrowth of *Candida* species have been reported in neurodegenerative conditions, where fungal cell wall components such as β-glucans can activate innate immune receptors and exacerbate neuroinflammation[23]. Although still underexplored, integrating bacterial, viral, and fungal interactions will be essential for a more comprehensive understanding of gut-derived signals in CNS homeostasis and neurodegeneration.

3. Gut Dysbiosis and Neurodegenerative Disorders

3.1 Alzheimer's Disease (AD)

Emerging data demonstrate distinct gut microbiota alterations in Alzheimer's disease (AD). AD patients exhibit reduced levels of beneficial taxa (e.g., Bifidobacterium, Faecalibacterium) and increased abundance of proinflammatory genera (e.g., Escherichia/Shigella, Bacteroides)[24]. This dysbiosis has been implicated in promoting neuroinflammation through increased systemic lipopolysaccharide (LPS) and decreased short-chain fatty acid (SCFA) levels.

Murine studies have provided causal links between gut microbial alterations and AD pathology. For instance, germ-free 5xFAD mice display reduced amyloid-beta (Aβ) plaque load and microglial activation, an effect reversed by recolonization with AD-associated microbiota[25,26]. Similarly, antibiotic-induced depletion of gut microbes leads to reduced Aβ aggregation and alterations in hippocampal microglial morphology, suggesting microbiota-mediated modulation of immune responses involved in Aβ clearance.

Tau pathology is also influenced by microbial metabolites and immune signaling. Tau transgenic mice exhibit increased hyperphosphorylation and neuroinflammation following colonization with dysbiotic microbiota derived from AD donors[7]. Mechanistically, microbial-derived tryptophan catabolites such as kynurenine promote excitotoxicity and oxidative stress, activating kinases including GSK-3β and CDK5, which are key drivers of tau phosphorylation[27]. Similarly, systemic bile acid dysregulation has been linked to abnormal tau aggregation, with certain secondary bile acids (e.g., deoxycholic acid) crossing the BBB and triggering neuronal stress pathways[28]. Inflammatory mediators induced by dysbiosis—particularly IL-6, TNF-α, and IL-1β—further exacerbate tau pathology by enhancing kinase activity and impairing tau clearance[29].

Compared to Aβ mechanisms, which are strongly linked to impaired microglial phagocytosis and altered SCFA signaling, tau pathology appears to be more tightly associated with metabolic and inflammatory pathways involving tryptophan metabolism, bile acid signaling, and proinflammatory cytokine cascades. This highlights how distinct microbial metabolites and immune axes converge on separate yet complementary arms of AD pathology—Aβ aggregation and tau hyperphosphorylation—together driving neurodegeneration.

3.2 Parkinson's Disease (PD)

In Parkinson's disease (PD), gastrointestinal symptoms often precede motor deficits by years, implicating the gut–brain axis in disease initiation. Braak's hypothesis posits that pathological α-synuclein aggregates originate in the enteric nervous system (ENS) and ascend via the vagus nerve to the brainstem. Animal models have recapitulated this pattern, with gut inoculation of α-synuclein fibrils inducing progressive neurodegeneration along the vagal pathway[30].

Clinical studies provide partial support for

this mechanism. A Danish nationwide cohort reported that individuals who underwent truncal vagotomy had a reduced risk of developing PD, suggesting interruption of vagal transmission may be protective[31]. Similarly, a Swedish registry study observed reduced PD incidence following truncal vagotomy, whereas selective vagotomy did not confer protection[32]. However, other population-based studies have failed to confirm this association[33], indicating that vagal involvement may vary by patient subtype, genetic background, or environmental exposures.

Microbial dysbiosis in PD includes increased abundance of proinflammatory *Proteobacteria* and reduced SCFA-producing bacteria. These shifts correlate with intestinal barrier dysfunction, systemic inflammation, and activation of microglia in the substantia nigra[34]. SCFAs, while generally considered neuroprotective, display a dual role in PD. At physiological concentrations, butyrate and propionate enhance gut barrier integrity, promote regulatory T cell differentiation, and support microglial maturation[35]. However, experimental evidence indicates that excessive SCFA exposure, or SCFAs acting in a proinflammatory environment, can exacerbate α-synuclein aggregation and microglial overactivation, thereby worsening motor pathology[35]. This suggests that the effects of SCFAs are highly context-dependent—protective in maintaining homeostasis but potentially harmful once neurodegenerative processes are initiated.

Fecal microbiota transplantation (FMT) has been explored as an experimental therapy. Transplanting PD patient microbiota into α-synuclein–overexpressing mice worsens motor symptoms and dopaminergic neurodegeneration, whereas microbiota from healthy controls confer neuroprotection[36]. These findings highlight the causal impact of microbial communities on PD progression and underscore the therapeutic potential of microbiota-targeted interventions.

3.3 Multiple Sclerosis, ALS, and Other Disorders

In multiple sclerosis (MS), gut microbiota influences immune cell polarization, particularly the balance between proinflammatory Th17 cells and anti-inflammatory Tregs. Dysbiosis in MS patients often includes reduced levels of Prevotella and Faecalibacterium, both implicated in SCFA production and Treg induction[37,38]. Colonization of germ-free mice with MS-derived microbiota enhances demyelination and CNS infiltration of Th17 cells, suggesting a microbiota-driven amplification of autoimmune pathology.

Amyotrophic lateral sclerosis (ALS) also shows evidence of gut-brain interactions. In SOD1-G93A mouse models, gut microbial depletion or dysbiosis accelerates disease progression, while specific taxa such as Akkermansia muciniphila are associated with improved motor performance and metabolic profiles[39]. Although human studies are limited, altered microbial compositions have been reported in ALS cohorts, potentially linked to neuroinflammation and intestinal permeability.

Table 1: Disease-specific gut microbial alterations and proposed CNS consequences in neurodegeneration

Disorder	Enriched taxa	Depleted taxa	Key consequences for CNS
Alzheimer's disease (AD)	*Escherichia/Shigella*, *Bacteroides*[24]	*Bifidobacterium*, *Faecalibacterium*[24]	↑ systemic LPS, ↓ SCFAs → microglial priming, impaired Aβ clearance, tau hyperphosphorylation (via kynurenine, bile acids, cytokines)[7,27-29].
Parkinson's disease (PD)	*Proteobacteria*, *Enterobacteriaceae*[34]	SCFA-producers (*Faecalibacterium*, *Roseburia*)[34]	Gut barrier dysfunction, systemic inflammation, dual role of SCFAs (protective vs. pro-aggregatory), α-synuclein aggregation, vagal propagation[30,35].
Multiple sclerosis (MS)	Proinflammatory taxa (*Akkermansia*)[37]	*Prevotella*, *Faecalibacterium*[37,38]	Skewed Th17/Treg balance, ↑ demyelination, ↑ CNS infiltration of autoreactive T cells
Amyotrophic lateral sclerosis (ALS)	Variable, some evidence of *Escherichia* overgrowth	Reduced *Akkermansia muciniphila*[39]	Gut barrier disruption, altered metabolism, accelerated motor neuron loss, systemic inflammation

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4. Gut Microbiota in Neurogenesis and Neural Repair

Short-chain fatty acids (SCFAs), particularly butyrate, cross the blood–brain barrier and modulate histone acetylation in neural stem cells. This epigenetic effect enhances the expression of neurogenic and plasticity-associated genes such as BDNF and Neurog2, thereby supporting basal hippocampal neurogenesis under homeostatic conditions[11]. Germ-free and antibiotic-treated mice exhibit reduced proliferation of neural progenitors in both the dentate gyrus and SVZ, highlighting the necessity of a healthy microbiota for sustaining adult neurogenesis. Restoration of microbial balance via colonization or SCFA supplementation rescues these deficits [9, 40].

In contrast, during injury or disease, microbiota influence neurogenesis through both systemic immune modulation and local CNS cues. After traumatic brain injury (TBI) or stroke, dysbiosis accelerates systemic inflammation and worsens neurological outcomes. Murine stroke models show that oral *Lactobacillus rhamnosus* GG enhances neural progenitor proliferation and increases BDNF, thereby improving recovery[41]. Conversely, antibiotic-induced dysbiosis impairs post-stroke neurogenesis, while fecal microbiota transplantation (FMT) from young or healthy donors restores hippocampal neurogenesis, enhances synaptic density, and improves behavior[42]. Probiotics such as *Bifidobacterium longum* have also been associated with better neurocognitive recovery post-injury[43].

Microbiota-derived signals further shape microglial polarization, which critically determines neurodegeneration versus repair. Germ-free mice exhibit immature or hyperactivated microglia, a phenotype normalized by SCFA administration[6,44]. Butyrate and propionate promote anti-inflammatory M2 polarization, characterized by enhanced phagocytosis, trophic factor release, and remyelination in models of spinal cord injury. By contrast, LPS and other microbial-associated molecular patterns drive proinflammatory M1 polarization, marked by TNF-α, IL-1β, and ROS production, thereby amplifying neuronal injury. Tryptophan metabolites also modulate polarization: kynurenine pathway activation favors M1-like states, while indole derivatives engage aryl hydrocarbon receptor (AhR) signaling to promote M2 phenotypes and neuroprotection[3,18]. Astrocytes, likewise, integrate microbial cues. Probiotics enhance astrocytic production of neurotrophic factors such as GDNF and BDNF, facilitating synaptic recovery after TBI[43]. Moreover, astrocytic modulation of glutamate uptake and BBB integrity in response to microbial metabolites underscores the broad role of gut-derived signals in orchestrating CNS homeostasis and repair.

5. Experimental and Clinical Insights

Germ-free (GF) and antibiotic-treated rodents exhibit significant alterations in CNS development, including reduced hippocampal neurogenesis, altered microglial morphology, and impaired stress reactivity[9,11]. These models demonstrate that the absence or depletion of microbiota impacts synaptic plasticity and gene expression related to neurodevelopment and plasticity. More recently, GF mice showed blunted BDNF expression in the prefrontal cortex and hippocampus, affecting emotional resilience[40].

Large-scale microbiome analyses in Alzheimer's and Parkinson's cohorts have identified disease-specific microbial signatures. For instance, *Prevotella* and *Faecalibacterium* were consistently depleted in PD patients, correlating with motor severity and inflammatory cytokine profiles[45]. Similar microbial alterations in AD patients have been linked to increased serum LPS and impaired Aβ clearance[46]. Integration with neuroimaging data suggests that dysbiosis correlates with hippocampal atrophy and cortical thinning[23].

Fecal microbiota transplantation (FMT) from healthy or young donors has improved cognitive performance and synaptic integrity in aged or AD-model mice[42]. Small clinical trials have also reported mild cognitive or motor improvements in PD patients receiving FMT or targeted probiotics, although findings are preliminary and require validation in larger cohorts [47, 48]. Limitations include interindividual microbiota variability, absence of standardized protocols, and inconsistent clinical endpoints.

6. Therapeutic Potential and Translational Strategies

Targeted microbial supplementation has shown promise in modulating neuroinflammatory markers and cognitive function. Specific

strains such as *Lactobacillus plantarum* PS128 improved motor symptoms and reduced anxiety in Parkinson's patients in a double-blind randomized trial[49]. Similarly, synbiotic supplementation attenuated cognitive impairment and oxidative stress in murine Alzheimer's models[50]. Prebiotics—non-digestible dietary fibers that selectively stimulate beneficial bacteria—are emerging as complementary tools. For instance, inulin and fructo-oligosaccharides (FOS) increase SCFA production, modulate immune tone, and improve cognitive outcomes in preclinical AD models[51]. Synbiotics, combining probiotics with prebiotics, provide synergistic effects by improving microbial survival and enhancing metabolite production, representing a distinct therapeutic avenue beyond general dietary modulation.

Dietary patterns such as the Mediterranean and ketogenic diets have been associated with neuroprotective microbiota shifts and reduced systemic inflammation[52,53]. In murine models, supplementation with sodium butyrate or polyphenol-rich extracts (e.g., resveratrol) restored synaptic plasticity and enhanced hippocampal BDNF levels[54]. Tryptophan supplementation also modulated serotonergic signaling and gut–brain axis responses post-stroke[55]. Importantly, environmental factors—including early life exposures, stress, sleep, and exercise—also shape microbiome composition and function, intersecting with neurodegeneration risk and resilience[1]. Chronic stress alters microbial diversity and increases gut permeability, while aerobic exercise enhances SCFA production and hippocampal neurogenesis, highlighting the need to view microbiome interventions within broader lifestyle contexts.

Emerging technologies such as CRISPR-based microbiota engineering allow for targeted manipulation of microbial genes affecting metabolite production[56]. Precision medicine approaches are being explored using metagenomic profiling to tailor probiotic or dietary interventions to individual microbiome features, as demonstrated in a pilot personalized nutrition study for Parkinson's patients[57]. However, practical challenges remain: metagenomic sequencing is still costly, time-intensive, and requires advanced bioinformatics pipelines, which limit its feasibility for routine clinical use. Moreover, the interpretation of microbial signatures is confounded by interindividual variability and a lack of standardized reference databases. While these tools hold significant potential, current application is largely confined to research or small pilot studies.

7. Challenges and Future Directions

Despite compelling associations between gut microbiota and brain health, distinguishing causation from correlation remains a central obstacle in translational neuroscience. While rodent models provide mechanistic insights, they often lack clinical fidelity, and human studies remain confounded by diet, genetics, medications, and lifestyle factors. Methodological variability—including inconsistent fecal sampling protocols, differences in sequencing pipelines, heterogeneity in disease staging, and confounds from polypharmacy—further complicates interpretation across cohorts.

A persistent translational challenge lies in defining microbial "dose–response" relationships. Unlike conventional drugs, probiotics or microbial consortia may not follow linear pharmacodynamics; their efficacy depends on concentration, host microbiome context, and competitive niche adaptation. Moreover, the long-term persistence or engraftment of supplemented strains is inconsistent, raising questions about the durability of therapeutic effects and the need for repeated interventions.

Establishing causality requires carefully controlled experimental designs. Germ-free models, targeted gnotobiotic colonization, and humanized microbiome mice have proven invaluable in testing direct microbe–host interactions and their relevance to human disease. Translating these findings to humans will require longitudinal, multi-omics approaches that integrate metagenomics, metabolomics, neuroimaging, and behavioral profiling to identify mechanistic biomarkers of disease progression and therapeutic response.

Ethical concerns also warrant deeper consideration. Fecal microbiota transplantation (FMT) and engineered microbial interventions present particular challenges in obtaining informed consent from cognitively impaired populations, such as patients with advanced Alzheimer's disease or Parkinson's dementia. Safeguards for autonomy and family/guardian involvement must be strengthened to ensure

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ethical trial conduct.

Finally, future research will benefit from leveraging big data analytics and artificial intelligence (AI) to unravel complex microbiome–host interactions. Machine learning models are already being applied to predict disease risk, stratify patient subgroups, and forecast response to microbiome-targeted therapies. Integrating AI with precision microbiome science holds the promise of accelerating biomarker discovery and guiding personalized interventions for neurodegenerative diseases.

Interindividual variability—linked to age, sex, comorbidities, and baseline microbiome composition—remains a fundamental barrier to generalization. The inclusion of diverse populations in research design will be essential for moving toward precision microbiome-based therapies that are capable of enhancing brain resilience and repair.

8. Conclusion

The gut–brain axis represents a fundamental and underappreciated regulator of central nervous system integrity, modulating neuroinflammation, neurogenesis, and circuit repair. Disruptions in microbiota composition and function—collectively termed dysbiosis—have emerged as key contributors to the pathophysiology of Alzheimer's disease, Parkinson's disease, multiple sclerosis, and other neurodegenerative conditions.

Importantly, the gut microbiome is modifiable. This opens therapeutic possibilities through diet, probiotics, targeted metabolite supplementation, or advanced bioengineering approaches. Integrative, cross-disciplinary strategies that combine microbiome science with neuroimmunology, regenerative neuroscience, and systems biology are poised to redefine our understanding of brain resilience and recovery.

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A Full Mouth Rehabilitation Restoring Function And Aesthetics: A Case Report

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ABSTRACT

Background: *This case report describes a middle-aged patient with pain in the right posterior mandibular molar and multiple missing teeth. The treatment plan involved a multidisciplinary approach, including periodontal therapy, non-surgical extraction, restorative and endodontic treatments, and then prosthetic rehabilitation.*

Purpose: *The treatment plan focused on achieving proper occlusion and improving the patient's aesthetic appearance.*

Methodology: *The treatment plan for the patient started with clinical and radiographic assessment, including panoramic and multiple periapical X-rays. Following the diagnosis, a comprehensive treatment plan was developed, which involved functional crown lengthening, several restorative procedures were performed. Additionally the placement of zirconia full-coverage restorations and porcelain-fused-to-metal fixed partial dentures.*

Conclusion: *An integrated treatment approach is essential for achieving optimal results in full-mouth rehabilitation. Effective treatment planning necessitates a thorough evaluation of the underlying causes, medical history, and factors related to the patient's oral health. Significant improvements were observed in both aesthetics and function.*

KEYWORDS

Full Mouth Reconstruction, Minimal Invasive Approach, Restorative and Prosthetic Rehabilitation, Digital Technology.

1. Introduction

Oral rehabilitation is a term that broadly describes efforts to restore the functionality and aesthetics of a person's oral health. While it is often associated with the restoration of all the teeth in patient's mouth, it can also apply to situations where only damaged or problematic teeth are treated (1). The aim is to improve the patient's quality of life by achieving a harmonious balance between functionality and aesthetics (1).

Poor oral hygiene is a primary contributor to various oral health problems, including gingival and periodontal diseases, as well as tooth decay, all of which can significantly impact an individual's quality of life (2,3,4). Among these conditions, dental caries is a common chronic infectious disease with a multifactorial nature. It develops when cariogenic bacteria adhere to the tooth surface and metabolize dietary sugars, producing acids that gradually demineralize the tooth structure and may eventually lead to tooth loss (2). The severity of dental caries and the patient's oral hygiene practices both play a critical role in determining disease progression and prognosis.

Comprehensive rehabilitation of a mouth with multiple carious lesions can greatly improve a patient's quality of life. The success of treatment relies heavily on selecting the appropriate restorations for both decayed teeth and those teeth that have undergone endodontic therapy (5,6).

The multidisciplinary approach involves a thorough examination, diagnostic mounting, and a step-by-step planning process (5). The combination of adhesive cementation, advanced ceramic materials, and computer-aided design/computer-assisted manufacture (CAD/CAM) technology offers a reliable method for full-mouth rehabilitation. The digital workflow has proven to enhance clinical efficiency by reducing impression time, increasing patient satisfaction, and improving time management. However, the outcomes vary depending on the time required for adjustments (7,8,9).

This case report describes the use of digital technology in full-mouth reconstruction for a patient with significant oral health issues. Oral health was compromised by tooth loss, decay, and smoking. Preventing recurrence requires addressing lifestyle and socioeconomic factors. (10).

CASE BACKGROUND

A 27-year-old male patient sought treatment at the outpatient clinic of the Arab Academy for Science, Technology, and Maritime Transport, College of Dentistry, presenting with pain in the right mandibular third molar and missing teeth. The patient works and lives in El-Alamein, Egypt. The medical history revealed no significant illnesses; his dental history included previous amalgam restorations at tooth number. #36 #47.

EXTRA-ORAL EXAMINATION

The extra-oral examination revealed facial symmetry, absence of palpable lymph nodes, and normal mandibular movement. The frontal view displayed balanced facial thirds with parallel horizontal and vertical lines. The lateral view showed a slightly convex profile and a normal nasolabial angle within the range of 165-175 degrees. According to the E-line concept, the upper lip was positioned 4 mm behind the line, and the lower lip was 2 mm behind the line. The oblique profile revealed no midface deformities, though prominent nasolabial folds were noted **(Fig. 1).**

All extra- and intra-oral photographs were taken using a mobile phone camera with an external professional twin light, utilizing retractors and mirrors for assistance.

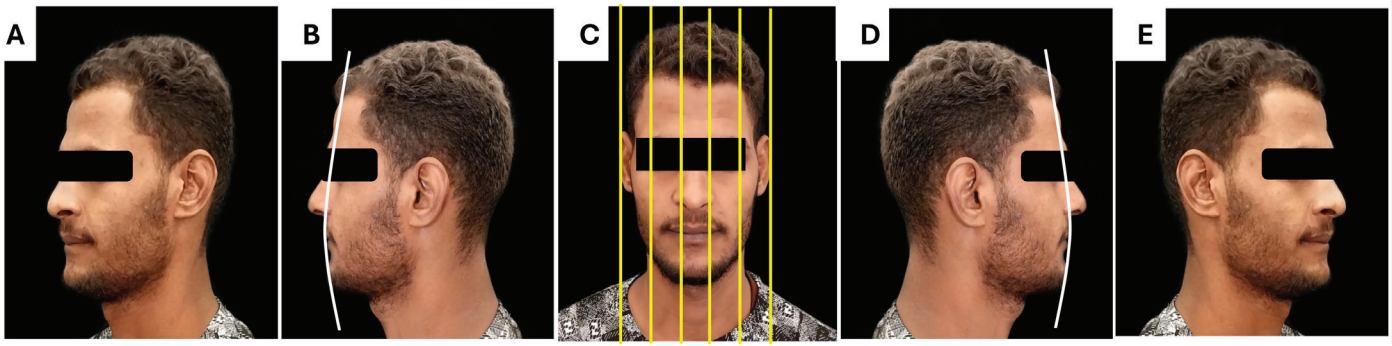

Figure 1: Extra-oral Examination Photographs.
(A): Left oblique view (B): Left lateral view. (C): Frontal view. (D): Right lateral view. (E): Right oblique view.

INTRA-ORAL EXAMINATION

The frontal view revealed generalized marginal redness with rolled margins, blunt interdental papillae, generalized marginal softness and edema, thick scalloped biotype, and matt surface texture. Brown staining was evident, alongside mild fluorosis as categorized by Dean's fluorosis index (3).

The occlusal view revealed a U-shaped arch with notable findings, including multiple remaining roots (#15 and #46) and several edentulous spaces.

The periodontal examination revealed the highest probing depth of 5 mm and a clinical attachment loss of 4 mm in tooth #34 and tooth #38, which exhibited grade I mobility (Fig. 2).

Radiographic analysis, including panoramic and periapical X-rays, demonstrated horizontal bone loss associated with the mandibular anterior teeth and premolars. Additional findings included angular bone loss related to tooth #27, as well as periapical radiolucency at tooth #35 (Fig. 3).

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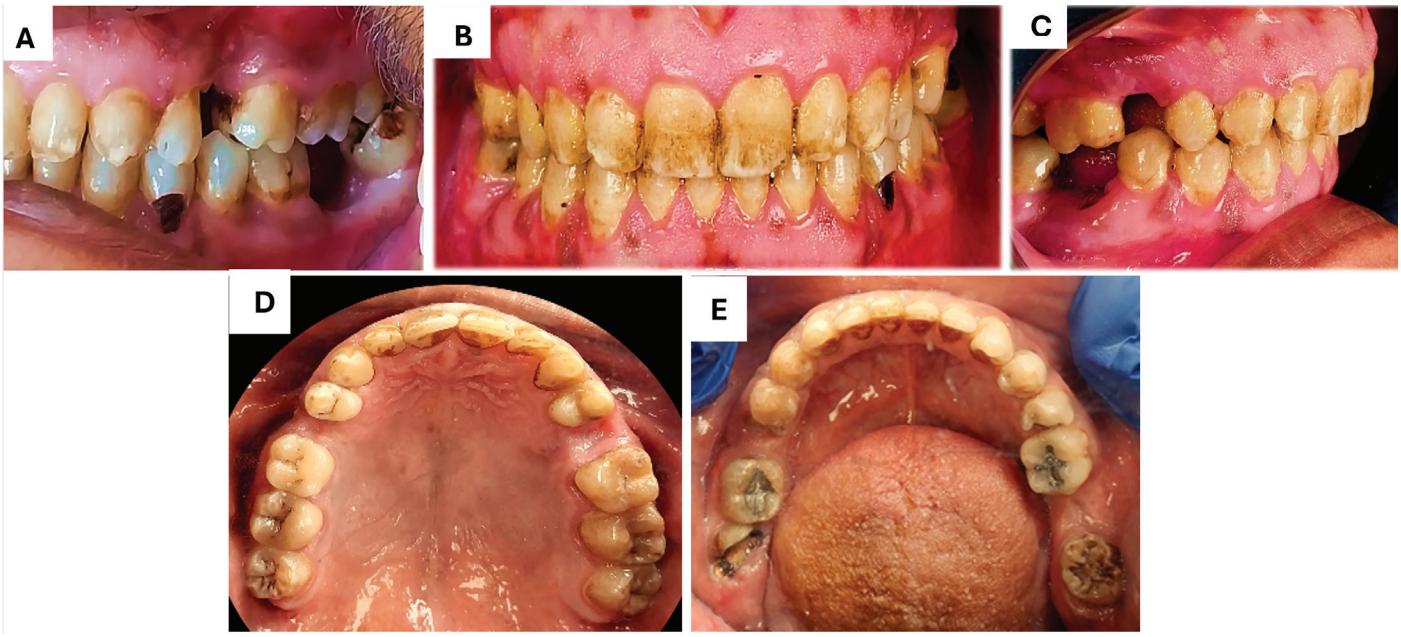


Figure 2: Intra-oral Examination Photographs.
(A): Left lateral view. (B): Frontal view. (C): Right lateral view. (D): Maxillary occlusal view. (E): Mandibular occlusal view.

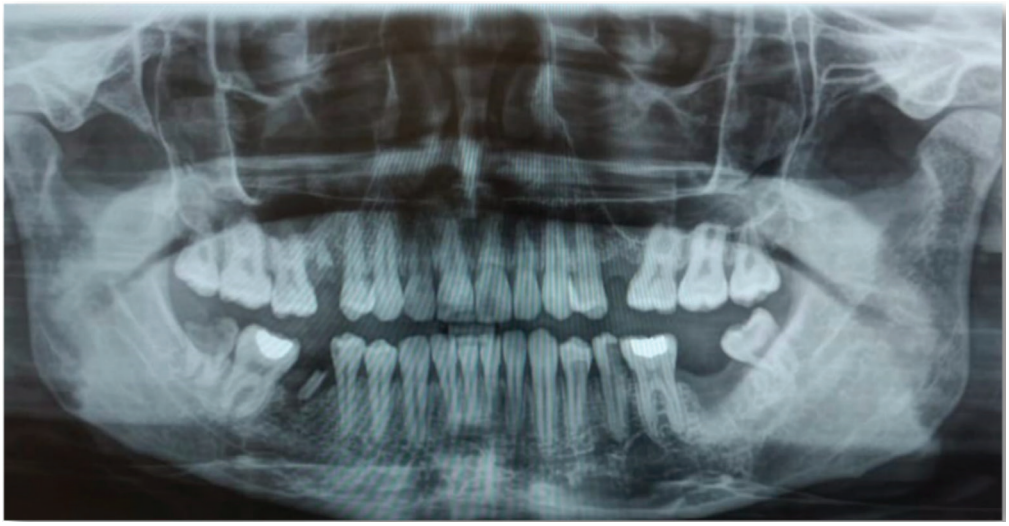


Figure 3: Panoramic x-ray showing: Horizontal bone loss related to mandibular anterior teeth and premolars, angular bone loss at #27, and periapical radiolucency at #35.

2. Diagnosis and Analysis

The periodontal diagnosis was generalized periodontitis, classified as stage II, grade C, with smoking identified as the grade modifier. A generalized bleeding score of 39% was found. Based on these findings, the overall prognosis was determined to be fair.

The endodontic evaluation of tooth #35 indicated a non-vital pulp, accompanied by a periapical lesion. The restorative assessment, based on G.V. Black's classification, identified carious lesions as follows: Class I in teeth #17, #27, #28, and #38; Class II in teeth #14, #16, #24, #26, and #45; and Class V in tooth #34. Additionally, recurrent caries with defective amalgam restorations were noted in tooth #36.

The prosthetic evaluation identified multiple missing teeth (#15, #25, and #46) requiring replacement. A comprehensive case analysis included a caries risk assessment using the DMF and DMFS indices, which revealed a DMFT score of 18 and a DMFS score of 24. According to CAMBRA analysis, the caries index was determined to be +8, indicating a high risk of caries. All dental and periodontal charting data were systematically documented using digital software (Derec, Switzerland). Primary impressions were made for both arches, and the casts were then mounted on a mean-value articulator. The case analysis was carried out using the diagnostic casts, panoramic X-ray, and several periapical X-rays.

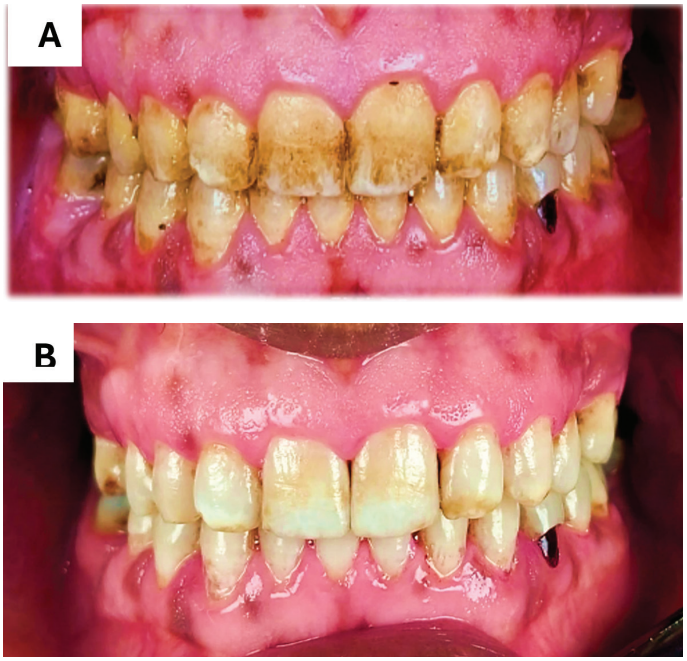
3. Clinical Management

Phase I: Stabilization Phase:

Objective: Control infection, inflammation, and eliminate sources of pain or pathology.

1.1 Periodontal Therapy:

Non-surgical periodontal therapy including both supra- and subgingival scaling and root planing using manual scalers (Sickle and Jacquette scalers) and universal curettes (2r-2l and 4r-4l) were performed. (Nordent, USA). Pocket irrigation with tetracycline, dissolved in saline, was performed to reduce inflammation **(Fig. 4).** The patient was instructed to brush twice daily and use chlorhexidine mouth wash (Hexitol, Egypt) twice daily for one week. Oral hygiene practices were reinforced, and plaque levels were reassessed after two weeks.



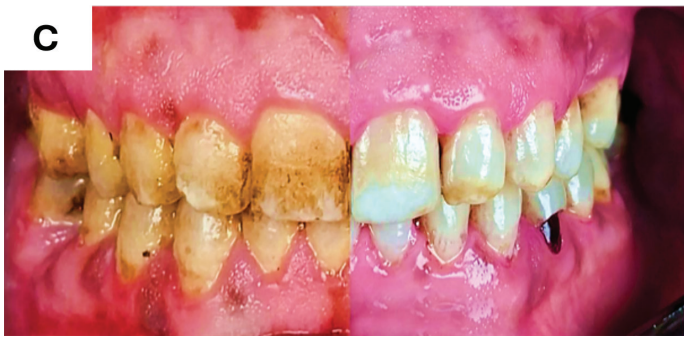


Figure 4: (A): Preoperative photograph. (B), (C): After scaling and irrigation with tetracycline HCL.

1.2 Non-Surgical Extraction

Non-restorable teeth #48 and #18 were extracted using bayonet forceps (Medesy, Italy distributed by Henry Schein Dental) . The remaining roots of teeth #15 and #46 were removed using a straight elevator (GDC, Germany – sourced via Al Mokawloon Dental Supply, Egypt). After a healing period of six weeks, the extraction sites for teeth #15 and #46 were fully healed and ready for the fabrication of a fixed partial denture.

Caries Control and Temporary Restorations

All deep caries in teeth #14, #16, #17, #26, #24, #27, #28, #34, and #38 were removed using ceramic burs (CeraBur, Germany) with a low-speed handpiece. For tooth #45, cavity preparation was carried out by using round diamond bur (KG Sorensen, Brazil) to remove all decay and temporization was performed using intermediate restorative material as RMGI (Fuji II LC, GC Corporation, Japan) **(Fig.5 & Fig.6)** . The defective amalgam restoration in tooth #36 was sectioned using carbide burs.

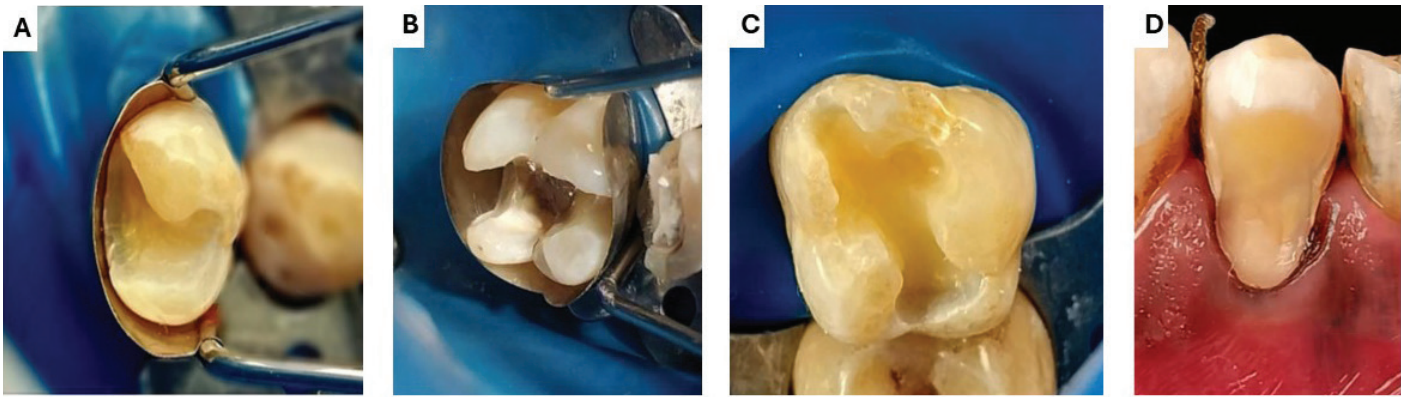


Figure 5: (A) Class II cavity preparation. (B) MOD cavity preparation. (C) class I cavity preparation. (D) class V cavity preparation

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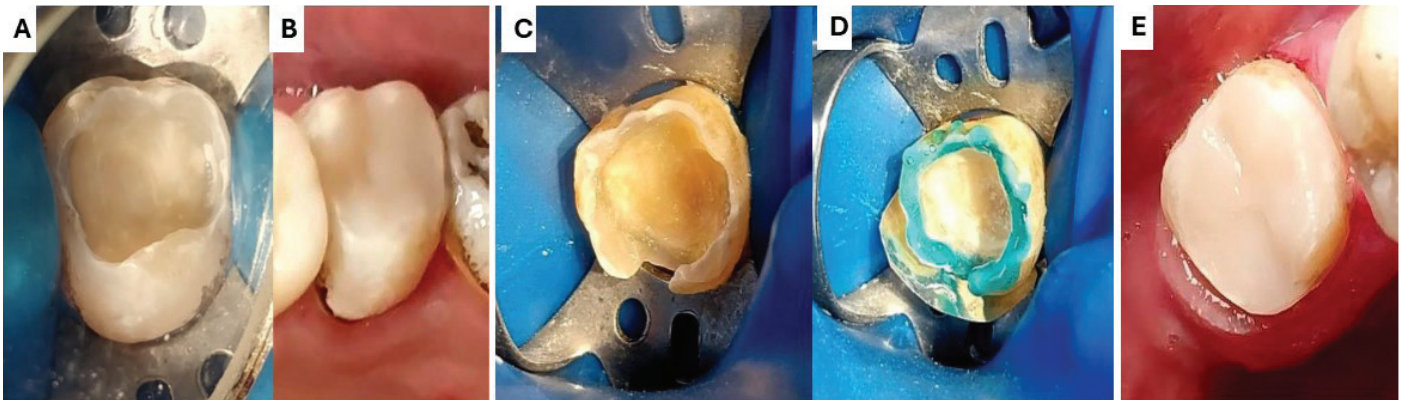

Figure 6 : (A), (C): Class I cavity preparations. (B), (E): final composite restorations. (D): selective etching technique

2. Phase II "Preparatory Phase"

Objective: Prepare the foundation for definitive treatment, including endodontic, surgical, and restorative build-up.

2.1 Endodontic treatment (Tooth #35).

This phase began with the endodontic treatment of tooth #35. The caries were removed, and an access opening was created using a rose head bur, safe-end burs, and an endo probe (DG16, Nordent) to scout the canals. The first step involved restoring the deep missing lingual wall using the 'A Deep Margin Elevation Protocol.' A circumferential stainless-steel matrix (TOR. VM, Russia) was placed around the tooth to seal the cervical margin. The deep margin was then elevated with a highly-filled flowable and condensable composite restoration. The initial file used was 15 mm, and the working length was determined to be 21.5 mm. The actual working length was confirmed both with an apex locator (Dpex V, Woodpecker, China) and radiographically via a periapical X-ray under complete isolation.

Using the crown-down technique with the ProTaper manual system, the irrigation protocol was performed with 2.5% sodium hypochlorite (Chorox, Egypt) for 5 minutes, delivered with a side-vented needle. This was followed by a 5-minute irrigation with 17% EDTA solution, saline, and a final irrigation using chlorhexidine (CHX). Master cones were verified using three techniques: clinical tactile sensation, the 'true tag-back' method, and radiographically. Obturation was completed using the cold lateral compaction technique with a resin sealer (Nexobio T SEAL, Korea) **(Fig. 7).**

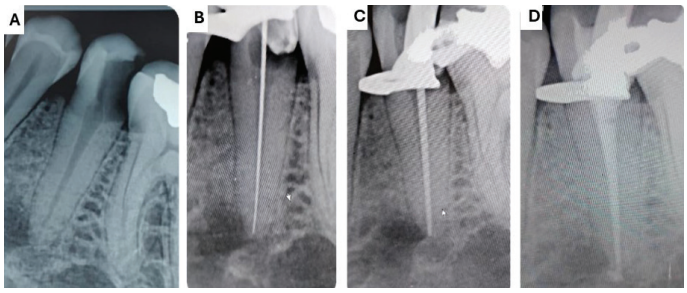

Figure 7: (A): Preoperative X-ray. (B): working length. (C): Master cone.(D): Obturation.

2.2 Crown Lengthening Surgery

Functional crown lengthening at tooth no. 35 was performed following a rolling test, to confirm an adequate zone of attached gingiva. An internal bevel gingivectomy using a 15C blade was performed followed by a full thickness mucoperiosteal flap. Distal bone removal was carried out with a round surgical bur and copious irrigation. The procedure was completed with simple interrupted sutures. **(Fig.8).**

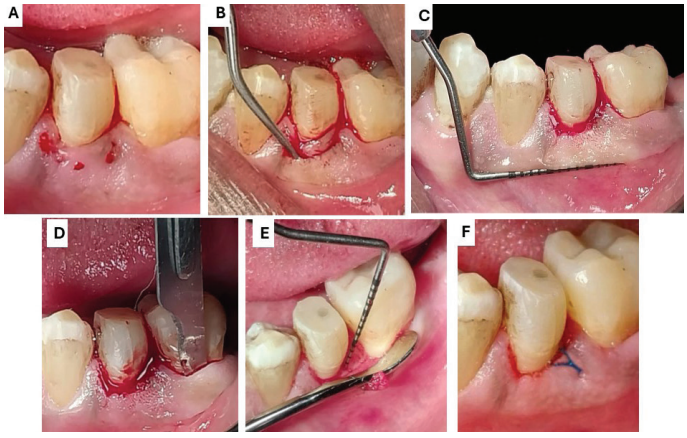

Figure 8: (A): bleeding points. (B): removal of the gingival collar (C): keratinized tissue width . (D): full-thickness mucoperiosteal flap. (E): flap reflection. (F): simple interrupted sutures.

2.3 Post Placement and Core Build-Up

For tooth #35, a glass fiber post was placed following post space preparation using Gates-Glidden drills and Peeso reamers to select a post matching the final drill size of 25. The bonding technique was employed, and the post was cemented using dual-cure resin cement. Subsequently, the core was built up using a dual-cure core material (Core It Dual Yellow – Automix). (**Fig.9**)

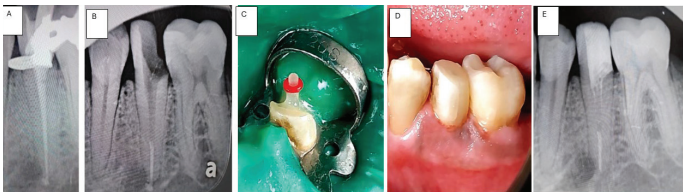

Figure 9: Glass fiber post following endodontically treated tooth #35
(A): Obturation. (B): post space preparation (C): post cementation
(D): core build up (E): x-ray shows post-cementation and core build up

Phase III "Definitive Phase"

Objective: Perform final restorations and esthetic and functional rehabilitation.

3.1 Final Composite Restorations

Completion of Class II, MOD, and Class V cavity preparations with composite restorations was performed under complete rubber dam isolation **(Fig. 10).**

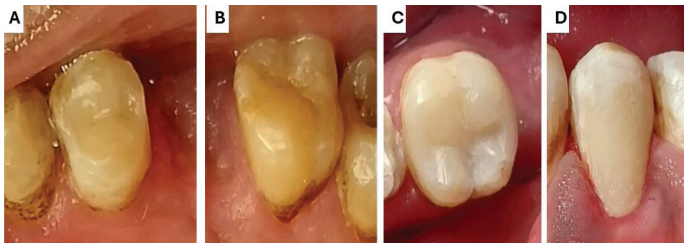

Figure 10: Class II, MOD, and class V composite restorations

3.2 Fixed Prosthodontics

Zirconia FPDs and Crowns:

Impressions were obtained using the "putty and wash technique" with condensation silicone material (Zhermack, Italy). A digital workflow (Exocad, Germany) was employed for the design of the restorations, which included a three-unit fixed partial denture to restore teeth #14 and #16 for tooth #15, as well as teeth #24 and #26 for tooth #25. Additionally, teeth #45 and #47 were restored for tooth #46 using the CAD/CAM workflow. The design also incorporated a single crown for tooth #35 following endodontic treatment.

The zirconia crown was milled from VITA YZ-HT (Germany), and a zirconia cleanser (ZirClean, Bisco, USA) was used to enhance bonding strength and remove salivary contamination prior to cementation The restoration was then cemented using dual-cure resin cement **(Fig. 11).**

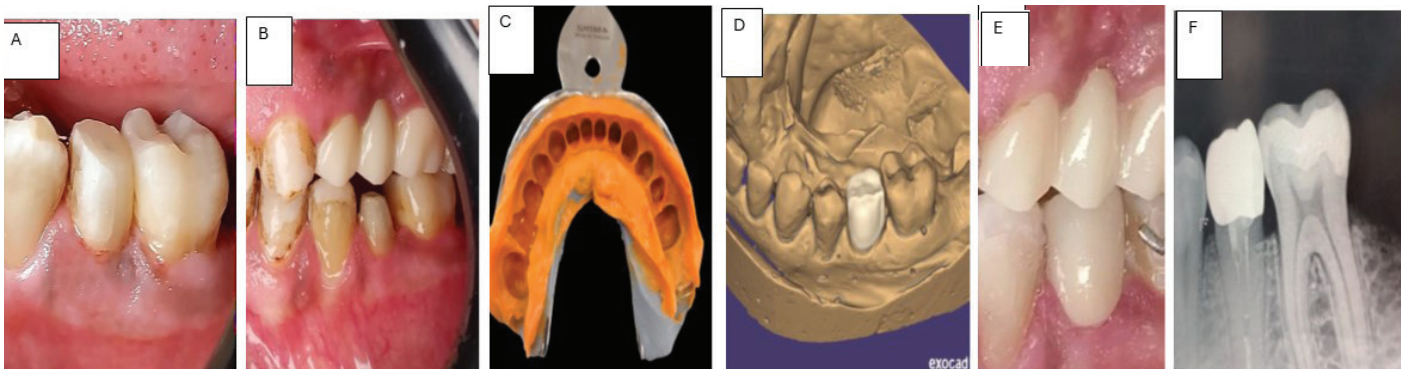

Figure 11: Zirconium Crown on #35.
(A): preoperative view (B): Lateral view showing adequate clearance. (C): Secondary impression.
(D): Digital design on "Exocad". (E): After milling. (F): Post-operative radiograph.

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The crown-to-root ratio of the zirconia fixed partial dentures was evaluated through periapical X-rays to examine the abutments' condition and the restoration's long-term stability. Prior to preparation, shade selection was made using the Vita shade guide (Germany). The abutments (#14, #16, #24, and #26) were then prepared by reducing them to provide sufficient space for the zirconia fixed partial denture, with an occlusal reduction of 2 mm.

The axial reduction was 1.5 mm, with a deep chamfer finish line, ensuring sufficient space at the cervical region. **(Fig. 12 & 13).**

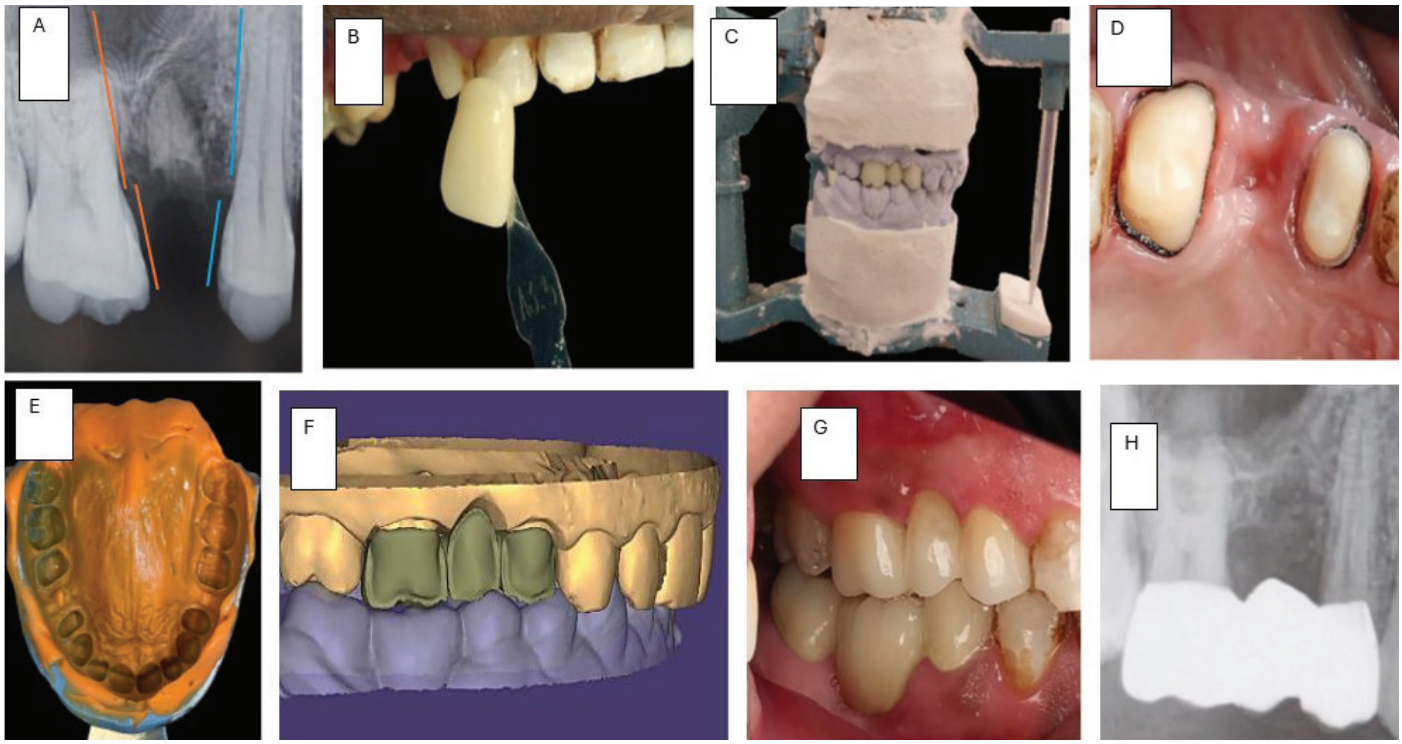

Figure 12: Zirconium Fixed Partial Denture on #14 and #16 to restore #15.
(A): Periapical radiograph. (B): Shade selection.
(C): provisional PMMA FPD. (D): Abutments preparation. (E): Secondary impression. (F): Digital design on "Exocad. (G): Cementation. (H): Postoperative radiograph.

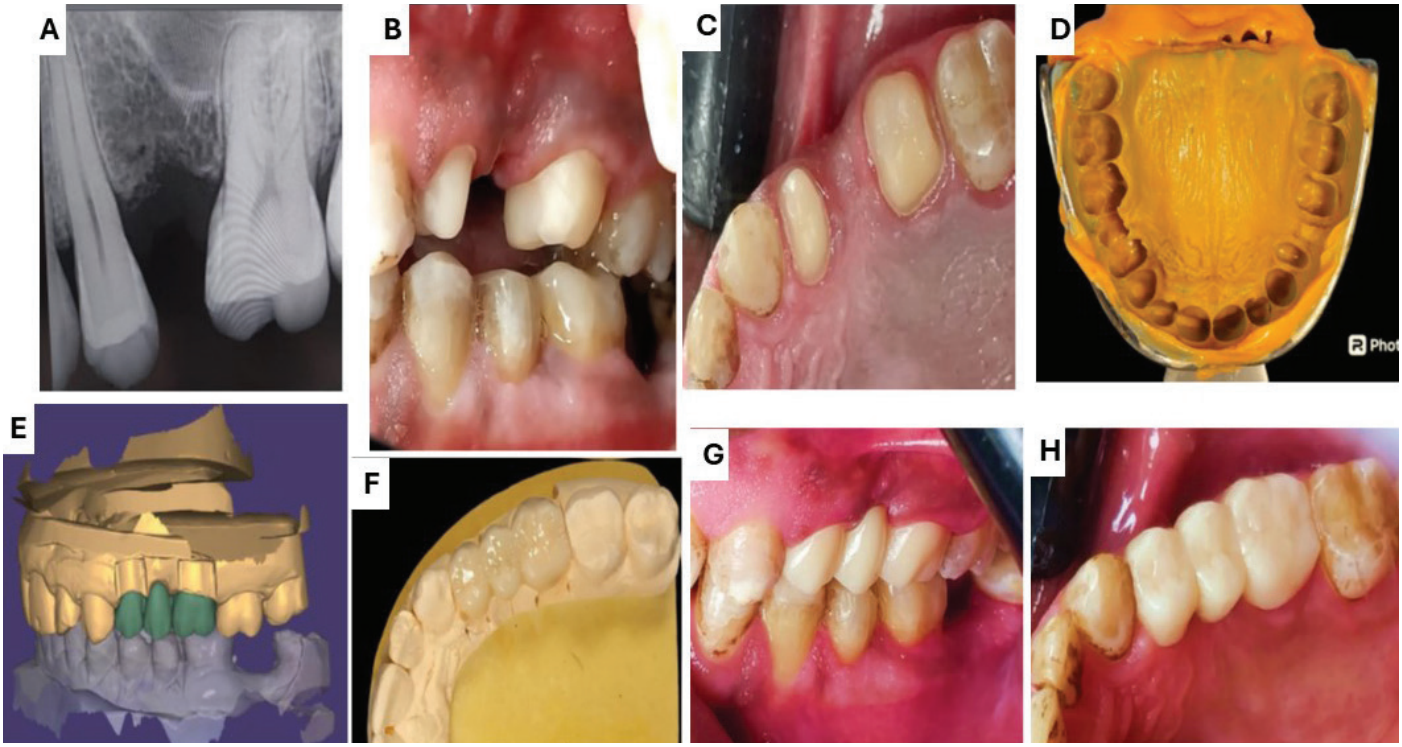

Figure 13: Zirconium Fixed Partial Denture on #24 and #26 to restore #25.

Porcelain-Fused-to-Metal FPDs:

For a porcelain-fused-to-metal (PFM) fixed partial denture, the clinical procedure began with precise occlusal and axial reduction. The occlusal reduction was carefully performed to achieve a uniform clearance of approximately 1.5 mm, ensuring adequate space for the metal and porcelain layers while preserving the structural integrity of the tooth. Following tooth preparation, metal frameworks were fabricated to fit the prepared teeth accurately. These frameworks were then tried intraorally to verify proper fit, marginal adaptation, and occlusal alignment. Once confirmed, the frameworks were veneered with porcelain to achieve optimal esthetics and functional contours. Subsequent occlusal adjustments were made to ensure proper articulation and minimize any potential occlusal interferences. Finally, detailed post-operative instructions were provided to the patient, emphasizing oral hygiene practices and the importance of regular follow-up appointments to maintain the longevity and functionality of the prosthesis. **(Fig. 14).**

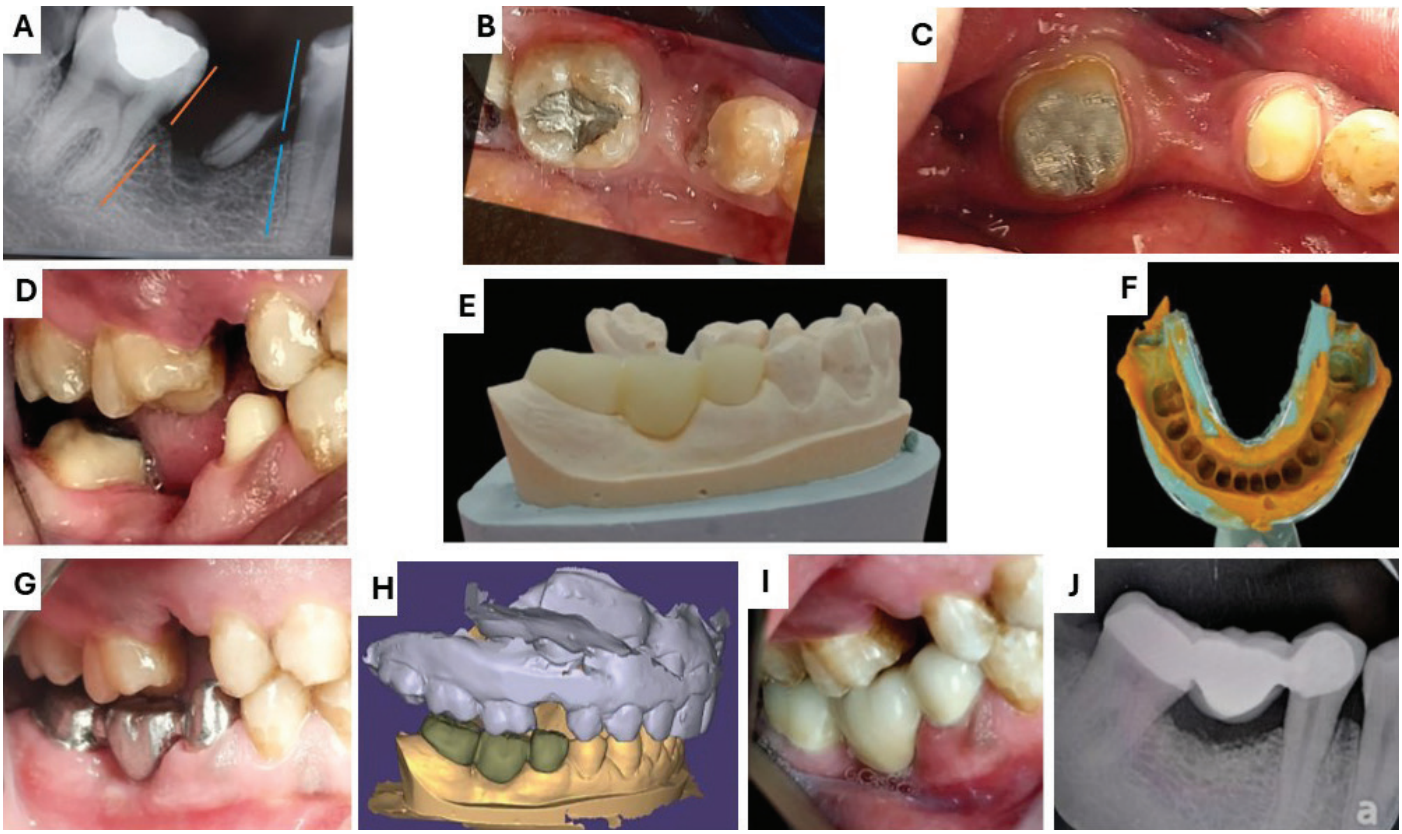

Figure 14: Porcelain Fused to Metal Fixed Partial Denture on teeth #45 and #47 to restore #46.

3.3 Removable Partial Denture (RPD)

Acrylic RPD was fabricated to restore single tooth #37 and to act as space preservation to prevent over eruption of the opposing second molar and further drifting of adjacent teeth. **(Fig.15)**

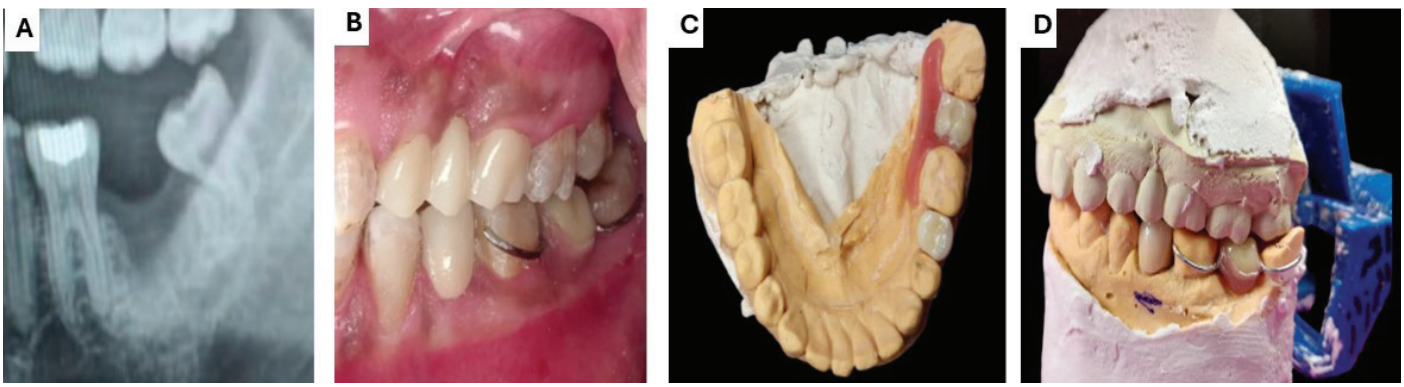

Figure 15: Acrylic RPD for restoring tooth #37

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Phase IV "Maintenance Phase"

The patient was advised to use dental floss or alternative interdental aids, such as interdental brushes or water flossers, to improve interproximal cleansing and reduce plaque accumulation (4). They were instructed to rinse twice daily for one minute with a 0.12% chlorhexidine gluconate mouthwash for one week and on the recall visits mouth wash will be re-instructed (11). Additionally, dietary modifications were recommended to lower the consumption of high-carbohydrate foods, aiming to reduce the substrate available for cariogenic bacteria (2).

4. Results

Post-treatment evaluation revealed a significant reduction in the measurements of probing depth; teeth that underwent endodontic therapy and restorations showed no evidence of periapical pathology or mobility. The functional and aesthetic results of the rehabilitation were positive, with proper marginal adaptation. The patient reported improved chewing efficiency, leading to enhanced self-esteem and overall satisfaction. In the patient's words, he regained his confidence and the ability to smile again. Three weeks postoperatively, regular follow-up appointments were scheduled for clinical and radiographic assessments, which indicated a high success rate. **(Fig. 16).**

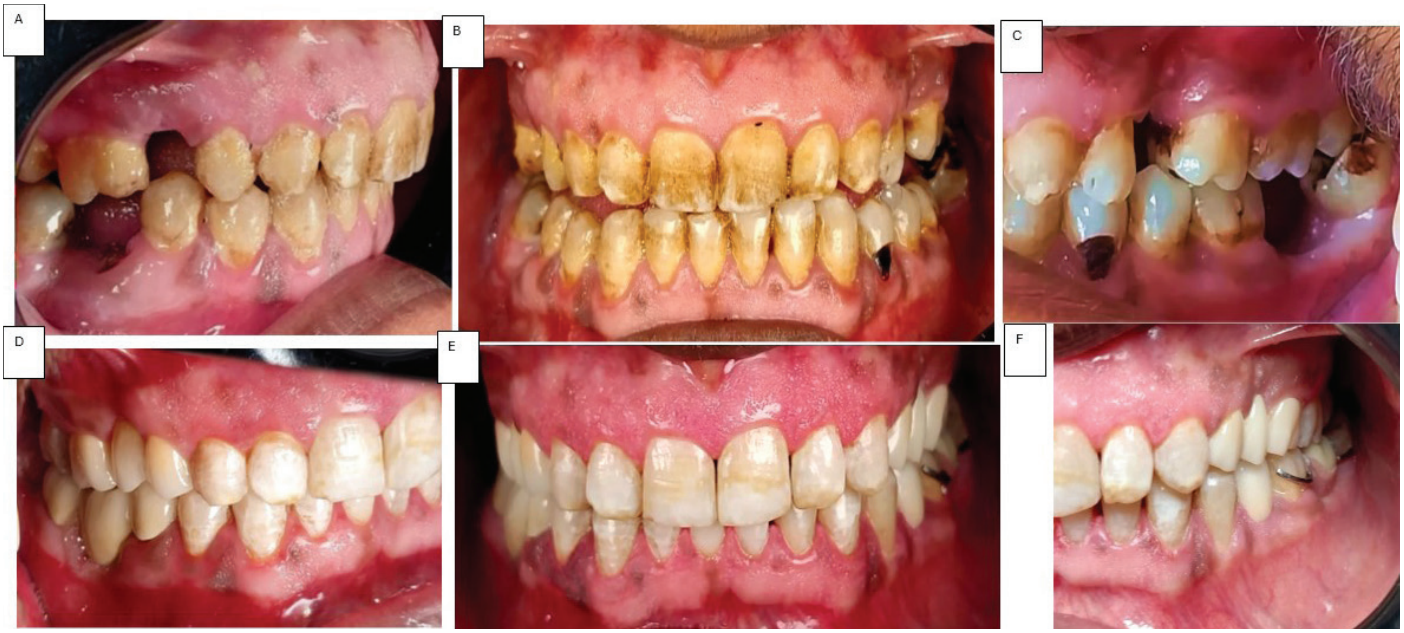

Figure 16 : (A): pre-operative right lateral view (B): pre-operative frontal view (C): pre-operative left lateral view (D): post-operative right lateral view (E): post-operative frontal view (F): post-operative left lateral view

5. Discussion

The successful outcome of this full-mouth rehabilitation emphasizes the importance of a holistic treatment approach. This collaborative effort addressed all aspects of the patient's oral health, including disease management and functional and aesthetic restoration (5).

The patient's chronic periodontitis posed a significant challenge. Non-surgical periodontal therapy was essential in stabilizing the condition, particularly with the use of tetracycline irrigation. The primary benefit of irrigating periodontal pockets with tetracycline-HCl lies in its localized concentration at the sites of disease activity. Tetracycline effectively penetrates gingival crevicular fluid and soft tissues, providing an enhanced antibacterial effect compared to mechanical debridement alone (11).

Dental caries is a multifactorial lifestyle disease, where adherence to medical advice regarding nutrition, lifestyle, and oral hygiene is essential. It can impact overall health through various mechanisms, with tooth loss affecting masticatory function and leading to changes in food selection and nutrition (2). The patient presented with multiple missing teeth (#15, #25, #37, and #46) and extensive cavities in teeth (#14, #16, #17, #18, #24, #26, #27, #28, #35, #36, #38, #45, and #48), which posed significant

restorative challenges. Additionally, the patient's high caries index prompted the recommendation for full-coverage restorations as part of the treatment plan (5).

Endodontic treatments successfully preserved the remaining teeth, enabling them to function as reliable abutments for crowns, particularly tooth #35. The treatment demonstrated a high success rate, with no signs of periapical pathology, indicating initial success, and follow-up visits were scheduled to confirm the 100% success rate. Multi-visit endodontic therapy was performed on tooth #35 to ensure an aseptic condition and absence of symptoms prior to obturation, leading to better healing and fewer postoperative complications (5).

Regarding the final prosthetic restorations, zirconia was selected due to its superior mechanical properties among dental ceramics and its low bacterial adhesion, which is crucial for the longevity of the prosthesis (8). The deep chamfer finish line was chosen to enhance fracture resistance and promote even distribution of occlusal forces along the margin of the restoration (8).

The cementation procedure for zirconia restorations is influenced by the thickness of the material, as it impacts light curing and the polymerization of dual-cure resin cements used beneath the restorations. A negative correlation exists between the thickness of the zirconia and the degree of polymerization, with thicker zirconia potentially reducing the effectiveness of the light cure and polymerization process (12).

6. Conclusion

Full-mouth rehabilitation is a multifaceted and challenging procedure that necessitates the careful integration of interdisciplinary principles to achieve both functional and aesthetic outcomes.

The treatment led to improved occlusal stability, enhanced masticatory efficiency, and a notable increase in the patient's self-confidence. The use of deep margin elevation, a conservative approach, effectively raised the cervical margin. Ongoing follow-ups and maintenance were emphasized to ensure long-term success. This case demonstrated a high success rate, with advanced restorative techniques leading to substantial improvements in the patient's oral health and quality of life. A well-rounded diagnostic evaluation and treatment plan are crucial for sustained success.

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A Neglected Confluent Middle Mesial Canal in an Infected Pretreated Mandibular First Molar - A Case Report

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ABSTRACT

In terms of root canal treatments, it is mandatory to detect all the orifices found on the pulp chamber floor in order to diminish the bacterial load ensuring proper chemo-mechanical debridement of the root canals and creating a three-dimensional space for an inert filling material preventing further infections; therefore, All clinicians must be aware of the Middle Mesial Canal (MMC), as these canals correlate significantly with apical periodontitis and failure of treatment representing a challenge in diagnosis. A female patient presented with throbbing pain and swelling in the lower left side of her face; after clinical and radiographic examinations, an infected, pretreated mandibular left first molar was found to be responsible for the symptoms. A neglected confluent middle mesial canal was discovered, cleansed, and treated. To date, the tooth has not developed renewed symptoms or any signs of failure. Since detecting and treating all root canals in multi-rooted teeth are fundamental in terms of endodontic therapy to minimize bacterial growth, provide proper sealing of root canal systems, and increase the success rate of root canal treatments, clinicians must always have a clear conscience and take sufficient time to seek all root canals present in the tooth, not only the well-known and recurrent ones.

KEYWORDS

Case report, Middle mesial canal, Root canal treatment, Missing canals, Retreatment.

1. Introduction

In terms of root canal treatments, it is mandatory to detect all the orifices found on the pulp chamber floor to diminish the bacterial load ensuring proper chemo-mechanical debridement of the root canals and creating a three-dimensional space for an inert filling material preventing further infections [1]; however, it is important not to excessively prepare the canals using rotary files in order to maintain fracture resistance [2, 3].

The root canal anatomy is diverse and complex consisting of numerous foramina, isthmuses, fins, apical deltas, loops, and intercanal passages [4]. A low-dose Cone Beam Computed Tomography radiograph could be necessary for obtaining the root anatomy, facilitating decisions, sparing time, detecting complications and difficulties improving future outcomes [5].

The mandibular first molar is the most root canal-treated tooth [6], where in 90% of cases, the mesial root contains two main canals that end in two separate foramina; whereas, in the rest (10%), the two canals fuse just before the foramen. The distal root contains one large oval or kidney-shaped canal (65%) or two canals (35%) [7, 8]. Overall, the mandibular first molar possesses a variety of abnormalities, for instance, Middle Mesial Canal (MMC), Middle Distal Canal (MDC), Radix Endomolaris, Radix Paramolaris, and Taurodontism, which all clinicians should be aware of before performing root canal therapies [9].

The presence of MMC differs according to ethnicity and ranges from 0.26 to 45.8 % [10] and was first reported in 1974 [11]. This canal is always present in younger and middle-aged patients and is significantly more relevant in younger ones [12]. In the mandibular first molar, the MMC incidence on both sides and in both genders is insignificant [13].

MMCs can be classified into three types: Type (I) fin, an isthmus is present between the MMC and

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the mesiobuccal canal from the orifice to the apex or an instrument can pass easily between the MMC and ML or MB canal; type (II) confluent, a separate orifice that joins the MB or ML canal through intracanal connection and isthmuses; type (III) independent, three independent canals from the orifices to the foramina [14].

2. Case Presentation

On 11th December 2024, a 20-year-old mentally healthy married female patient was referred by a colleague, accompanied by her mother. In terms of medical history, she had no systemic disorders, allergies, or infections, and takes no medications that could interfere with future dental treatments. Intraorally, she had good oral hygiene with no relevant caries or restorations visible; however, she still had an orthodontic appliance on the upper teeth. She had a chief complaint of throbbing pain and a focal swelling on the lower left side of her mandible for over three weeks. She started using an analgesic (paracetamol 500 mg) three times a day and a broad-spectrum antibiotic (Cefix 400 mg) once a day for 5 days, trying to relieve the acute and disturbing symptoms, yet she experienced stomachache as a side effect. Clinical and intraoral radiographic examinations were performed to detect the tooth responsible for the chief complaint.

Clinically, the mandibular left first molar was previously restored with composite resin (MO) and was hypersensitive on both palpation and percussion with a negative cold test result, along with a hard palpable swelling localized on the buccal mucosa with no fistula; in addition, signs of micro-leakage around the restoration were also observed. No significant findings were observed on the other teeth on the same side. The combination of hypersensitivity, swelling, and micro-leakage strongly suggests that the mandibular left first molar may be experiencing periapical issues. A preoperative diagnostic periapical radiograph was warranted to assess the extent of any underlying pathology and guide appropriate treatment. Surprisingly, the radiograph displayed well-condensed root canal fillings with full working lengths in all four canals with a large J-shaped radiolucent lesion on the mesial root apex and a small lesion on the distal one (figures 1 and 2).

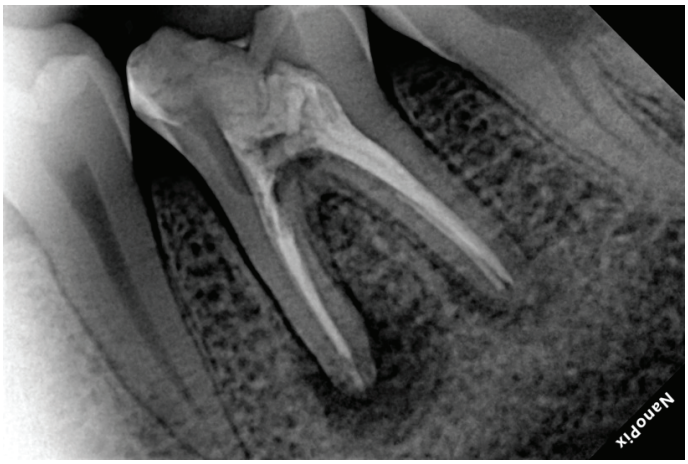

Figure 1. Preoperative radiograph of the first molar

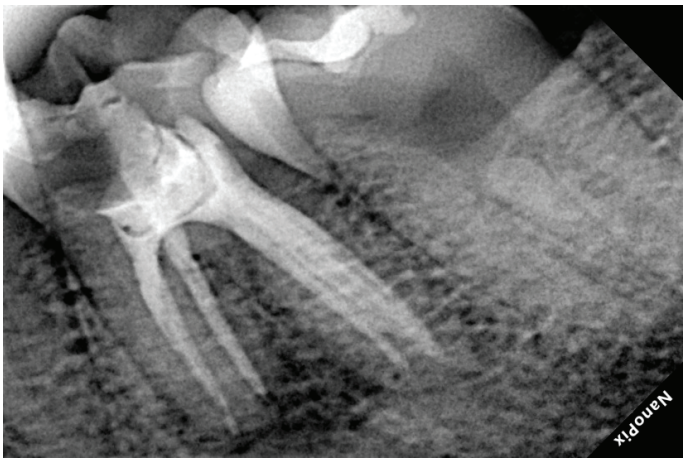

Figure 2. Angulated preoperative radiograph of the molar

After diagnosis was established, the treatment plan was to retreat the first molar by non-surgical endodontic intervention, seeking the possible cause of failure as follows: The restoration and base layer were completely removed using a size 023 coarse round diamond bur (DIASWISS, Switzerland), and the pulp chamber was examined with a sharp endodontic explorer #DG16 (Nordent, USA) to eliminate accidental perforations and to ensure all orifices are accessible. Since the middle mesial canal is mostly present in the first molar in younger individuals [13] and the lesion is focalized on the mesial root, this undetected and neglected canal was thought to be responsible for the failure.

The patient was a little concerned and uncooperative, refusing to put the rubber dam during sessions as she was a mouth breather and was concerned about having the orthodontic appliance damaged by the clamp. Cotton rolls and a saliva ejector were used as alternatives. The root fillings were dissolved and removed manually by injecting Sep Xylol

(Septa, Syria) for 2 minutes, and the canals were carefully negotiated with #25 H files (MANI, Japan) to provide a safe path to the apices and remove the fillings and remnants of the sealer. The MMC was detected by the explorer and found to be neither negotiated nor treated. The working lengths were determined for the four pretreated canals using #10 K files (MANI, Japan) for the mesial canals and #15 for the distal ones. These working lengths were determined to be (17 mm) for the mesial canals and (19 mm) for the distal ones; unfortunately, the radiograph of working lengths could not be retrieved from the system; finally, the two mesial canals were cleaned and prepared by M3 Pro Gold rotary files taper 0.04% (Bondent, Germany) mounted on a rotary handpiece (Cicada, China) up to #25 for the medial canals and up to #30 the two distal canals under 5.25% sodium hypochlorite irrigation. The MMC was negotiated gently with #6 K files to the full working length (17 mm), then mechanically prepared up to #25 taper 0.04%. Clinically, the MMC appeared to have a separate orifice located equidistant between the MB and ML canals, as shown in Figure 3 (Figure 4), and its path joins the MB canal, ending in the same apex, forming a confluent type, whereas the ML canal had a separate path from the orifice to a different apex.

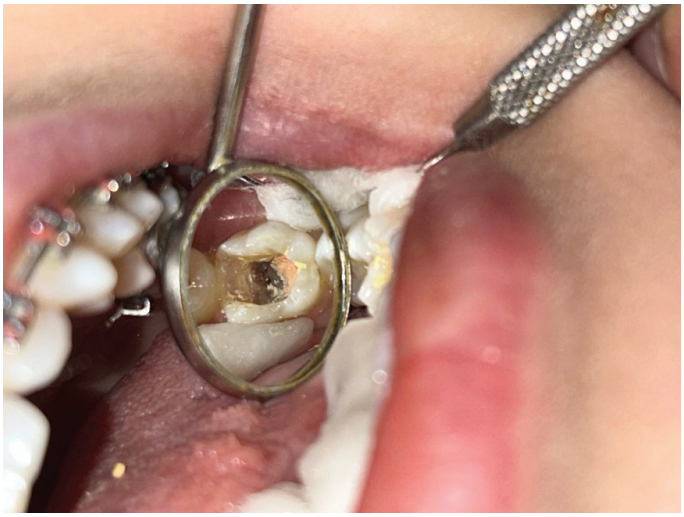

Figure 3. The three mesial orifices

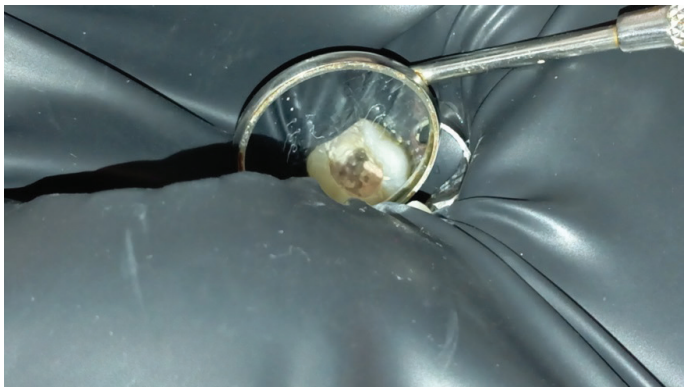

Figure 4. The three mesial orifices under the rubber dam

Each canal was irrigated with 5 mL of sodium hypochlorite, dried with paper points, and then filled with CaOH dressings with iodoform (Calplus™, India) with the help of a lentulo spiral; this process was performed once a week for 4 weeks in a row to ensure disinfection of all canals before obturation. After the final dressing, a thorough evaluation of the canals was conducted to confirm the absence of any purulent or serous exudates. Mastercones (Dia-ProISO.04, Diadent, South Korea) corresponding to the size of the prepared canals were fitted and assessed radiographically with mesial angulations to ensure full working lengths and the feeling of tug-back. The radiograph of mastercones of the distal canals was also lost from the system.

The patient was convinced to put the rubber dam only during the session of filling the distal canals (Figure 4). After ensuring dryness of all canals by paper points and excluding any serous or purulent exudate, the obturation was established in two separate sessions by cold lateral compaction technique using Gutta-percha mastercones taper 0.04 % compacted with secondary cones taper 0.02 % along with epoxy-based resin sealer system (Dia-proseal, Diadent, South Korea) to provide proper and better sealing for the three thirds of each canal. Restoration of the access and MO cavities was achieved using bulk-fill composite resin (ESPE Filtek™ Bulk-Fill, 3M, USA) on 22nd February 2025 (Figure 5). Postoperative instructions were provided to the patient, emphasizing the importance of follow-up visits to monitor the healing process and the overall success of the treatment. The patient was scheduled for reassessment after at least 3 months to evaluate the lesion and for Retreatment.

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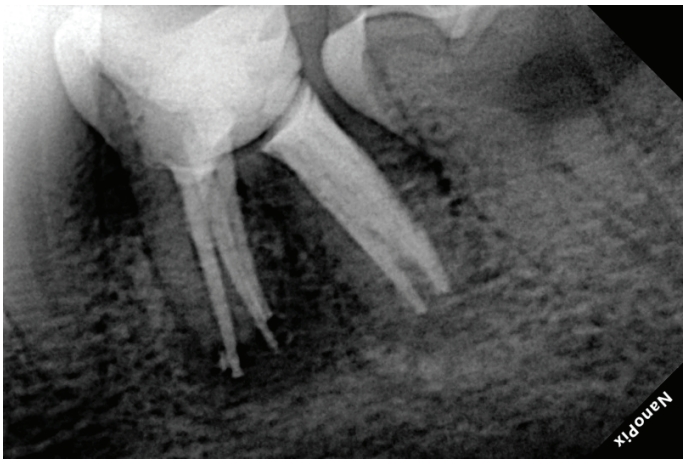

Figure 5. Postoperative radiograph of the first molar

So far, on 20th May 2025, the tooth has not developed any further symptoms or signs of failure, and the prognosis seems to be good so far. No adverse and unanticipated events were observed. The patient was satisfied with the treatment offered, as the disturbing symptoms subsided gradually and became asymptomatic; in addition, the patient could preserve the tooth without having to undergo an extraction procedure, as she was planning to have a fixed orthodontic appliance on the lower jaw.

A reevaluation radiograph was obtained on 29th June 2025, where no further resorption of the mesial root or increase in the size of the lesion was observed. A metal band for a fixed orthodontic appliance was applied to the molar by the patient's orthodontist, as shown in Figure 6.

Apologies to the readers for the accidental loss of some radiographs.

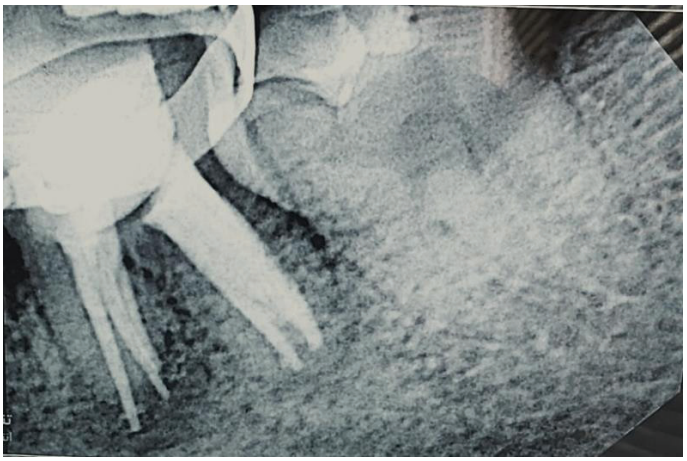

Figure 6. Reevaluation radiograph of the molar

3. Discussion

This case highlights the crucial role of the MMC in the success rate of root canal treatments in mandibular molars and, in particular, the first molars. According to Fabra-Campos, 2.6% of lower molars have three passages in the mesial root, 1.7% of which have merged canals [15]. The mesial root of the mandibular first molar has one large canal until the age of 11, but due to secondary dentin deposition, the root canal system alters in the apical two-thirds at the age of 30-40 [16]; therefore, acknowledging these age-related differences in configuration assists in locating and negotiating all canals present [17].

In the patient's case, no dentinal projection along the mesial flange of the pulp chamber was observed because it was probably removed by the previous clinician; however, this projection is present in the majority of cases [18]. No dental loupes or any other magnification methods were used in managing this case; nevertheless, the dental operating microscope could have provided better illumination and visibility as it detects slight color alterations and enhances comprehension of pulp chamber floor anatomy [18].

All clinicians must be aware of MMC variations to provide complete cleaning and sealing of the canals, as these missing canals correlate significantly with apical periodontitis [19]; these findings were observed in the patient's case, where a large radiolucent lesion formed on the mesial root apex.

A little deliberate extrusion in the mesial canals was intended, as a case series of 220 root canal treatments reported that overfilling does not negatively affect the long-term performance of root canal treatments if adequate disinfection and three-dimensional seal of the apical one-third were provided [20].

As strengths associated with this case report, no surgical interventions or novel diagnostic methods were resorted to in diagnosing and treating this case. Some limitations were encountered during the manipulation of this case report, for instance, the rubber dam was applied only during the obturation process, as the patient normally suffers from renal disorders; no MTA plugs were applied at the apices of the absorbed mesial root, as a time-sparing procedure was mandatory to avoid further contamination.

4. Conclusions

Since detecting and treating all root canals in multi-rooted teeth are fundamental in terms of endodontic therapy to minimize bacterial growth, provide proper sealing of root canal systems, and increase the success rate of root canal treatments, clinicians must always have a clear conscience and take sufficient time to seek all root canals that may be present in a tooth, not only the well-known and recurrent ones.

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Conflict of Interest

The authors declare no potential conflict of interest concerning the patient's case, authorship, and/or publication of this case report.

Informed Consent and Ethical Approval

Written informed consent was obtained from the patient. Ethical approval was not required.

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Investigating Potential Antihypertensive Bioactive Agents In Hibiscus Sabdariffa Through Molecular Docking, Pharmacokinetic, And Admet Prediction Studies

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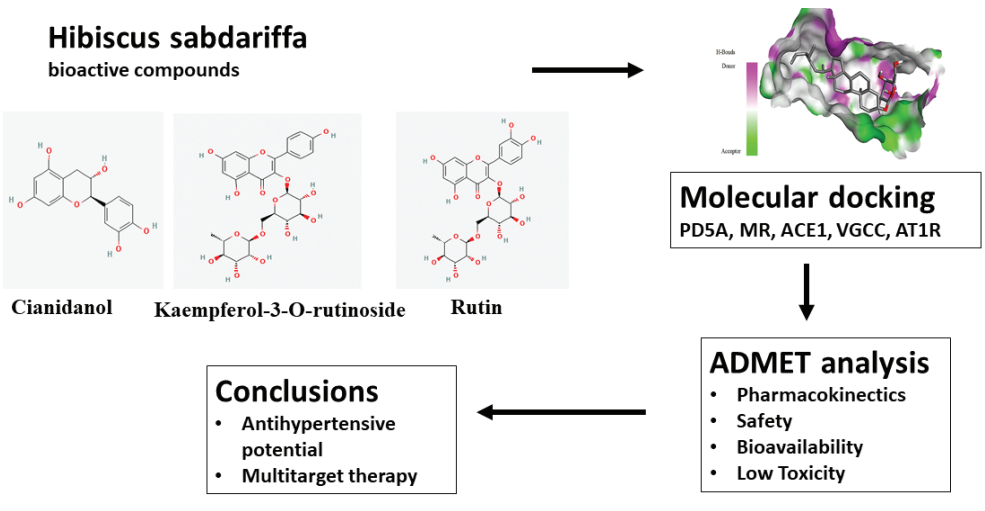
ABSTRACT

Hypertension remains a critical global health concern, contributing significantly to cardiovascular morbidity and mortality. In different cultures, traditional remedies, such as plant-based therapeutics, have been widely employed to manage this condition. However, the limited scientific understanding of their mechanisms hinders their integration into standardized treatment frameworks. This study investigated the antihypertensive potential of Hibiscus sabdariffa (HS) by employing molecular docking and ADMET analyses to elucidate its bioactive compounds and their molecular interactions. Seventy-four compounds from HS were docked against five antihypertensive protein targets. ADMET and pharmacokinetic analyses were done on the top-ranking compounds. Molecular docking analysis revealed promising interactions between key bioactive compounds of HS, including kaempferol-3-O-rutinoside, beta-sitosterol 3-O-beta-D-galactopyranoside, cianidanol, and Rutin, and the following crucial antihypertensive targets: phosphodiesterase 5A (PDE5A), angiotensin II type 1 receptor (AT1R), angiotensin-converting enzyme I (ACE 1), mineralocorticoid receptor (MR), and voltage-gated L-type calcium channel (VGCC). The best docking scores for the receptors ranged from -9.4 to -10.6. Complementary ADMET analysis provided valuable insights into the pharmacokinetic properties and safety profiles of these compounds, underscoring their therapeutic potential. Notably, cianidanol exhibited favorable docking scores and pharmacokinetic attributes, including high bioavailability and low toxicity. These findings establish a molecular basis for the traditional use of Hibiscus sabdariffa as a multitarget therapy for the management of hypertension and support its potential development as a natural therapeutic agent. Future experimental studies are essential to validate and optimize these bioactive compounds for antihypertensive drug development.

KEYWORDS

ADMET analysis, Bioactive compounds, Hibiscus sabdariffa, Hypertension, Molecular docking.

Graphical Abstract



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1. Introduction

Hypertension, a cardiovascular disorder characterized by persistently elevated blood pressure, is a leading preventable cause of cardiovascular diseases and premature death worldwide. Affecting over one billion adults globally, it imposes a significant health burden, particularly in low- to middle-income countries, where nearly 46% of cases are undiagnosed, owing to its often asymptomatic nature [1-4].

Current treatment strategies typically combine lifestyle modifications, such as dietary changes and increased physical activity, with pharmaceutical interventions. However, the high cost and side effects of these medications have spurred interest in alternative natural therapies, which may offer safer, cost-effective solutions [5]. Among these natural therapies, *Hibiscus sabdariffa* (HS), known for its rich array of bioactive compounds, has emerged as a promising candidate [6].

Commonly referred to as Roselle, Jamaica sorrel, or red sorrel, HS is an annual, flowering subshrub from the *Malvaceae* family. Its vibrant red calyces (sepals) and reddish stems are widely recognized and cultivated across subtropical and tropical regions, including Nigeria, Mexico, and Thailand. Its long history of traditional use across various cultures highlights its potential health benefits [7-8].

Preclinical and clinical studies have shown that HS contains bioactive components that are beneficial for managing various health conditions, including hypertension, inflammation, and diabetes, while maintaining a favorable safety profile and minimal side effects [7, 9-10]. Its widespread availability, cost-effectiveness, and proven efficacy in hypertension management have contributed to its popularity as a natural therapeutic agent in diverse cultural and socioeconomic settings [6, 11].

Despite its long history of use in hypertension management, the specific phytochemicals responsible for its antihypertensive effects and its mechanisms of action are not fully understood. Bridging these knowledge gaps is crucial for integrating HS into standardized treatment protocols, as understanding its molecular interactions is key to confirming its therapeutic efficacy in hypertension management.

This study utilizes in silico methods — molecular docking and ADMET profiling — to examine the antihypertensive potential of bioactive compounds found in HS. By assessing the binding affinities, physicochemical properties, and toxicological properties of these compounds in the context of hypertension, this research contributes to the development of natural treatments for hypertension. Building on existing research that has highlighted the general therapeutic potential of HS [7-9], this study focused on identifying specific bioactive compounds to elucidate their molecular mechanisms in hypertension management. These findings will also help bridge the gap between traditional herbal medicine and modern drug discovery, potentially expanding treatment options for this prevalent health concern.

2. Material and Methods

2.1 Target Selection

All the targets used in this study were sourced from the *Open Targets platform* [12] (https://platform.opentargets.org/). Protein targets relevant to hypertension pathways and those with the highest potential for therapeutic intervention were selected. The X-ray crystallographic 3D structures of the selected targets were downloaded from the Research Collaboratory for Structural Bioinformatics (*RCSB*) online protein data bank repository [13] (https://www.rcsb.org/). The following protein targets were used: phosphodiesterase 5A (PD5A; PDB ID: 1xp0), angiotensin II type 1 receptor (AT1R; PDB ID: 4zud), angiotensin-converting enzyme I (ACE 1; PDB ID: 7z70), mineralocorticoid receptor (MR; PDB ID: 6gev), and voltage-gated L-type calcium channel (VGCC; PDB ID: 8we8).

2.2 Target preparation

Protein structures were prepared via the BIOVIA Discovery Studio visualizer. Water molecules were removed, and hydrogen atoms were added to stabilize the protein structures. The cocrystalized ligands were used to determine the binding sites of the targets before removal. Protein chain A, which contained the active site for each target, was used in the analysis.

2.3 Ligand Selection

The 74 ligands used in this study were sourced from the *PubChem* database [14] (https://pubchem.ncbi.nlm.nih.gov/). Silicon-containing compounds were excluded because of

incompatibility with the PyRx software used for molecular docking.

2.4 Ligand Preparation

The 2D/3D structures of the bioactive compounds used were retrieved from *PubChem*. *PyRx* was used in the 3D transformation, optimization, and energy minimization of the ligands to determine their most stable pose.

2.5 Molecular analysis

Docking was performed using the *AutoDock Vina* module within *PyRx*, followed by visualization of ligand–target interactions via *BIOVIA Discovery Studio*. The results obtained were ranked on the basis of their root mean square deviation values and binding energies in kcal/mol. Ligands with the lowest binding energies, indicative of strong target interactions and favorable poses, were selected for further analysis.

2.6 Setting the Grid Dimension for AutoDock Calculations

The grid was generated to define the position and size of the protein's active site for ligand docking. The search space coordinates were provided by *AutoDock Vina* via *PyRx*. The target's active site was determined via the position of the cocrystallized ligand at the binding site and validated via the web-based tool *Castp* [15] (http://sts.bioe.uic.edu/castp/index.html?1ycs). The grid settings used in the PyRx interface for the site-defined docking analysis are as follows:

Phosphodiesterase 5A: Center (Angstrom): X: -20.2738, Y: 31.8800, Z: 65.3799; Dimensions (Angstrom): X: 18.2444, Y: 20.8713, Z: 25.4134

Mineralocorticoid receptor: Center (Angstrom): X: 7.5056, Y: 16.5037, Z: 15.9444; Dimensions (Angstrom): X: 17.9585, Y: 20.3215, Z: 27.7966

Angiotensin converting enzyme 1: Center (Angstrom): X: 8.8189, Y: 4.4763, Z: 23.5860; Dimensions (Angstrom): X: 29.4904; Y: 34.9122; Z: 32.0535

Angiotensin II type 1 receptor: Center (Angstrom): X: -40.7332, Y: 67.5631, Z: 28.7825; Dimensions (Angstrom): X: 23.7163, Y: 18.0038, Z: 26.2902

L-type voltage-gated calcium channel: Center (Angstrom): X: 159.4740, Y: 166.1762, Z: 147.6636; Dimensions (Angstrom): X: 25.0000, Y: 25.0000, Z: 27.2547

Default settings were applied for all other parameters to ensure consistency across the analyses.

2.7 Docking Validation Protocol

To validate the docking protocol, the cocrystallized ligands for each target protein were extracted and re-docked into their respective binding sites using Discovery Studio 2021 and PyRx's *AutoDock Vina* module. The same docking parameters applied to the test compounds were used. The accuracy of redocking was assessed by calculating the root-mean-square deviation (RMSD) between the docked pose and the experimental crystallographic pose of the ligand. An RMSD ≤ 3.0 Å was considered indicative of a valid docking protocol.

2.8 Pharmacokinetic and Toxicity Analysis

The ADMET properties of the selected lead compounds were evaluated using in silico predictive models to assess their potential efficacy, safety, and bioavailability. The *SwissADME* web application (16) (http://www.swissadme.ch/) was used to assess the ADME properties of the compounds - lipophilicity (mean Log Po/w), aqueous solubility (via the estimated SOLubility (ESOL) model), human gastrointestinal absorption, and interactions with metabolizing enzymes. The drug likeness of the compounds was evaluated on the basis of bioavailability scores and adherence to Lipinski's Rule of Five. Compounds meeting these criteria are more likely to exhibit favorable oral bioavailability and therapeutic potential [16]. The *ProTox-III* online server [17] (https://comptox.charite.de/protox3/) was used to predict toxicological endpoints, including acute toxicity class, lethal dose (LD50), hepatotoxicity, carcinogenicity, mutagenicity, cytotoxicity, and immunotoxicity of the lead compounds.

3. Results and Discussion

3.1 Molecular Docking Analysis

Hypertension continues to pose a significant global health challenge, with its prevalence and associated burden projected to escalate in the coming years. Several enzymes and receptors have been identified as key in the development and progression of hypertension and are often the targets of common antihypertensive medications. Effective modulation of these targets

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is essential in managing this prevalent condition [18]. The use of combination pharmacotherapy, which has demonstrated superior efficacy compared with single-pill therapy, may be hindered by its higher costs, increased risk of side effects, and complexity of multidrug regimens. Consequently, the exploration of alternative therapeutic strategies, particularly those derived from natural sources, is imperative. HS offers a promising avenue for the development of safe, cost-effective antihypertensive agents [5, 10].

This study elucidates the molecular interactions between key bioactive compounds from HS and hypertensive targets, revealing several compounds with binding affinities surpassing those of established drugs. These findings highlight the therapeutic potential of HS-derived compounds, particularly their capacity to modulate critical enzymes and receptors involved in hypertension pathophysiology.

3.1.1 Target 1: Phosphodiesterase 5A (PD5A)

Table 1 summarizes the docking scores for the top five bioactive compounds and the standard drug sildenafil against PD5A. Kaempferol-3-O-rutinoside, HS03, and Rutin showed similarly high predicted binding affinities (-10.2 to -10.1 kcal·mol^{-1}), while epigallocatechin gallate and quercitrin scored slightly lower (-10 to -9.6 kcal·mol^{-1}).

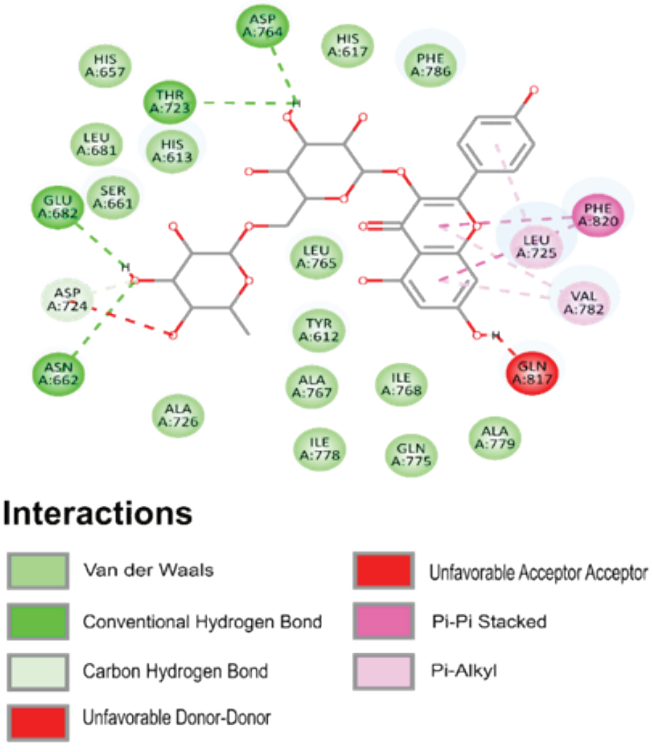
Kaempferol-3-O-rutinoside formed several hydrogen bonds with residues ASN662, GLU682, THR723, and ASP764 within the active site of the target. Pi–alkyl and pi–pi stacking interactions were also observed with the hydrophobic amino acid residues LEU725 and VAL782 and the aromatic amino acid residue PHE820 (Fig. 1). Taken together, the docking poses and interaction patterns suggest these compounds warrant further investigation as potential PD5A binders. The interaction between the reference drug, sildenafil, and PD5A is shown in Fig. 2.

The docking protocol reproduced the crystallographic binding pose of the co-crystalized ligand, Vardenafil, with an RMSD of 0 Å and binding affinity of −9.0 kcal/mol, which is within the acceptable cutoff for validation.

Table 1: Docking scores of the five top-scoring compounds of HS and standard drug against the antihypertensive target phosphodiesterase 5A (PD5A)

Compound (ligand)	Structural class	Docking score (Kcal/mol)
Kaempferol-3-O-rutinoside	Trihydroxyflavone	-10.2
HS03	Terpenoid	-10.1
Rutin	Tetrahydroxyflavone	-10.1
Epigallocate-chin Gallate	Flavans	-10
Quercitrin	Tetrahydroxyflavone	-9.6
Sildenafil (Standard)	PD5A Inhibitor	-9.6

HS03 = (3R,5S,9S,10R,13S,14R,17S)-17-[(E,2S,5S)-5-ethyl-6-methylhept-3-en-2-yl]-10,13-dimethyl-2,3,4,5,6,9,11,12,14,15,16,17-dodecahydro-1H-cyclopenta[a]phenanthren-3-ol



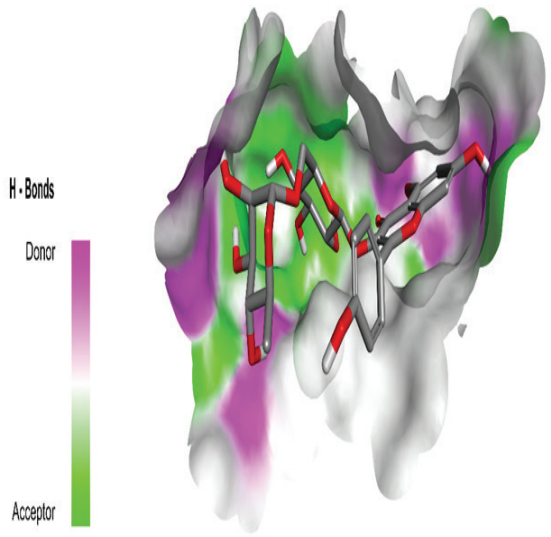


Figure 1: 2D (top) and 3D (bottom) interactions of kaempferol3-O-rutinoside with the phosphodiesterase A5 active site

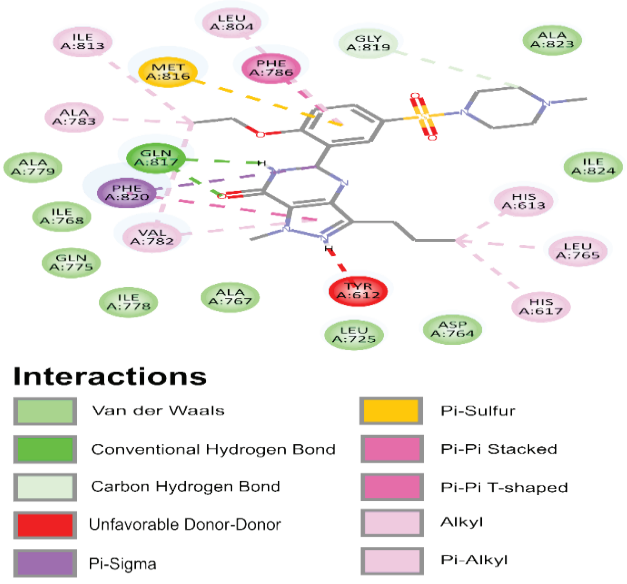


Figure 2: 2D ligand interaction of sildenafil (standard) with the phosphodiesterase A5 active site

PD5A is an enzyme involved in the degradation of cyclic guanosine monophosphate (cGMP) in vascular smooth muscle cells. The inhibition of this enzyme by HS-derived flavonoids, including kaempferol-3-O-rutinoside and structurally related compounds, could increase cGMP levels, promote vasodilation, and reduce blood pressure. These results are consistent with studies that highlighted the antihypertensive effects of kaempferol-3-O-rutinoside (19-21). This study demonstrates that kaempferol-3-O-rutinoside and similar HS compounds may serve as promising antihypertensive agents through PD5A inhibition.

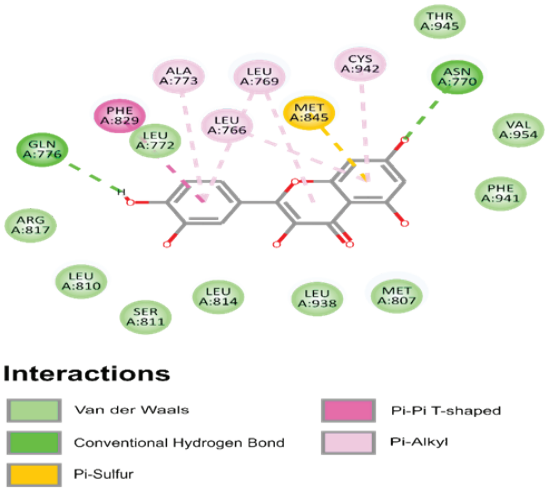
3.1.2 Target 2: Mineralocorticoid Receptor (MR)

Table 2 summarizes the docking scores for the top five bioactive compounds and the standard drug spironolactone against the MR. Quercetin, gossypetin, and cianidanol formed multiple hydrogen bonds with key residues GLN776 and MET807, alongside pi–sulfur interactions at MET845, pi–alkyl interactions with hydrophobic residues, and pi–pi stacking interaction with PHE829. These interactions, depicted in Figs. 3–5, contributed to their strong predicted binding affinity (–9.3 kcal/mol), which is comparable to that of spironolactone (–10.4 kcal/mol, Fig. 6). Therefore, these flavonoids may be considered to have a similar binding potential to the standard drug.

Redocking yielded a perfect overlap with the crystallographic pose for the co-crystalized ligand, RSCB ligand ID: EWN, with an RMSD of 0 Å, and a binding affinity of –12.2 kcal/mol, indicating excellent docking reliability.

Table 2: Docking scores of the five top-scoring compounds of HS and standard drug against the antihypertensive target mineralocorticoid receptor (MR)

Compound (ligand)	Structural class	Docking score (Kcal/mol)
Spironolactone (Standard)	MR antagonist	-10.4
Quercetin	Pentahydroxyflavone	-9.3
Gossypetin	Hexahydroxyflavone	-9.3
Cianidanol	Flavan-3-ols	-9.3
Hibiscetin	Flavonoids	-9.2
Apigenin	Trihydroxyflavone	-9.2



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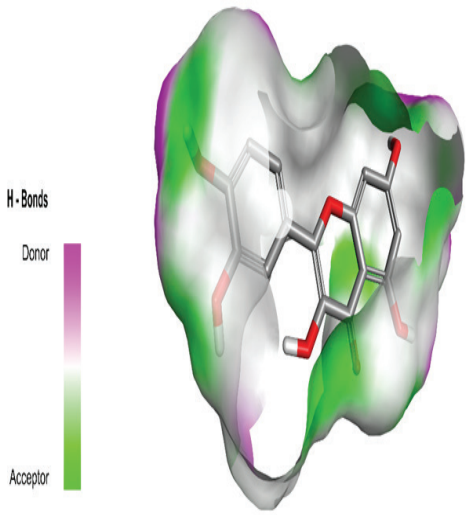


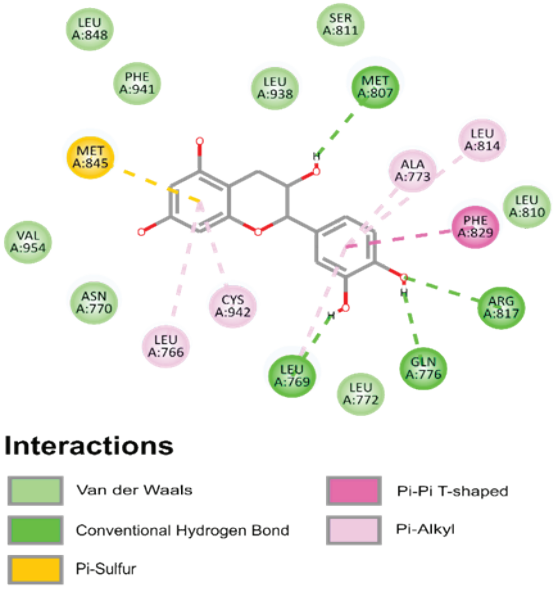
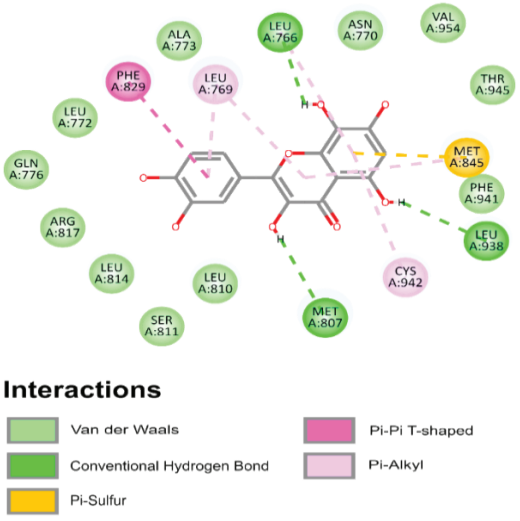


Figure 3: 2D (top) and 3D (bottom) interactions of quercetin with the MR active site



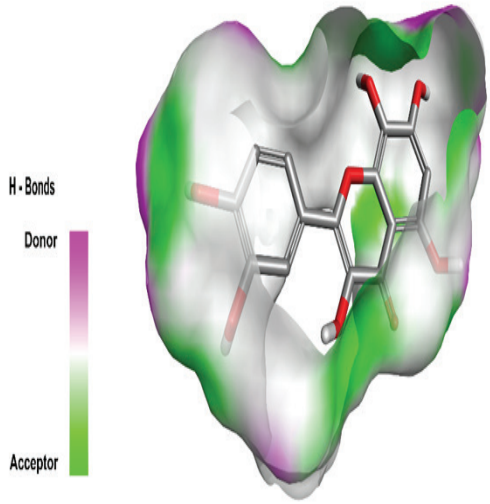


Figure 4: 2D (top) and 3D (bottom) interactions of gossypetin with the MR active site

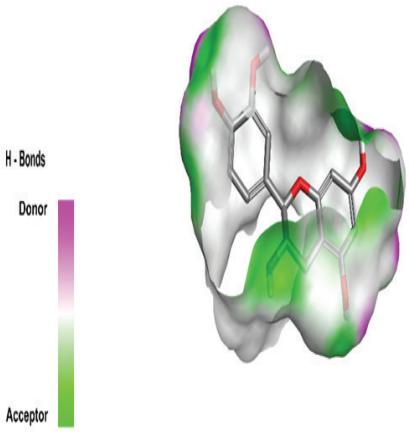


Figure 5: 2D (top) and 3D (bottom) interactions of cianidanol with the MR active site

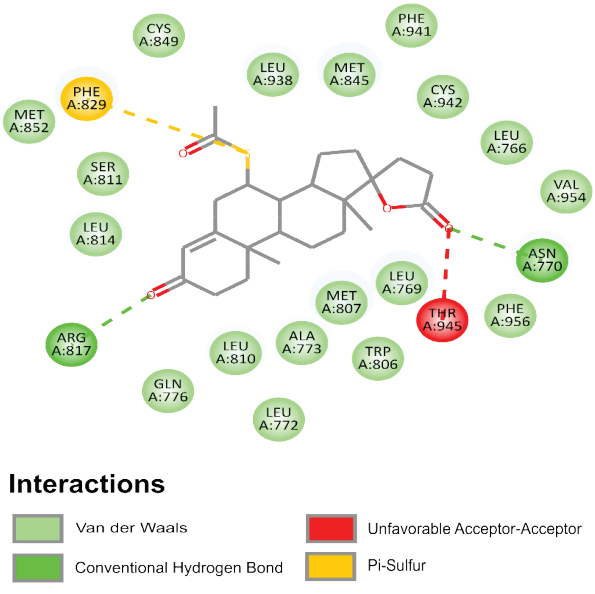


Figure 6: 2D ligand interaction of spironolactone (Standard) with the MR active site

The MR, a nuclear receptor central to sodium and water homeostasis, plays a key role in blood pressure regulation. This study revealed that quercetin, gossypetin, and cianidanol scored slightly lower than the standard drug spironolactone. However, these compounds exhibited structural similarities and favorable binding profiles, highlighting their potential as scaffolds for developing novel MR antagonists. The diverse biological activities of quercetin, including its antidiabetic, neuroprotective, and antihypertensive effects, are well documented [22-23]. The antihypertensive actions of quercetin have been attributed to multiple mechanisms, including attenuation of oxidative stress, modulation of the renin–angiotensin system, and improvement of endothelial function [24]. Similarly, cianidanol (catechin) has shown therapeutic potential in regulating lipid metabolism and stimulating nitric oxide production — mechanisms that collectively contribute to its antihypertensive effects [25-27].

Consistent with prior studies reporting the diuretic effects of quercetin and its analogs [28-29], our findings suggest that these compounds may modulate MR activity. By potentially influencing aldosterone-mediated sodium and water retention, these HS-derived flavonoids may contribute to the observed diuretic and antihypertensive effects of HS.

3.1.3 Target 3: Angiotensin II Type I Receptor (AT1R)

Table 3 summarizes the docking scores for the top five bioactive compounds and the standard drug Valsartan against AT1R. Several HS-derived compounds, including beta-sitosterol 3-O-beta-D-galactopyranoside, gossypol, and kaempferol-3-O-rutinoside, demonstrated high predicted binding affinities (–10.1 to –9.6 kcal/mol), which were comparable or slightly more favorable than valsartan (–9.0 kcal/mol). These binding affinities suggest that multiple structurally diverse HS compounds may serve as potential AT1R modulators. Beta-sitosterol 3-O-beta-D-galactopyranoside formed hydrogen bonds with key residues such as PRO19, ALA21, and ARG23, alongside pi–pi interactions with ILE288 and TRP84 (Fig. 7). These interactions suggest that multiple structurally diverse HS compounds may serve as potential AT1R modulators.

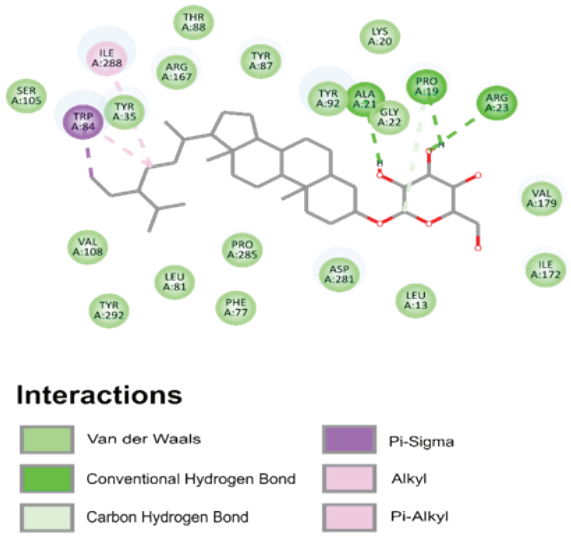
Redocking of the cocrystallized ligand, Olmesartan, yielded RMSD values of 2.62 Å (lower bound) and 3.87 Å (upper bound) for the best pose. The lower-bound RMSD indicates a near-native alignment, while the upper-bound value is slightly above the commonly used 3.0 Å threshold. Thus, the redocking result is borderline: a near-native pose was recovered, but conservative alignment metrics suggest some deviation from the crystallographic orientation. The resulting pose showed a molecular overlay RMSD of 0.94 Å compared to the crystallographic conformation via Discovery Studio.

Table 3: Docking scores of the five top-scoring compounds of HS against the antihypertensive target angiotensin II Type 1 receptor (AT1R)

Compound (ligand)	Structural class	Docking score (Kcal/mol)
Beta-Sitosterol 3-O-beta-D-galactopyranoside	Plant steroid	-10.1
Gossypol	Sesquiterpenes	-9.6
Kaempferol-3-O-rutinoside	Trihydroxyflavone	-9.6
HS03	Flavonoids	-9.6
HS05	Flavonoids	-9.6
Valsartan (Standard)	AT1R antagonist	-9.0

HS03 = (3R,5S,9S,10R,13S,14R,17S)-17-[(E,2S,5S)-5-ethyl-6-methylhept-3-en-2-yl]-10,13-dimethyl-2,3,4,5,6,9,11,12,14,15,16,17-dodecahydro-1H-cyclopenta[a]phenanthren-3-ol

HS05 = 5-hydroxy-2-(4-methoxyphenyl)-8-(3-methylbut-2-enyl)-7-[(2S,3R,4S,5S,6R)-3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]oxy-3-[(2R,3R,4R,5R,6S)-3,4,5-trihydroxy-6-methyloxan-2-yl]oxychromen-4-one



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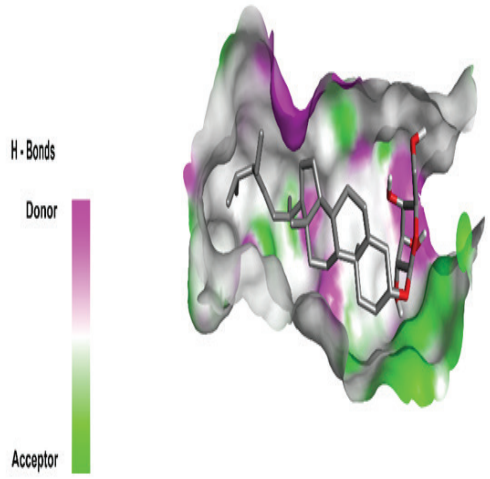

Figure7:2D(top)and3D(bottom)interactionsofbeta-sitosterol 3-O-beta-D-galactopyranoside with the AT1R active site

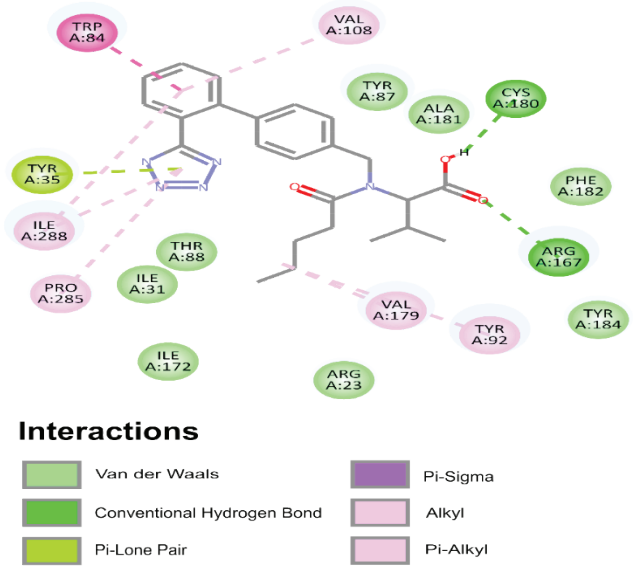

Figure 8: 2D ligand interaction of Valsartan (Standard) with the AT1R active site

AT1R is integral to hypertension pathophysiology, mediating vasoconstriction and sodium retention via the renin–angiotensin system. Several HS-derived compounds, including beta-sitosterol 3-O-beta-D-galactopyranoside, gossypol, and kaempferol-3-O-rutinoside, exhibited high predicted affinities for AT1R, suggesting potential for antagonistic activity. Such interactions could counteract angiotensin II-mediated effects and thereby contribute to blood pressure reduction. Notably, beta-sitosterol 3-O-beta-D-galactopyranoside has been reported to possess diverse biological activities, including antidiabetic and gastroprotective effects [30-31]. While its antihypertensive potential has been less explored, our findings suggest that this compound, alongside other HS phytochemicals with comparable docking profiles, may serve as promising candidates for AT1R-targeted therapies.

3.1.4 Target 4: Angiotensin-Converting Enzyme I

Table 4 summarizes the docking scores for the top five bioactive compounds and the standard drug lisinopril against ACE 1. Several flavonoids, including Rutin (−10.6 kcal/mol), kaempferol-3-O-rutinoside (−10.4 kcal/mol), and gossypitrin (−10.2 kcal/mol), demonstrated high predicted binding affinities, all more favorable than the standard drug lisinopril (−7.6 kcal/mol) (Fig. 10). For instance, Rutin formed hydrogen bonds with GLU162, THR282, and LYS511, along with a carbon–hydrogen bond with ASP377 (Fig. 9), contributing to its strong binding profile. These results suggest that multiple HS compounds may act as potential ACE1 inhibitors, offering scaffolds for the development of novel antihypertensive agents.

Redocking of the cocrystallized ligand, fosinoprilat, yielded RMSD values of 2.48 Å (lower bound) and 4.15 Å (upper bound) for the best pose. The lower-bound RMSD indicates a near-native alignment, while the upper-bound value is slightly above the commonly used 3.0 Å threshold. Thus, the redocking result is borderline: a near-native pose was recovered, but conservative alignment metrics suggest some deviation from the crystallographic orientation.

Table 4: Docking scores of the five top-scoring compounds of HS against the antihypertensive target angiotensin converting enzyme (ACE 1)

Compound (ligand)	Structural class	Docking score (Kcal/mol)
Rutin	Tetrahydroxyflavone	-10.6
Kaempferol-3-O-rutinoside	Trihydroxyflavone	-10.4
Gossypitrin	Flavonols	-10.2
Epigallocatechin Gallate	Flavans	-10.1
Quercitrin	Tetrahydroxyflavone	-9.8
Lisinopril (Standard)	ACE 1 inhibitor	-7.6

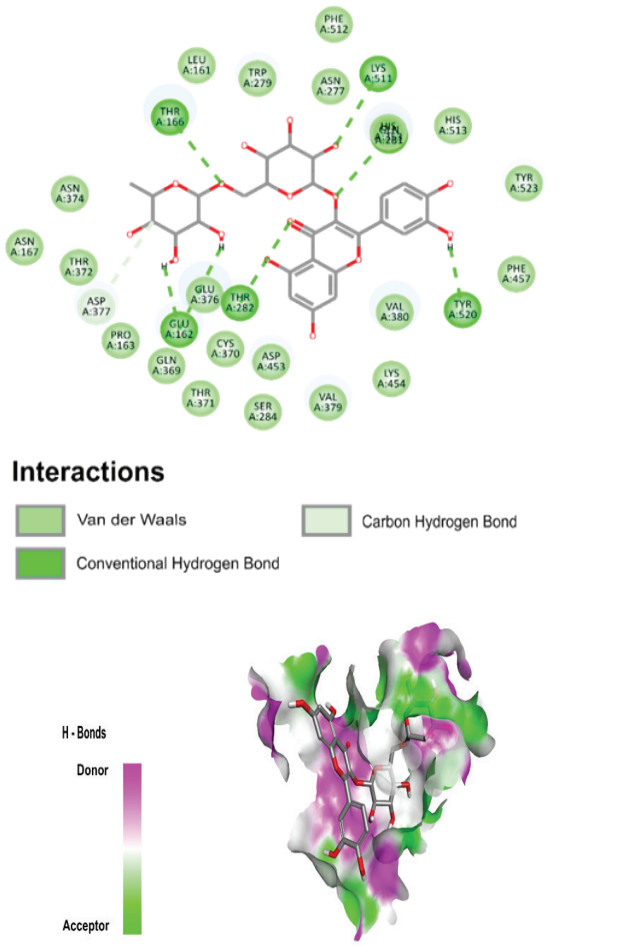

Figure 9: 2D (left) and 3D (right) interactions of Rutin with the ACE 1 active site

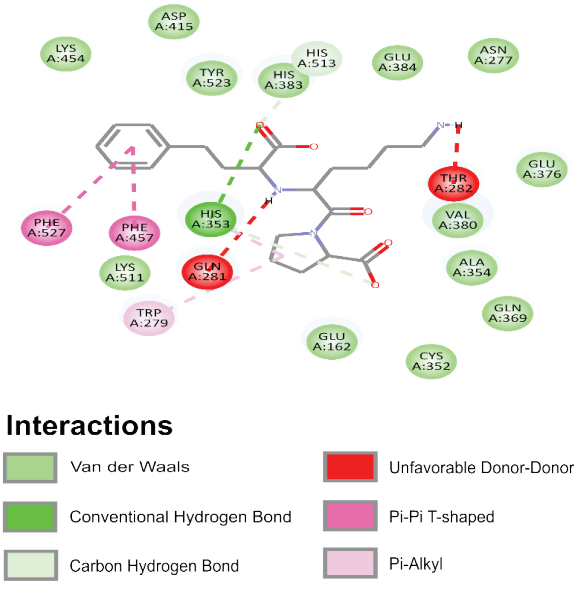

Figure 10: 2D ligand interaction of lisinopril (standard) with the ACE 1 active site

ACE 1 is a pivotal enzyme within the renin-angiotensin-aldosterone system that catalyzes the conversion of angiotensin I to the vasoconstrictive angiotensin II. Rutin exhibited a high predicted binding affinity for ACE 1, characterized by multiple hydrogen bonds and strong carbon–hydrogen bond interactions. These findings suggest that Rutin and structurally related compounds may act as potential ACE 1 inhibitors, thereby attenuating angiotensin II production and promoting blood pressure reduction. Rutin has been reported to exhibit various biological effects, including antioxidant, antidiabetic, anti-inflammatory, neuroprotective, and antihypertensive effects [30, 32]. Studies have linked its antihypertensive properties to mechanisms such as stimulation of the nitric oxide/guanylate cyclase pathway, ACE 1 inhibition, and antagonism of angiotensin II type 1 and mineralocorticoid receptors [32-34]. These actions are comparable to those of the known inhibitor lisinopril. Our findings further support Rutin's potential role as a multifaceted antihypertensive agent, with ACE1 inhibition forming a key mechanism underlying its efficacy.

3.1.5 Target 5: L-type Voltage-gated Calcium Channel (VGCC)

Table 5 summarizes the docking scores for the top five bioactive compounds and the standard drug amlodipine against VGCC. HS03 and beta-sitosterol 3-O-beta-D-galactopyranoside were the top-scoring compounds, each with a predicted binding affinity of −9.4 kcal/mol. Both formed multiple pi-interactions with hydrophobic residues, including VAL1053, ILE1046, MET1509, and PHE1513, within the active site. Beta-sitosterol 3-O-beta-D-galactopyranoside additionally engaged in two pi-interactions with PHE1181 and LEU1510 and a hydrogen bond with GLN1060, suggesting subtle differences in binding mode. These interactions, shown in Figs. 11 and 12, highlight their potential to modulate VGCC activity. The binding affinities of these compounds surpassed that of amlodipine, the known VGCC blocker (Fig. 13), which had a score of -6.9 kcal/mol.

Redocking of the VGCC cocrystallized ligand, amlodipine, did not reproduce the crystallographic pose within the docking protocol employed (closest RMSD = 3.071 Å). This likely reflects limitations of standard small-molecule docking for this ligand/target (e.g., large peptide ligand or induced-fit pocket).

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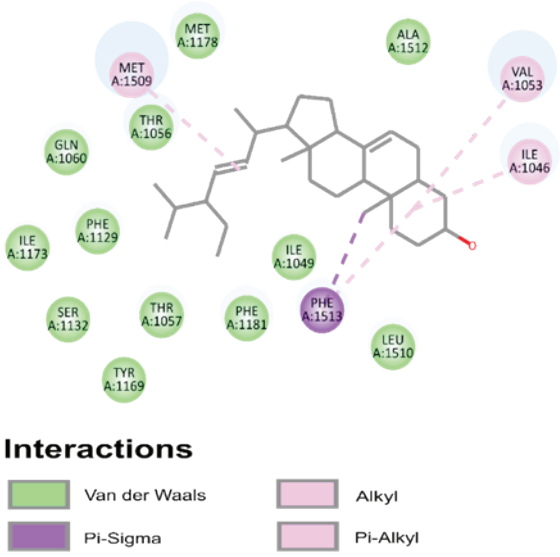
Table 5: Docking scores of the five top-scoring compounds of HS against the antihypertensive target VGCC

Compound (ligand)	Structural class	Docking score (Kcal/mol)
HS03	Terpenoids (Steroid)	-9.4
Beta-Sitosterol 3-O-beta-D-galactopyranoside	Plant steroid	-9.4
Cyanidin 3-(6''-acetyl-galactoside)	Anthocyanidin glycoside	-9.2
Epigallocatechin Gallate	Flavan	-9
HS07	Terpenoids (Steroid)	-8.9
Amlodipine (Standard)	VGCC Blocker	-6.9

HS03 = (3R,5S,9S,10R,13S,14R,17S)-17-[(E,2S,5S)-5-ethyl-6-methylhept-3-en-2-yl]-10,13-dimethyl-2,3,4,5,6,9,11,12,14,15,16,17-dodecahydro-1H-cyclopenta[a]phenanthren-3-ol

HS07 = (3S,8S,9R,10R,13S,14S,17S)-10,13-dimethyl-17-[(2R)-6-methylheptan-2-yl]-2,3,4,7,8,9,11,12,14,15,16,17-dodecahydro-1H-cyclopenta[a]phenanthren-3-ol



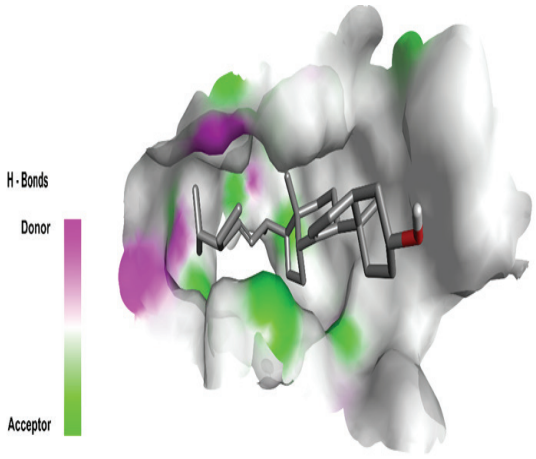


Figure 11: 2D (top) and 3D (bottom) interactions of HS03 with the VGCC active site

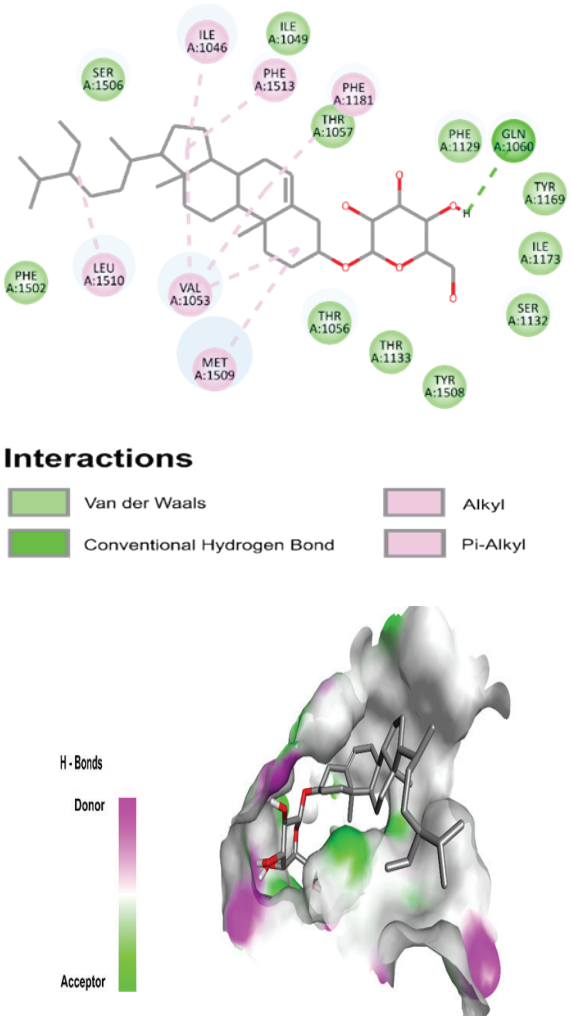


Figure 12: 2D (top) and 3D (bottom) interactions of beta-sitosterol 3-O-beta-D-galactopyranoside with VGCC active site

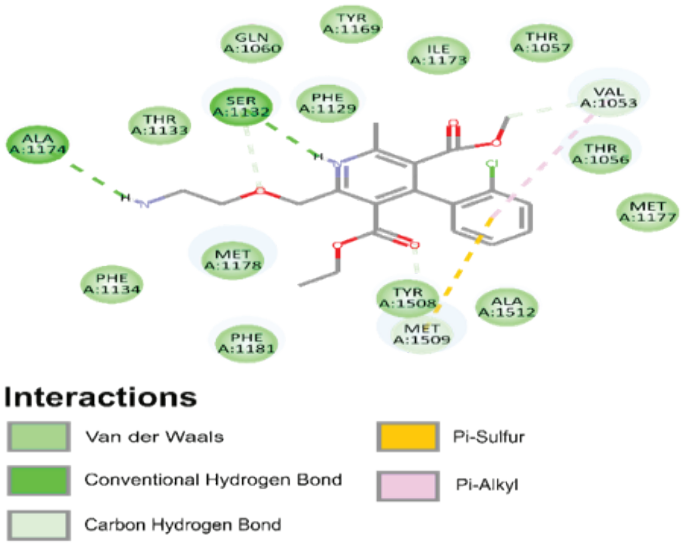


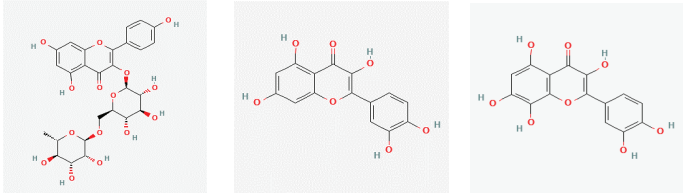
Figure 13: 2D ligand interaction of amlodipine (Standard) with VGCC active site

VGCC is a critical regulator of intracellular calcium levels and is essential for vascular smooth muscle contraction and cardiac function. HS03 and beta-sitosterol 3-O-beta-D-galactopyranoside exhibited significantly high predicted binding affinities for VGCC, with interactions primarily involving hydrophobic residues within the binding site. By modulating calcium influx, these compounds may induce vasorelaxation, contributing to the observed antihypertensive effects of HS. HS03, structurally similar to campesterol, has been previously reported to exhibit hypotensive effects, although the precise mechanisms remain unclear (35). Our findings indicate a potential role for HS03 and HS phytochemicals with comparable docking profiles in VGCC modulation, providing a plausible pathway for their antihypertensive effects. Similarly, beta-sitosterol 3-O-beta-D-galactopyranoside showed promise as a VGCC inhibitor, suggesting that a multifactorial mechanism underlies its observed antihypertensive effect.

Overall, the results of the molecular docking analyses revealed promising interactions between several bioactive compounds from HS and the selected antihypertensive targets. Kaempferol-3-O-rutinoside, beta-sitosterol 3-O-beta-D-galactopyranoside, and Rutin displayed notable binding affinities with phosphodiesterase 5A, angiotensin II T1 receptor, and angiotensin-converting enzyme 1, respectively. The formation of multiple hydrogen bonds and pi interactions suggests that these compounds are potential lead molecules. These findings provide molecular insights into the antihypertensive mechanisms of HS, which align with previous experimental observations [5-6, 10]. These findings also suggest a plausible molecular basis for the observed efficacy of *Hibiscus sabdariffa* in traditional medicine and could pave the way for the development of multitargeted natural therapeutics for hypertension management. The differences in docking scores of ≤1.0 kcal/mol fall within the margin of error for most docking algorithms. Therefore, compounds with similar scores were interpreted as having comparable predicted binding affinities, and emphasis was placed on groups of promising ligands rather than strict numerical rankings. It is important to acknowledge the limitations of in silico studies, including the simplifications and assumptions inherent in computational modeling. Experimental validation through *in vitro* and *in vivo* studies will be crucial to confirm these computational predictions. The complete docking scores and interaction details are presented in the Supplementary Tables.

3.2 Pharmacokinetic and Toxicological Evaluation

The top lead compounds (Fig. 14) presented varied pharmacokinetic profiles. Quercetin and cianidanol demonstrated high gastrointestinal absorption with no Lipinski violations, indicating favorable oral bioavailability. In contrast, glycosylated compounds such as Rutin and kaempferol-3-O-rutinoside were predicted to have poor absorption and multiple Lipinski violations due to their higher molecular weights and lower lipophilicity (Table 6). Toxicological analysis revealed that most of these compounds, including beta-sitosterol 3-O-beta-D-galactopyranoside, presented high safety margins with no significant cytochrome P450 (CYP450) enzyme inhibition. However, they were flagged for immunotoxicity, indicating potential risks to immune system function. Quercetin and gossypetin, despite their promising docking affinities, were identified as specific CYP450 inhibitors and flagged for potential carcinogenicity (Table 7).



Kaempferol-3-O-rutinoside Quercetin Gossypetin

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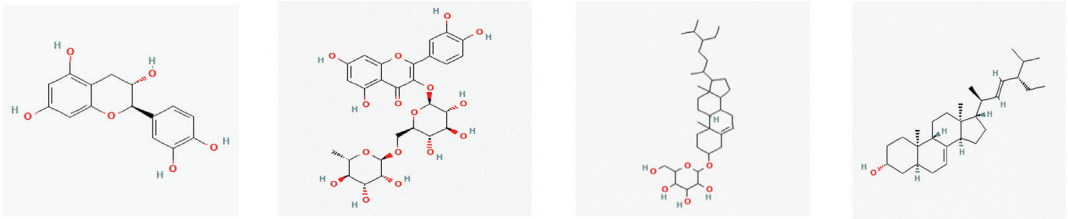

Figure 14: Chemical structure of the top-scoring compounds of Hibiscus sabdariffa against five selected antihypertensive targets

Table 6: Physicochemical Properties and Pharmacokinetics of top-scoring HS compounds

Compounds	Molecular Weight	Log Po/w	GI Absorption	Lipinski Violations	Bioavailability Score
Kaempferol-3-O-rutinoside	594.52 g/mol	-0.73	Low	3	0.17
HS03	412.69 g/mol	6.87	Low	1	0.55
Rutin	610.52 g/mol	-1.29	Low	3	0.17
Beta-Sitosterol 3-O-beta-D-galactopyranoside	576.85 g/mol	5.51	Low	1	0.55
Quercetin	302.24 g/mol	1.23	High	0	0.55
Gossypetin	318.24 g/mol	0.96	Low	1	0.55
Cianidanol	290.27 g/mol	0.85	High	0	0.55

Table 7: Toxicological and Metabolic Highlights of the top-scoring HS compounds

Compounds	Lethal Dose 50	CYP Inhibition	Notable Toxicity
Kaempferol-3-O-rutinoside	5000 mg/kg	None	Immunotoxicity
HS03	2000 mg/kg	None	Immunotoxicity
Rutin	5000 mg/kg	None	Immunotoxicity
Beta-Sitosterol 3-O-beta-D-galactopyranoside	8000 mg/kg	None	Immunotoxicity
Quercetin	159 mg/kg	CYP1A2, CYP3A4, CYP2D6 Inhibitor	Carcinogenicity, Immunotoxicity
Gossypetin	159 mg/kg	CYP1A2, CYP3A4, CYP2D6 Inhibitor	Carcinogenicity, Immunotoxicity
Cianidanol	10000 mg/kg	None	None

ADMET analysis revealed critical insights into the pharmacokinetic parameters of the bioactive compounds from *HS*. Despite their favorable binding affinities, lead compounds such as Rutin and kaempferol-3-O-rutinoside exhibited suboptimal gastrointestinal absorption, bioavailability, and multiple Lipinski violations. This could limit their efficacy *in vivo* by hindering their ability to reach therapeutic concentrations at their target site. While our results indicate potential bioavailability challenges for certain compounds, the literature suggests a more nuanced perspective. Ma et al. (2017) reported favorable gastrointestinal absorption permeability for kaempferol-3-O-rutinoside [36], and Ganeshpurkar and Saluja (2016) reported approximately 10% oral bioavailability for Rutin [37]. Rahman et al. (2021) further emphasized Rutin's robust biological activities [38]. These findings suggest that these compounds and their analogs may retain therapeutic potential despite challenges associated with their predicted bioavailability.

Interestingly, beta-sitosterol 3-O-beta-D-galactopyranoside and its analogs exhibited satisfactory bioavailability despite their poor gastrointestinal absorption. Strategies such as nanoparticle-based delivery, liposomal encapsulation, prodrug design, or cocrystals have been proposed to enhance the oral bioavailability of potential phytocompounds [39]. Incorporating such approaches may improve the translational potential of these promising compounds. In contrast, quercetin and its analogs demonstrated favorable pharmacokinetic properties, including high gastrointestinal absorption and bioavailability, making them strong candidates for further development as antihypertensive agents.

The low likelihood of CP450 enzyme inhibition for most compounds minimizes the risk of drug–drug interactions, a crucial factor in combination therapies. However, quercetin and its analogs were identified as potential CYP450 inhibitors, necessitating careful consideration in clinical applications.

Toxicological evaluation revealed generally acceptable acute toxicity profiles but raised concerns regarding potential immunotoxicity. These findings necessitate further investigation into their potential immunomodulatory effects and long-term safety. Quercetin and several of its analogs were identified as potential mutagens and carcinogens, highlighting the need for structural optimization to improve safety while preserving their therapeutic efficacy.

While this study provides a detailed assessment of individual bioactive compounds, the pharmacokinetic and safety profiles within the *Hibiscus sabdariffa* matrix may be influenced by complex synergistic interactions. These interactions warrant further investigation to fully understand the holistic therapeutic potential of this medicinal plant.

4. Conclusion

This in silico study provides evidence for the antihypertensive potential of HS through its ability to modulate multiple molecular targets associated with blood pressure regulation. The identification of key bioactive compounds, such as Rutin, kaempferol-3-O-rutinoside, and beta-sitosterol 3-O-beta-D-galactopyranoside, which have favorable binding affinities for critical enzymes and receptors, provides a robust foundation for the development of natural antihypertensive therapeutics. These findings underscore the potential for HS as a complementary or alternative therapeutic approach to conventional antihypertensive medications. While these results offer valuable molecular insights, further validation through *in vitro* and *in vivo* studies is essential to confirm their therapeutic efficacy and elucidate their detailed mechanisms of action. Moreover, addressing the pharmacokinetic challenges associated with certain compounds and optimizing their safety profiles will be crucial for the successful translation of these findings into clinical applications. By integrating computational methodologies with existing experimental evidence, this study represents a pivotal step forward in natural product-based drug discovery and cardiovascular research. Such interdisciplinary efforts hold promise for developing novel, multitargeted treatments for hypertension, ultimately improving patient outcomes and addressing the global burden of this condition.

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Synergistic Impact of Polyherbal Formulations on Metabolic Syndrome: A Comprehensive Review of Mechanisms and Therapeutic Potential

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ABSTRACT

Obesity, dyslipidemia, hypertension, and insulin resistance are all components of metabolic syndrome, which significantly increases the risk of cardiovascular diseases and type 2 diabetes. Current conventional pharmacological therapies typically have limited efficacy and might induce side effects, highlighting the need for innovative therapeutic strategies. This mini-review looks at polyherbal formulations' potential as an effective multi-target therapy for metabolic syndrome, which may offer a more suitable alternative to the current monotherapy agents. Drawing from current Research, our team investigates how these formulations provide anti-obesity, antihyperglycemic, and hypolipidemic effects. Bioactive compounds in herbs such as Citrus reticulata, Momordica Charantia, and Glycine max collaborate to enhance lipid metabolism, reduce adipogenesis, and strengthen antioxidant defense. This Research also checks the safety profiles and therapeutic potential of these formulations to relieve the complicated symptoms resulting from metabolic syndrome. This study reinforces the opportunity of using herbal medications as a comprehensive approach to manage metabolic syndrome, paving the way for further clinical studies creating standardized herbal medications.

KEYWORDS

"Polyherbal Formulation"; "Metabolic Syndrome"; "Anti-Hyperglycemia"; "Anti-Obesity"; "Herbal Medicine"; "Hypolipidemic"; "Antihypertensive" .

1. Introduction

1.1 Metabolic Syndrome Definition and Diagnosis Criteria:

Metabolic syndrome (MetS) is identified as a group of interdependent metabolic conditions. The most important five components are: insulin resistance, visceral obesity, hypertension, increased LDL, and decreased HDL (Figure 1).

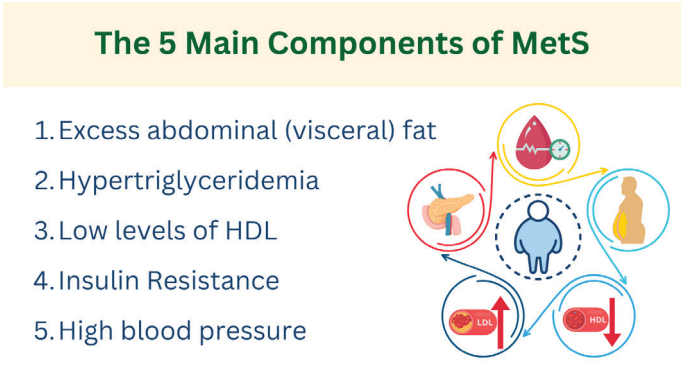

Figure 1: The Five Main Components of Metabolic Syndrome

significantly increase the incidence of cardiovascular diseases (CVDs) and type 2 diabetes mellitus (T2DM) (1)

The new approaches and Research focus on holistic therapeutic strategies to target several, dependent, and complicated metabolic pathways in order to reach better outcomes than conventional therapies targeting single pathways.

During the last years, diagnostic criteria of metabolic syndrome have been subjected to variation. In 2001, the National Cholesterol Education Program's (NCEP) Adult Treatment Panel III (ATP III) stated standard criteria for metabolic syndrome diagnosis, which are based on

These criteria were based on standard clinical measurements such as blood pressure, HDL-C, triglycerides, waist circumference, and fasting glucose level, where these criteria have been widely used by epidemiological researchers and in clinical practice.

To be diagnosed with metabolic syndrome, abnormalities in 3 of 5 clinical parameters should be determined. The criteria paved the way for focusing on more than one cause. In The AHA and NHLBI are going forward to use and clarify these criteria with new adjustment to meet the scientific variability where according to guidelines for insulin resistance, the threshold for counting elevated fasting glucose is lowered from ≥110 mg/dL to ≥100 mg/dL; permitting blood pressure, HDL-C, and triglycerides to be counted as abnormal when an individual is taking medication for these conditions; and permitting waist circumference to be adjusted to lower thresholds when individuals or ethnic groups are predisposed to insulin resistance. (2–4).

1.2. Prevalence and global burden

MetS affects about 25% of the global population, with a prevalence rate of 35% in the Middle East and North Africa (MENA). Dietary choices, genetic predispositions, financial issues, and sedentary lifestyles are all factors contributing to metabolic syndrome. These high prevalence rates increase the risk of premature mortality, reduce quality of life, and significantly increase the burden of noncommunicable illnesses. (5,6)

1.3. Recent Treatment Challenges

Traditional pharmacological therapies—such as antihypertensives, statins, and antidiabetic medications manage specific MetS components separately, but they are limited by side effects, high prices, and poor patient non-adherence. In addition, the interrelated mechanisms of MetS components impair the effectiveness of monotherapies, where addressing hyperglycemia doesn't mean that they target the inflammatory or dyslipidemia elements of MetS, necessitating multitargeted therapies. (7,8)

1.4. Novel approach of Polyherbal Formulations

This innovative method uses a variety of medicinal plants with bioactive components that work synergistically to deliver a more comprehensive solution than conventional medications. Herbs that alter multiple metabolic pathways, such as AMP-activated protein kinase (AMPK) activation, NF-κB inhibition, and insulin receptor sensitization, include Berberis vulgaris (berberine), Cinnamomum verum (Cinnamon), and Curcuma longa (turmeric). These mechanisms make (PHFs) more effective and safer than conventional ones. (9,10)

1.5. Future Perspectives

Emerging delivery system strategies involve increasing bioavailability and effectiveness by generating PHF as nanoparticles and within phospholipid complexes. To improve long-term efficacy and safety, future studies and clinical trials will focus on defining standard formulations and dosing protocols. MetS care may change if PHFs are included in normal therapy procedures, providing long-term, cost-effective solutions to this expanding health issue. (11)

1.6. Aim of Study

This study aims to evaluate the different pharmacological mechanisms of action, therapeutic potential, and types of polyherbal formulations used in managing metabolic syndrome, focusing on their effects on glucose metabolism, lipid profiles, and inflammation.

2. Methods

To assess the therapeutic potential of polyherbal formulations for MetS, a comprehensive literature search was conducted using PubMed, Scopus, and Google Scholar databases from 2001 to 2023. The following keywords were used: "metabolic syndrome," "polyherbal formulations," "herbal medicine," "glucose regulation," and "lipid profiles."

2.1. Inclusion Criteria:

- **Study Types:** Only peer-reviewed clinical trials, in vivo, and in vitro studies were included.
- **Focus:** Studies investigating the effects of polyherbal formulations on at least one of the three components of MetS (e.g., insulin resistance, dyslipidemia, hypertension).

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- **Herbs:** Emphasis was placed on herbs with well-established evidence of efficacy, such as berberine, Cinnamon, garlic, and turmeric.

2.2. Data Extraction and Analysis:

- **Outcome Measures:** The primary outcomes assessed included changes in glucose metabolism (e.g., fasting blood glucose, HbA1c), lipid profiles (e.g., LDL, HDL, triglycerides), blood pressure, and inflammatory markers.

- **Herbal Mechanisms of Action:** Pharmacological actions of each herb were identified, and their synergistic potential within PHFs was analyzed.

- **Formulation Characteristics:** The formulation type (e.g., capsules, powders, nanoparticles) and bioavailability-enhancing strategies were recorded.

3. Results

For managing metabolic syndrome (MetS), several herbal alternative formulations have shown significant promise. These formulations often include a combination of medicinal plants that target multiple pathways involved in MetS, such as reducing oxidative stress, improving lipid profiles, regulating glucose metabolism, and enhancing cardiovascular health as shown in Figure 2.

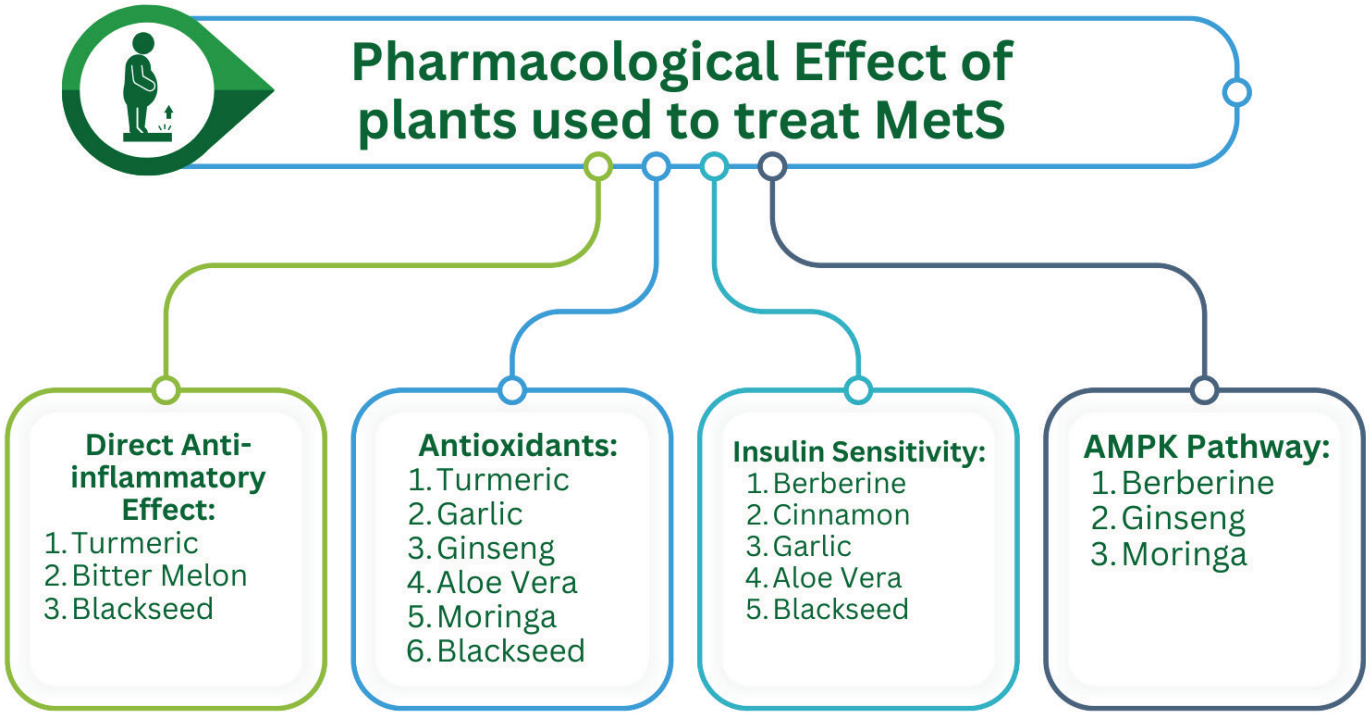

Figure 2: Different Mechanisms of action (pharmacological effect) showed by different herbs used in treating MetS.

3.1. Berberine:

It is an alkaloid extracted from Berberis vulgaris, which has metabolic regulatory effects, where several RCT shows that it effectively decreases fasting blood glucose (25%) and HbA1c by (0.5-1.2%) (12) These results are similar to those obtained by conventional drugs like (metformin), which enhances skeletal muscle uptake and impedes gluconeogenesis by activating AMP-activated protein kinase (AMPK). Thus, decreasing plasma glucose levels. Additionally, another RCT shows that it reduces LDL cholesterol by up to 31.7% (13), where it downregulates proprotein convertase subtilisin/kexin type 9 (PCSK9), leading to decreased LDL receptor degradation and enhanced clearance of LDL cholesterol, also due to its action on AMPK. It affects lipid metabolism, inhibiting lipid synthesis pathways and overall decreasing TG by 19.5%. In addition, it modulates adipokine secretion. Third RCT shows after 3-months of treatment decrease in (SBP) from 123 ± 7 mmHg to 115 ± 9 mmHg, triglycerides: Reduced from 2.4 ± 0.7 mmol/L to 1.4 ± 0.5 mmol/L, glucose Area Under the Curve (AUC): Decreased from 1182.1 ± 253.6 to 1069.5 ± 172.4 mmol/L and Matsuda Insulin Sensitivity Index (ISI Matsuda): Improved from 2.1 ± 1.0 to 3.1

± 1.6 (14). So, it can be used in dyslipidemia, T2DM, and various conditions of metabolic syndrome.

Formulations:

It has poor bioavailability through the oral route, so berberine is either available as:

1-capsules or

2-combined with bioavailability enhancers like silymarin (15).

3-New delivery methods, including berberine-loaded nanoparticles, which are promising in enhancing its absorption and therapeutic efficacy(16,17).

3.2. Cinnamon:

RCTs show an efficient decrease in fasting blood glucose levels by 8.84% after 12 weeks of treatment with 2g cinnamon and HbA1c by 0.5–0.83% (18). Among patients with type 2 diabetes, after 90 days of treatment with cinnamon capsules. Cinnamaldehyde, which is a main constituent in Cinnamon, increases the phosphorylation of insulin receptors, thus enhancing insulin signaling; thus, tissue muscle and adipose tissues glucose uptake increases. Secondly, Cinnamon inhibits key enzymes like HMG-CoA reductase, impeding hepatic cholesterol synthesis. So, Cinnamon has lipid-lowering effects, where it decreases LDL cholesterol by a range (7–27%). Also, decreasing SBP from (132.6 to 129.2) mmHg while DBP decreased from (85.2 to 80.2) after 12 weeks of treatment with 2g cinnamon (19), making it useful to use in both glycemic and cardiovascular parameters. Finally, it decreases systemic inflammation associated with metabolic syndrome by modifying the nuclear factor-kappa B (NF-κB) pathway.

Formulations: Cinnamon is formulated as

1-Powder

2-Capsules

3-Essential oil extract rich in cinnamaldehyde is used therapeutically, but with caution, with dosing to limit variability.

3.3. Turmeric:

Turmeric contains polyphenols, the most important of which is Curcumin, which exhibits anti-inflammatory and antioxidant effects, mainly through inhibition of NF-κB. In addition, it inhibits Alpha tumor necrosis factor (TNF-α). Thus, it decreases inflammation and oxidative damage, making it a good candidate for managing chronic inflammation associated with metabolic syndrome(20,21). Additionally data from RCT Shows after 12 weeks treatment with curcumin extract that TGs Reduced from 226.10 ± 64.99 mg/dL to 160.79 ± 75.46 mg/dL (28.8%), total reduced from 195.10 ± 42.47 mg/dL to 175.86 ± 30.63 mg/dL(9.8%), LDL cholesterol reduced from 120.55 ± 36.81 mg/dL to 106.51 ± 25.02 mg/dL. (11.64%) , and HDL increased from 40.96 ± 8.59 mg/dL to 43.76 ± 9.54 mg/dL (6.18%) (22). These lipid-lowering effects are due to the reduction of lipid absorption by downregulating NPC1L1 expression, and it increases bile acid synthesis by increasing 7Alpha hydroxylase activity. Another RCT demonstrates a reduced risk of conversion of prediabetics to diabetics by 16.4 %, insulin resistance (HOMA-IR) from (3.22±0.4), and c-peptide from(1.7±0.3ng/ml), and an increase in HOMA-β scores (61.58±3.1). So overall, it improves beta cell function and insulin sensitivity by reducing inflammatory markers, oxidative stress, and increasing the level of adiponectin(23), making it a good candidate for metabolic syndrome management.

Formulations:

Curcumin is often mixed with piperine extracted from black pepper, which improves its absorption by up to 2000% (24). Formulations like:

1- curcumin capsules.

2- phospholipid complexes

3-Curcumin nanoparticles for sustained release(25,26).

3.4. Garlic:

RCT shows that after a month of treatment with a twice-daily dose of raw crushed garlic, the circumference of the waist reduced(from 101.41 ± 8.59 cm to 97.95), and triglycerides decreased from 170.49 ± 40.24 mg/dL to 146.46 ± 36.21 mg/dL. In addition to the increase of HDL cholesterol from 40.36 ± 4.51 mg/dL to 46.27 ± 3.92 mg/dL, this indicates that garlic has a lipid-lowering effect as it contains sulfur compounds like Allicin, which converts to Allicin with crushing, where the latter inhibits HMG-CoA reductase, impeding fatty acid synthesis enzymes. Also, this trial shows a decrease in fasting blood glucose (FBG) from 165.02 ± 25.10 mg/dL to 120.14 ± 10.18 mg/dL

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and a decrease in both systolic blood pressure, from 150.25 ±14.65 mmHg to 140.22 ± 5.39 mmHg, and diastolic blood pressure from 96.40 ± 9.21 mmHg to 84.10 ± 3.65 mmHg. Antidiabetic effect as sulfur compounds act as an insulin secretagogue, also due to their antioxidant effect as they activate the AMPK pathway, thus improving insulin sensitivity and protecting against oxidative stress. Antihypertensive effect due to decreased peripheral vascular resistance by prostaglandin-like activity, active compounds like gamma-glutamylcysteine, which inhibit angiotensin-converting enzymes and promote vasodilation by the NO pathway (27). Other RCT shows similar results. Also shows a decrease in insulin resistance where HOMA-IR decreased by 0.5±0.5(28). Third RCT shows a reduction in LDL cholesterol by 32.9 mg/dl in men and by 27.3 mg/dl in women. (29). Finally, another RCT shows that garlic prevents platelet aggregation. (30). All these features make garlic suitable for use in metabolic syndrome.

Formulations:

1-Raw Garlic powder.

2-Extract formulations.

3-Aged garlic extract, sulfur compounds, like Allicin, which must be standardized to limit variability(31).

3.5. Ginseng:

Ginsenosides are the active principles in ginseng. One RCT demonstrates that American ginseng, after 8 weeks of ginseng administration, decreased fasting glucose levels by (0.71±0.34mmole/L) mg/dL and HbA1c by (0.29±0.1%), systolic blood pressure was reduced by (5.6±2.7mmHg), and finally decreased LDL cholesterol by 12.3% (32). Another RCT shows that Korean ginseng extract increases (ISI insulin sensitivity index) by (Δ+1.24) while American ginseng extract increases ISI by(Δ±0.87) (33), where different types of ginseng have several mechanisms to achieve these results, like activating the AMPK pathway, improving glucose uptake, and insulin sensitivity in peripheral tissues. Secondly, it activates GLUT4 translocation, which modulates insulin signaling. They also inhibit the NF-κB pathway, reducing. So, they reduce inflammation and cytokine production, suppressing oxidative stress. So, overall, ginsenosides contribute to improved metabolic and cardiovascular health and managing chronic inflammation(34,35).

Formulations:

1-Raw root.

2-extract formulations.

3-Capsules.

Ginseng products are standardized to limit variability. (36).

3.6. Fenugreek:

The use of fenugreek supplements in populations showed a Promising effect on both glycemic control and lipid profiles.

Glycemic control:

In individuals with type 2 diabetes, it showed a reduction in the FPG by 20.32 mg/dl (95% CI: -26.65 to -13.99) in one meta-analysis study (37), and a 15.10% reduction in the FPG in another study, which reflects a significant improvement in glycemic control. Fenugreek also lowered the HbA1c by 0.54% (95% CI: -0.80 to -0.28) for patients whose HbA1c levels typically range from 7% to 9% (38,39)

Lipid profile:

Also fenugreek supplementation lowered total cholesterol (TC) by 33.10 mg/dL (95% CI: -64.31 to -1.88), Low-density lipoprotein cholesterol (LDL) decreased by 29.14 mg/dL (95% CI: -55.45 to -2.83), high-density lipoprotein cholesterol (HDL-C) increased by 5.68 mg/dL (95% CI: 3.51 to 7.85), equivalent to a 10-12% rise, contributing to enhanced cardiovascular protection reflecting a significant improvement in lipid profiles.

Its effectiveness is attributed to the main bioactive components in it, including soluble fiber, 4-hydroxyisoleucine, and saponins, which help regulate blood glucose levels, enhance insulin sensitivity, and improve lipid absorption. (38)

Available formulations:

1. Standardized Extracts:

Standardized capsules that contain a dose of 100 mg saponins or 4-hydroxyisoleucin.

2. Powdered Seeds:

It is usually taken as tea or incorporated into diets

as it is widely available as bulk powders.

3. Combination Products:

Fenugreek is often included in multi-herbal supplements targeting diabetes and lipid management, where a study combining fenugreek (200 mg) and berberine (300 mg) in capsule form demonstrated a 15% reduction in insulin resistance and significant improvements in triglycerides and LDL cholesterol compared to placebo. (40)

3.7. Bitter Melon:

Glycemic Control:

Bitter melon supplementation reduced the fasting blood glucose levels of the participants significantly in an RCT. The participants' fasting blood glucose levels reduced by 21%, but there was no significant effect on HbA1c, which only reduced by 0.217% (41,42). Another RCT with 100 patients showed that taking bitter melon led to a reduction in fasting blood glucose levels by 28% after 3-7 weeks. (43), but another RCT involving dried bitter melon fruit tablets for 4 weeks showed no significant changes in blood glucose markers, suggesting that the form and duration of administration might influence outcomes (44)

Lipid Profile management:

Using bitter melon proved its potential to improve the lipid profile in humans after a study that showed a significant reduction in LDL cholesterol and total cholesterol levels in insulin-resistant rodents after consuming bitter melon extract, and supplementation with bitter melon in human trials showed a great reduction in patients' triglyceride levels and improved HDL cholesterol. (45,46).

Mechanism of Action:

Enhancing insulin sensitivity is due to Charantin, a cucurbitane-type triterpenoid in bitter melon, which increases the glucose uptake in skeletal muscles and inhibits hepatic gluconeogenesis. It is demonstrated that bitter melon may stimulate insulin receptor activity. Additionally, its anti-inflammatory properties work against oxidative stress, which is a very common issue among diabetics. (41,47)

Available formulations:

A variety of formulations of Bitter melon are available in the market, such as capsules, tablets, powdered extracts, and juices. In clinical trials, we usually use a dose of 500mg – 1000mg of dried fruit powder or dry extract per day. To ensure consistent amounts of active compounds like charantin and vicine, these formulations are often standardized. (48).

3.8. Aloe Vera

Aloe vera has significant benefits in the management of type 2 diabetes by reducing fasting blood glucose, HbA1c, and oxidative stress, as well as improving insulin sensitivity. It acts by improving glucose uptake, reducing the hepatic glucose production, and mitigating oxidative damage.

1. Fasting Blood Glucose Reduction:

An RCT involving 60 type 2 diabetes patients demonstrated that consuming Aloe vera gel (15 mL twice daily) for 12 weeks resulted in a significant reduction in fasting blood glucose (FBG) levels by 30%, compared to placebo (49) Another systematic review of clinical trials, reporting a reduction in FBG by up to 1.7 mmol/L among participants supplemented with Aloe vera juice or capsules over a similar period (50)A meta-analysis confirmed that Aloe vera consistently reduces FBG, with an average drop of -0.41 mmol/L (95% CI: -0.77 to -0.05) in prediabetic and diabetic patients across several trials. (51).

2. HbA1c Improvement

An RCT with 100 diabetic patients showed that Aloe vera gel supplementation for 3 months reduced HbA1c levels by an average of 0.5% compared to placebo. (52), a systematic review also concluded that Aloe vera supplementation in prediabetic patients reduced HbA1c by up to 0.8%, emphasizing its role in long-term glucose regulation (49).

3. Insulin Sensitivity Enhancement

Studies found that Aloe vera extract improved insulin sensitivity in a randomized trial involving insulin-resistant patients, resulting in a 22% improvement in HOMA-IR (Homeostatic Model Assessment for Insulin Resistance) scores over 8 weeks. (50)Also, another RCT demonstrated that Aloe vera significantly enhanced glucose uptake in peripheral tissues, evidenced by a

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15% improvement in fasting insulin levels after 12 weeks of supplementation. (51).

4. Antioxidant and Anti-inflammatory Effects

There is a study that observed that Aloe vera supplementation reduced the markers of oxidative stress, such as malondialdehyde (MDA), by 25%, indicating decreased lipid peroxidation. (52)Another study found that Aloe vera gel reduced pro-inflammatory markers, including C-reactive protein (CRP), in patients with diabetes, after 6 weeks of treatment with an average reduction of 20% (49).

Mechanisms of Action:

Polysaccharides and Lectins:

Studies showed that Aloe vera polysaccharides enhance insulin sensitivity, contributing to better blood glucose control by activating insulin receptor pathways in peripheral tissues, and by reducing hepatic glucose production (49,50).

Antioxidant Properties:

Antioxidant effects, including the reduction of reactive oxygen species (ROS), were confirmed in multiple RCTs (49,52).

Available Formulations:

1. Aloe Vera Gel (Oral) Capsules or Juice:

Capsules containing 300–500 mg of Aloe vera extract were effective in improving insulin sensitivity and lowering FBG in clinical trials. (49,50).

2. Topical Aloe Vera Gels and Creams:

Though less common for systemic glycemic control, topical aloe vera has been investigated for its wound-healing and antioxidant properties, which can be beneficial for managing diabetic ulcers (52).

3. Aloe Vera Extract Powders and Tablets:

Dried extracts of aloe vera gel are used in tablet form, often standardized for their active components. Studies have reported improvements in glycemic markers in individuals using standardized doses of these products daily. (49,51).

4. Combination Formulations:

Aloe vera is included in polyherbal or combination formulations targeting metabolic syndrome and diabetes. These products often blend aloe with other herbs like fenugreek or bitter melon, aiming to improve insulin sensitivity and glucose metabolism synergistically. (51).

3.9.Moringa Oleifera

Glycemic Control:

In a Pilot Clinical Trial, Moringa leaf capsules of a 400 mg twice daily dose showed reductions in fasting blood glucose (FBG) by 23.5% and HbA1c by 0.75% over 8 weeks in patients with metabolic syndrome. (53).

Niazirin, which is a bioactive compound from moringa seeds, reduced FBG by 30% and improved insulin sensitivity by 25% in diabetic mice through AMPK pathway activation. (54,55).

Ethanolic Extract in Rodents improved insulin sensitivity and reduced FBG in obese rats by 45% over 12 weeks, with upregulation of GLUT4 expression. (56).

Lipid Profile Management:

In a Clinical Study, moringa reduced LDL cholesterol by 19%, total cholesterol by 14%, and triglycerides by 17%, while increasing HDL cholesterol by 12% after 8 weeks of supplementation (57).

In a Rodent Study, Moringa ethanolic extract modulated lipid metabolism through **PPAR-α activation**, enhancing fatty acid oxidation and suppressing cholesterol biosynthesis. (58,59).

Mechanism of Action:

AMPK Pathway: Activation leads to increased fatty acid oxidation, glucose uptake, and inhibition of gluconeogenesis, as demonstrated in studies using niazirin (57,58)

Antioxidant Effects: Reduces oxidative stress markers like MDA by 45% and boosts antioxidant enzyme activities such as SOD and catalase (56,57).

Anti-inflammatory Effects: Suppresses cytokines (e.g., TNF-α, IL-6) and enhances anti-inflammatory markers like IL-10 (56,58).

Available Formulations:

1. **Capsules/Tablets:** Typically, 8 grams per day of Moringa leaf capsules; shown effective for glycemic and lipid control in clinical trials (60)

2. **Aqueous Extracts:** Rich in phenolic content, effective at **20–50 mg/kg/day** in rodent models(56,58)

3. **Seed Extracts:** Niazirin-enriched formulations; doses of **10–20 mg/kg/day** improved glucose and lipid metabolism in mice (54,55)

4. **Powdered Leaves:** Incorporated into diets at **6 g/day**, reducing FBG by **20–25%** in type 2 diabetes patients(53,55)

3.10. Black Seed:

Glycemic Control:

Black seed shows a significant potential in improving glycemic control, which is supported by clinical and preclinical studies:

Clinical Evidence: Black seed powder supplementation with 2 g/day showed its effect in reducing fasting plasma glucose (FPG) by 25% and HbA1c by 0.75% over 12 weeks in individuals with type 2 diabetes (61,62)

Animal Studies: Thymoquinone (TQ), a key active compound, reduced FPG by 30% in diabetic rodent models and improved insulin sensitivity by 25% through mechanisms such as beta-cell preservation and enhanced insulin secretion (62,63).

Lipid Profile Management:

Clinical Outcomes: Black seed supplementation (500–1000 mg/day) resulted in a 20% reduction in LDL cholesterol, an 18% decrease in total cholesterol, and a 15% reduction in triglycerides, along with a 10% increase in HDL cholesterol in hyperlipidemic patients over eight weeks(61,63)

Preclinical Studies: In animal models, thymoquinone reduced triglycerides and LDL cholesterol by 30% and increased HDL cholesterol, likely by inhibiting HMG-CoA reductase activity and promoting fatty acid oxidation(62)

Antioxidant and Anti-Inflammatory Properties:

Antioxidant Effects: Black seed reduces oxidative stress markers such as malondialdehyde (MDA) by 45–50% and enhances antioxidant enzyme activities (e.g., superoxide dismutase, catalase) (62)

Anti-Inflammatory Effects: The seed suppresses pro-inflammatory cytokines like TNF-α and IL-6 while enhancing anti-inflammatory markers such as IL-10. These effects contribute to reduced systemic inflammation and improved cardiovascular and metabolic health. (62,63).

Mechanism of Action:

Black seed activates the AMPK pathway, which boosts glucose uptake in peripheral tissues, inhibits hepatic gluconeogenesis, and regulates insulin signaling(62). Its lipid-lowering effects are attributed to antioxidant and anti-inflammatory properties, which enhance hepatic cholesterol metabolism and inhibit oxidative damage (62,63). Thymoquinone, a potent antioxidant in black seed, neutralizes free radicals, protects beta-cells from oxidative damage, and regulates the expression of anti-inflammatory mediators through the NF-kB pathway. (62,63).

Available Formulations:

Standardized Extracts:

Capsules containing 500–1000 mg/day of black seed powder or extracts with standardized thymoquinone concentrations are commonly used for glycemic and lipid improvements. (62)
Ethanolic Extracts:

Typically used in Research at doses of 20–50 mg/kg/day, effective for glucose and lipid modulations. (62)

Oil Preparations:

Black seed oil, rich in thymoquinone, is dosed at 0.2–0.5 ml/day in studies, showing benefits for lipid management and antioxidant effects. (63)

Powdered Seeds:

Widely consumed as dietary supplements or in teas, with doses of 1–3 g/day effective for reducing FPG and improving lipid profiles. (61,62)

3.11. Citrus reticulata (Mandarin Orange Peel)

Citrus reticulata, commonly known as mandarin orange peel, is rich in bioactive flavonoids

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such as hesperidin, naringenin, and tangeretin, which exhibit significant antioxidant, anti-inflammatory, and hypolipidemic properties. These compounds have gained increasing attention for their potential therapeutic effects in metabolic syndrome (MetS).

A randomized, double-blind, placebo-controlled clinical trial evaluated the effect of Eriomin®, a standardized citrus flavonoid extract primarily derived from Citrus reticulata, in 48 prediabetic individuals over 12 weeks. Participants who received 500 mg/day of Eriomin® demonstrated significant reductions in:

- Fasting blood glucose (↓5%)
- Fasting insulin levels (↓11%)
- HOMA-IR (↓17%)
- High-sensitivity C-reactive protein (↓12%)
- IL-6 (↓13%)
- TNF-α (↓11%)

These improvements were not observed in the placebo group, indicating the glycemic and anti-inflammatory efficacy of the extract in early metabolic dysregulation (64)

Additionally, a preclinical study using a rat model of MetS revealed that Citrus reticulata peel extract administration significantly improved insulin sensitivity, reduced hepatic steatosis, decreased oxidative stress markers such as malondialdehyde (MDA), and elevated antioxidant enzymes, including superoxide dismutase (SOD) and catalase. The therapeutic mechanisms were linked to AMPK pathway activation, NF-κB inhibition, and cytokine modulation (65)

Formulations:

Citrus reticulata is available as:

1-Standardized citrus peel extracts (e.g., Eriomin®)

2-Capsules and functional beverages

3-Polyherbal formulations, often combined with other insulin-sensitizing or anti-inflammatory herbs

3.12. Glycine max (Soybean)

Glycine max (soybean) is a leguminous plant widely used for its nutritional and medicinal properties, particularly due to its richness in isoflavones (e.g., genistein and daidzein) and soy proteins. These compounds exhibit significant antihyperglycemic, hypolipidemic, and anti-inflammatory effects, making Glycine max a promising botanical in the management of metabolic syndrome (MetS).

In a randomized controlled trial involving prediabetic postmenopausal women, supplementation with 15 g of soy protein and 100 mg of isoflavones daily for six months led to improvements in glucose tolerance and insulin sensitivity, although some outcomes showed borderline significance (66). Another clinical trial in type 2 diabetic patients showed that soy consumption led to significant reductions in serum CRP levels, improved lipid profiles, and enhanced endothelial function, reinforcing the anti-inflammatory and cardiometabolic benefits of soy-based interventions (67). Furthermore, a recent clinical study involving soy milk in patients with nonalcoholic fatty liver disease (NAFLD), a condition strongly associated with MetS, reported significant reductions in liver enzymes, body weight, and insulin resistance (68).

Preclinical studies have further confirmed these findings. Administration of Glycine max seed extracts in diabetic rodents improved glucose tolerance, increased insulin secretion, and reduced lipid peroxidation(69). Another study using soybean leaf extract demonstrated reduced hepatic fat accumulation, improved insulin resistance, and lower inflammation markers in obese mice(70). Mechanistically, the metabolic benefits are attributed to activation of PPAR-α and PPAR-γ, enhancement of adiponectin levels, and suppression of inflammatory cytokines, particularly TNF-α and NF-κB, contributing to improved insulin signaling and reduced systemic inflammation (71).

3.13. Clinical Evidence for Key Polyherbal Formulations in Metabolic Syndrome Management

Polyherbal formulations (PHFs) leverage pharmacodynamic and pharmacokinetic synergism to simultaneously target multiple pathological pathways in MetS—addressing insulin resistance, dyslipidemia, hypertension, and chronic inflammation (as shown in Table 1) more comprehensively than monotherapies (72,73)

Table 1: Comparison between the herbs used in treating MetS from the aspects of mechanisms of action, therapeutic potential and formulations.

Herb	Mechanism of Action	Therapeutic Potential	Formulations	Evidence (Reference + Study Type)
Berberine	Activates AMPK, enhances glycolysis, inhibits gluconeogenesis, improves insulin sensitivity, reduces LDL & TG.	Effective in T2DM, dyslipidemia, obesity, and NAFLD.	Extracts, capsules, combos with silymarin, nanoparticles.	RCTs (12,13,14); Systematic Review (9,10)
Cinnamon	Cinnamaldehyde enhances insulin receptor phosphorylation, ↑ glucose uptake, and inhibits HMG-CoA reductase.	Improves FBG, HbA1c, LDL, HDL, SBP/DBP.	Powder, capsules, extracts, and essential oils.	RCTs (18,19); Systematic Review (9)
Turmeric	Curcumin is anti-inflammatory & antioxidant; inhibits NF-κB, TNF-α, ↓ NPC1L1 (↓ lipid absorption).	↓ TG, LDL, TC, ↑ and HDL prevent T2DM progression.	Curcumin + piperine capsules, phospholipid complexes, nanoparticles.	RCTs (22,23); Clinical Trial (21)
Garlic	Allicin improves insulin secretion, ↑ the AMPK pathway, ↓ oxidative stress, and is an antihypertensive via ACE inhibition and the NO pathway.	↓ FBG, TG, LDL, ↑ HDL, ↓ SBP/DBP, anti-platelet.	Raw powder, extracts, and aged garlic supplements.	RCTs (27–30); Review (31)
Ginseng	Ginsenosides activate AMPK, GLUT4 translocation, improve insulin sensitivity, and inhibit NF-κB.	↓ FBG, HbA1c, LDL, BP; anti-inflammatory, antioxidant.	Raw root, extracts, capsules.	RCTs (32,33); Reviews (34,35)
Fenugreek	Soluble fiber slows carb absorption; 4-hydroxyisoleucine ↑ insulin secretion; saponins ↓ cholesterol absorption.	↓ FPG, HbA1c; improves lipid profile.	Capsules, seed powder, extracts, combo products.	Meta-analysis (37,39); RCT (38,40)
Bitter Melon	Charantin mimics insulin; ↑ glucose uptake; inhibits hepatic gluconeogenesis; and is anti-inflammatory.	↓ FBG has some effect on HbA1c, improves TG & HDL.	Capsules, tablets, extracts, juices.	RCTs (41–44); Meta-analysis (45); Review (47)
Aloe Vera	Polysaccharides ↑ affect insulin sensitivity, ↓ hepatic glucose production, antioxidant, and anti-inflammatory properties.	↓ FBG, HbA1c; improves insulin resistance; ↓ CRP, oxidative stress.	Capsules, juice, powders, combination formulas.	RCTs (49–52); Meta-analysis (51)
Moringa	Niazirin activates AMPK & PPAR-α, ↑ GLUT4, ↓ inflammation & oxidative stress.	↓ FBG, HbA1c, LDL, TG; ↑ HDL.	Capsules, extracts, powdered leaves.	Pilot Clinical Trial (53); RCT (60); Preclinical (54–59)
Black Seed	Thymoquinone activates AMPK, ↓ gluconeogenesis, antioxidant, and NF-κB inhibition.	↓ FPG, HbA1c; ↓ LDL, TC, TG; ↑ HDL.	Capsules, oils, seed powders.	Systematic Reviews (61–63); RCTs (61); Animal Studies (62)
Citrus reticulata	Flavonoids (hesperidin, naringenin) activate AMPK, inhibit NF-κB, ↓ and cytokines.	↓ FBG, insulin resistance, inflammation.	Standardized extracts (Eriomin®), capsules, and beverages.	RCT (64); Preclinical (65)
Glycine max	Isoflavones (genistein, daidzein) activate PPAR-α/γ, ↑ adiponectin, ↓ NF-κB, and TNF-α.	Improves glucose tolerance, lipid profile, and reduces NAFLD.	Soy protein, isoflavone capsules, soy milk.	RCTs (66–68); Preclinical (69,70); Review (71)

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3.13.1 Triphala: The Ayurvedic Standard

Composition: Equal parts Emblica officinalis (Amalaki), Terminalia bellerica (Bibhitaki), Terminalia chebula (Haritaki) (74).

Mechanisms:

Gut microbiota modulation: Increases Bifidobacteria and Lactobacillus while suppressing Escherichia coli, enhancing SCFA production (e.g., butyrate) to reduce systemic inflammation (74,75).

Enzyme inhibition: Inhibits α-amylase and α-glucosidase, reducing postprandial hyperglycemia (74).

Antioxidant effects: Tannins (gallic acid, ellagic acid) lower oxidative stress markers (MDA) by 45% (74).

Clinical Outcomes (12 RCTs, n=749):

Lipid profile: LDL-C reduced by 19–32.9 mg/dL; triglycerides by 17% (74,76).

Glycemic control: Fasting blood glucose (FBG) decreased by 12.7% in diabetics (74).

Anthropometrics: Significant reductions in waist circumference (5 cm) and BMI (1.8 kg/m²) after 12 weeks (76).

Safety: No serious adverse events; mild GI discomfort in <5% of subjects (74).

Key Study: A 12-week RCT in dyslipidemic patients (n=198) showed Triphala (634 mg/day) plus atorvastatin reduced LDL-C by 27% vs. 18% with statin alone ($p<0.01$) (76) as shown in Table 2.

Table 2: Clinical Outcomes of Key Polyherbal Formulations in MetS

Formulation	Dose/Duration	Key Outcomes	Study Design
Triphala	500–1000 mg/day; 12 wks	LDL-C ↓19%; FBG ↓12.7%; WC ↓5 cm	RCT, n=198
Cinnamon + Turmeric+ Garlic	2g+500mg+1g/day; 12 wks	HOMA-IR ↓15%; TG ↓28.8%; SBP ↓8 mmHg	RCT, n=120
Fenugreek+ Berberine	200mg+300mg/day; 8 wks	HOMA-IR ↓15% vs mono; LDL-C ↓29 mg/dL	RCT, n=75
Divya-WeightGo	500 mg/day; 10 wks	Weight ↓5.6 kg; TG ↓19.5%	RCT, n=60

3.13.2 Cinnamon-Turmeric-Garlic Synergy

Composition: Cinnamomum verum + Curcuma longa + Allium sativum.

Mechanisms:

Insulin sensitization: Cinnamaldehyde enhances insulin receptor phosphorylation; Curcumin activates PPAR-γ (77,78).

Lipid regulation: Allicin inhibits HMG-CoA reductase; Curcumin downregulates NPC1L1 expression (78).

Anti-inflammatory action: NF-κB inhibition by Curcumin and Allicin reduces TNF-α by 11–20% (77).

Clinical Outcomes:

Cinnamon (2g/day): HbA1c ↓0.83%; SBP ↓4.3 mmHg (77).

Turmeric (Curcumin + piperine): Triglycerides ↓28.8%; LDL-C ↓11.64% (78).

Garlic (raw, 1g/day): FBG ↓27.17%; waist circumference ↓3.46 cm (78).

Combined effect: A 3-month RCT in MetS patients (n=120) demonstrated 15% greater reduction in HOMA-IR with the trio vs. individual herbs ($p<0.05$) (77).

3.13.3 Fenugreek-Berberine Formulation

Composition: Trigonella foenum-graecum (200 mg saponins) + Berberis vulgaris (300 mg berberine) (79).

Mechanisms:

Glucose uptake: 4-Hydroxyisoleucine in fenugreek stimulates GLUT4 translocation.

Cholesterol clearance: Berberine downregulates PCSK9, increasing LDL receptor availability (72).

Clinical Outcomes:

HbA1c ↓0.54% (fenugreek) + 1.2% (berberine) (79)

Insulin resistance (HOMA-IR) was reduced by 15% more than monotherapy ($p<0.01$) (79).

LDL-C ↓29.14 mg/dL with combined therapy vs. 17 mg/dL with berberine alone (79).

3.13.4 Emerging Polyherbal Combinations

Ginseng-Bitter Melon-Aloe Vera:
Effects: FBG ↓30% (Aloe); HbA1c ↓0.75% (Ginseng); insulin sensitivity ↑25% (Bitter melon) (80).

Trial: 8-week study in T2DM patients (n=90) showed an additive reduction in postprandial glucose (22%) (80).

Divya-WeightGo (Ashwagandha-Guggul-Garcinia):

Outcomes: 5.6 kg weight loss; 19.5% triglyceride reduction in obese patients (n=60) (79).

3.13.5 Safety and Tolerability of PHFs

Triphala: No hepatorenal toxicity in 12-month studies (74).

Berberine combinations: Transient GI effects (e.g., bloating) in 10–15% of subjects; mitigated by enteric coating (72).

Contraindications: Garlic may potentiate anticoagulants; high doses of Cinnamon (>4g/day) are linked to coumarin toxicity (78).

3.13.6 Future Research Directions

Microbiome-focused formulations: Triphala increases Akkermansia muciniphila (8.2-fold), enhancing gut barrier integrity (75).

Nano-delivery systems: Berberine-loaded erythrocytes and curcumin nanoliposomes (LSBoost-optimized) improve bioavailability by 200–400% (72,81).

Dose standardization: Clinical trials are required for PHFs like Eriomin® (citrus flavonoids) to define optimal dosing (73).

4. Discussion

The findings of this review underscore the potential of polyherbal formulations (PHFs) as comprehensive and integrative therapies for managing metabolic syndrome (MetS). By targeting the interconnected metabolic abnormalities of MetS, PHFs offer a more holistic approach than conventional single-target therapies.

4.1. Key Findings and Mechanisms

Glucose Regulation

Berberine's AMPK activation and Cinnamon's enhancement of the insulin receptor Phosphorylation are complementary mechanisms that collectively improve glycemic control. Clinical studies have reported significant reductions in fasting blood glucose (25%) and HbA1c levels (up to 1.2%) with these compounds, demonstrating efficacy comparable to standard antidiabetic medications, where berberine demonstrates the most effect on HbA1c, as in (Figure 3), while aloe vera has the highest effect on FBG, as in (Figure 4).

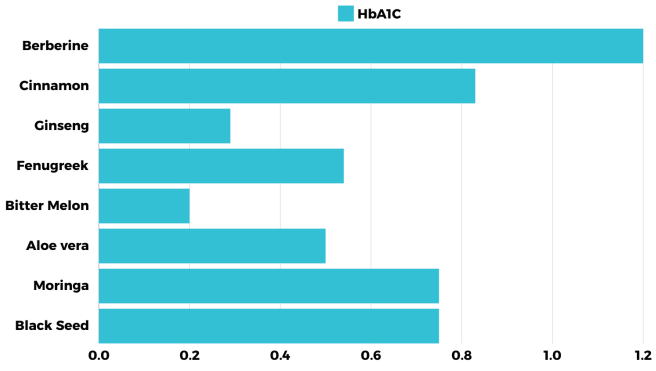

Figure 3: HbA1c reduction percentages across selected herbs. Values derived from single studies or meta-analyses.

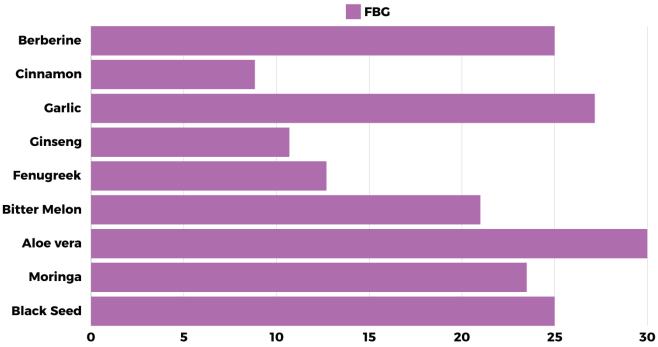

Figure 4: FBG reduction percentages based on representative clinical trials. Values derived from single studies or meta-analyses.

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Lipid Profile Improvement

Berberine, turmeric's curcumin, and garlic's allicin were effective in reducing LDL cholesterol and triglycerides while increasing HDL cholesterol, where turmeric demonstrates the highest reducing effect on TG while berberine demonstrates highest reducing effect on LDL cholesterol, as shown in Table 3 and Figure 5.

Table 3: Percentage reduction in HbA1c, FBG, TGs, LDL caused by the herbs used in treating MetS.

Herb	HbA1c Reduction	FBG Reduction	TG Reduction	LDL-C Reduction	Evidence (Reference + Study Type)
Berberine	0.5–1.2%	↓25%	↓19.5%	↓31.7%	RCTs (12–14); Systematic Review (9,10)
Cinnamon	0.5–0.83%	↓8.84%	NR	↓7–27%	RCTs (18,19); Review (9)
Turmeric	NR	NR	↓28.8%	↓11.64%	RCTs (22,23); Clinical Trial (21)
Garlic	NR	↓27.17%	↓14.09%	↓16.9% (men), ↓15.6% (women)	RCTs (27–30); Review (31)
Ginseng	0.29%	↓10.7%	NR	↓12.3%	RCTs (32,33); Reviews (34,35)
Fenugreek	0.54%	↓12.7%	NR	↓20.1%	Meta-analysis (37,39); RCT (38,40)
Bitter Melon	0.217%	↓21%	NR	NR	RCTs (41–44); Meta-analysis (45); Review (47)
Aloe Vera	0.5%	↓30%	NR	NR	RCTs (49–52); Meta-analysis (51)
Moringa	0.75%	↓23.5%	↓17%	↓19%	Pilot Clinical Trial (53); RCT (60); Preclinical (54–59)
Black Seed	0.75%	↓25%	↓15%	↓20%	Systematic Reviews (61–63); RCTs (61); Animal Studies (62)

NR: Not reported in the referenced studies Not reported in the referenced studies

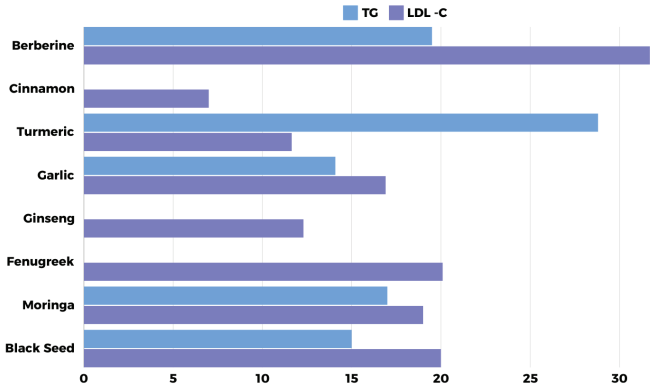

Figure 5: Lipid profile improvements (TG and LDL-C) across herbs. Missing data points indicate parameters not reported or assessed.

The lipid-lowering effects of these herbs are attributed to their modulation of bile acid synthesis, inhibition of HMG-CoA reductase, and downregulation of NPC1L1 expression. The combination of these effects significantly reduces cardiovascular risks associated with MetS.

Anti-Inflammatory and Antioxidant Properties

Turmeric and garlic were also highlighted for their potent anti-inflammatory and antioxidant effects. By inhibiting NF-κB and reducing pro-inflammatory cytokines like TNF-α, these herbs mitigate systemic inflammation, a key

contributor to insulin resistance and MetS. Curcumin's reduction of oxidative stress markers and enhancement of antioxidant enzymes further support its role in improving metabolic parameters.

Synergistic Benefits

The synergistic actions of combined herbs in PHFs amplify their individual therapeutic effects. For instance, the co-administration of berberine and turmeric enhances AMPK activation and antioxidant defenses as shown in Table 4 , providing a robust metabolic and anti-inflammatory response. Similarly, combinations like garlic and cinnamon address both glycemic and lipid abnormalities, offering a comprehensive solution for MetS management.

Table 4: Mechanisms of Synergy in PHFs

Synergy Type	Example	MetS Impact
Pharmacodynamic	Berberine (AMPK) + Curcumin (NF-κB)	Enhanced glucose uptake + inflammation reduction
Pharmacokinetic	Piperine + Curcumin	Bioavailability ↑2000%
Microbiome-mediated	Triphala polyphenols → SCFA production	Gut barrier integrity; inflammation ↓

4.2. Advancements in Formulation Technologies

Recent innovations, such as nanoparticle encapsulation and bioavailability enhancers like piperine, have addressed the limitations of poor solubility and stability in herbal compounds. These advancements have not only improved the therapeutic efficacy of PHFs but also ensured consistent clinical outcomes and enhanced patient compliance.

4.3. Limitations and Recommendations

Despite promising findings, limitations in the current evidence include small sample sizes, short follow-up durations, and variability in herbal compositions across studies. Future Research should focus on large-scale, multicenter randomized controlled trials to validate these findings and standardize formulations. Additionally, exploring the pharmacokinetics and pharmacodynamics of combined herbs will provide deeper insights into their mechanisms and optimize formulation strategies.

Challenges

a. Toxicity and Safety Aspects

Unlike conventional pharmaceuticals, which typically contain one or a few well-characterized active ingredients, herbal medicines often comprise hundreds of bioactive compounds. This complexity poses significant challenges for toxicological evaluation, as isolating and assessing each constituent requires extensive time and resources (82). In many regions, herbal products are permitted on the market without undergoing rigorous safety testing or standardized toxicological screening. As a result, consumers may be exposed to potentially harmful substances, including toxic plant metabolites, contaminants such as heavy metals or pesticides, and adulterants (83). The lack of harmonized regulatory oversight and variability in herbal composition further complicates risk assessment and increases the likelihood of adverse effects (84).

b. Lack of Quality Control

Quality control is a cornerstone of modern pharmaceutical production, ensuring that medicinal products meet standards of purity, identity, safety, and efficacy. However, the quality assurance of herbal medicines remains a significant challenge due to the complex nature of plant-based formulations. Factors such as environmental conditions (e.g., temperature, light exposure), soil nutrients, water availability, and variability in harvesting and post-harvest practices (drying, packaging, storage, and transportation) can drastically affect the phytochemical composition and therapeutic consistency of herbal products (85). Without standardized cultivation and processing protocols, batch-to-batch variability undermines both clinical reliability and consumer trust.

c. Lack of Governmental Legislation

In many countries, herbal products are marketed as dietary supplements or wellness aids without stringent regulatory oversight. These formulations are often promoted for their supportive, preventive, or health-enhancing properties, yet they may bypass rigorous

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pre-market evaluation. While some regions require labeling and authenticity certificates that disclose active compounds and intended medicinal use, enforcement is inconsistent and often lacks harmonization across jurisdictions (86). The absence of unified legislation and regulatory frameworks contributes to variability in product quality and raises concerns about consumer safety and confidence.

d. Scientific and Clinical Assessment

Despite their natural origin, not all herbal ingredients are inherently safe. Several compounds used in traditional medicine have demonstrated toxicity to vital organs such as the liver, kidneys, and heart (82). Therefore, rigorous scientific evaluation—including pharmacological profiling, toxicological screening, and clinical trials—is essential to ensure the safety, efficacy, and reproducibility of herbal products. International guidelines issued by the World Health Organization (WHO), the U.S. Food and Drug Administration (FDA), the International Council for Harmonisation (ICH), and the United States Pharmacopeia Convention (USPC) emphasize the importance of botanical identification, chemical characterization, and safety pharmacology in herbal drug development (87,88)

4.4. Implications for Clinical Practice

PHFs offer a viable, cost-effective alternative to conventional therapies, particularly in resource-limited settings. Their multitargeted mechanisms and safety profiles support their potential integration into routine MetS management, provided standardized, high-quality formulations are used.

5. Advances in Optimization

5.1. Berberine Loading in Erythrocytes

A study successfully optimized berberine loading into erythrocytes for anti-inflammatory effects using the Taguchi method. The approach identified optimal conditions for enhanced drug loading while preserving erythrocyte integrity, significantly reducing inflammatory cytokines like TNF-α and IL-1β (89)

5.2. Curcumin-Nanoliposomal Formulations

Machine learning techniques, such as ensemble learning (LSBoost), were applied to optimize curcumin-loaded liposomes for high entrapment efficiency (EE). Variables like molar ratios, particle size, and pH were systematically analyzed, providing insights into creating stable, effective formulations. (90–93)

6. Conclusion

PHFs represent a promising holistic treatment for MetS, addressing its complex metabolic abnormalities through multitargeted mechanisms. Advances in formulation technologies and optimization techniques enhance their efficacy and safety, making them suitable for integrative healthcare. Standardization and further Research are essential to establish their role in mainstream clinical practice.

Authorship

Peter M. Besada, Ahmed M. Abdelaziz, and Ahmed R. Rabie contributed equally to its development, actively participated in the conception and design of the study, data analysis, interpretation, drafting, and critical review of the manuscript. Dina M. Mahdy supervised and revised the manuscript. All authors have read and approved the final version of the manuscript submitted for publication.

Conflicts of Interest: The authors disclose all relationships or interests that could have direct or potential influence or impart bias on the work.

Abbreviations:

MetS: Metabolic Syndrome

CVDs: cardiovascular diseases

T2DM: type 2 diabetes mellitus

ISI: insulin sensitivity index

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Repurposing of Selected Antivirals and Anthelmintics for Treatment of COVID-19 (In Silico Study)

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ABSTRACT:

The 2019 Coronavirus Disease (COVID-19) is a highly contagious viral illness that has infected a lot of people, causing high rates of morbidity worldwide, turning mostly into a pandemic. The disease is caused by the SARS-CoV-2 virus, resulting in severe respiratory and hyper-inflammatory responses. Finding an immediate, effective cure for the recently discovered disease has drawn the interest of researchers to repurpose existing drug candidates, including different pharmacological classes such as antiviral and anti-inflammatory medicines. Numerous therapy modalities have been investigated for COVID-19 treatment. Niclosamide and Nitazoxanide, the FDA-approved anthelminthic medications, have been shown to be effective against several viral infections. This finding suggests the drugs' potential as antiviral agents. Also, many antiviral drugs with different mechanisms have shown great efficacy in treating COVID-19, such as Molnupiravir and Remdesivir. The primary objective of this brief study is to enhance the pharmacokinetics of the FDA-approved anthelmintic drugs Niclosamide and Nitazoxanide through in silico structural modification, aiming to confirm their repurposing as antiviral candidates against COVID-19. Computer-aided drug design (CADD) has contributed to the acceleration of drug discovery and the development of new analogs. Using molecular docking simulations (CDocker algorithm) and ADMET predictions, several analogs of both drugs were generated and assessed. Among the tested analogs, Niclosamide modification 2 demonstrated the most promising profile, with the least C-docker interaction energy, superior binding affinity compared to the parent Niclosamide and Nitazoxanide analogs, and comparable results to the benchmark antiviral Molnupiravir. This analog also exhibited improved hydrophilic interactions (and favorable pharmacokinetic properties, including enhanced solubility and absorption. These findings suggest that Niclosamide modification three and Nitazoxanide modification one could serve as a promising lead candidate for further synthesis and experimental validation in the development of new COVID-19 therapies.

KEYWORDS:

COVID-19 treatment, drug optimization, drug repurposing, in silico modeling, Molnupiravir and Remdesivir, Niclosamide and Nitazoxanide.

Introduction

At the end of 2019, the world encountered the most challenging pandemic of the modern era. A novel coronavirus was identified as the cause of a cluster of pneumonia cases in Wuhan, China. [The COVID-19 outbreak has posed a serious threat to public health worldwide. Coronaviruses are a family of positive ribonucleic acid (RNA) viruses, leading to severe acute respiratory syndrome (SARS) and a variety of other respiratory infections.

Vaccines have reduced the transmission and severity of COVID-19, but there remains a lack of efficacious treatment for drug-resistant strains and more susceptible individuals. The aim of this study is to repurpose existing FDA-approved drug alternatives. Drug repurposing is the process of identifying new therapeutic uses for existing drugs. This strategy significantly reduces the time, cost, and risk associated with traditional drug discovery, as repurposed drugs have already undergone extensive safety and toxicity testing. In the context of the COVID-19 pandemic, where urgent therapeutic solutions are needed, repurposing offers an efficient and scientifically robust approach for accelerating drug development. The FDA-approved anthelmintics, Niclosamide and Nitazoxanide, have shown a promising ability to inhibit viral infections, including SARS-CoV, MERS-CoV, ZIKV, HCV, and human adenovirus, in nanomolar to micromolar potency with activity comparable to

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the currently used medications.[,]

Niclosamide and Nitazoxanide have been associated with some pharmacokinetic drawbacks, such as poor water solubility and, consequently, low oral bioavailability.[2] The aim of this study is to execute computer-aided structural modification on both drugs in order to maximize their antiviral efficacy and overcome their structure-related setbacks. The activity and efficacy of the selected agents have been evaluated and compared to their proposed structural alterations and benchmark antiviral agents, Molnupiravir and Remdesivir, in terms of testing the improvement in their efficacy, enhancement of their absorption, with the aim of developing new optimized drug candidates.

Methods

Construction of 3D drug molecules using BIOVIA Discovery Studio software. The structure of SARS-CoV-2 main protease (PDB ID: 6LU7, resolution: 2.16 Å) and RNA-dependent RNA polymerase (PDB ID: 7BV2, resolution: 2.5 Å) were retrieved from the protein data bank (www.rcsb.org). Virtual screening was implemented by the docking module of the BIOVIA Discovery Studio, Dassault Systèmes, Academic Standard Base, PR14361, San Diego: Dassault Systèmes, 2024, using the CDocker technique. The docking was performed using the full potential CDocker algorithm and the CHARMm forcefield for the highest accuracy and minimal result biases. The binding sites were defined according to the co-crystallized ligands. For 6LU7, the binding pocket was centered at (-10.75, 12.39, 68.98 Å) with a radius of 15.7 Å. For 7BV2, the binding sphere was centered at (96.50, 117.04, 83.03 Å) with a radius of 5 Å. Ten random conformations per ligand were generated, followed by simulated annealing dynamics (2000 heating steps and 5000 cooling steps). The top docking poses were ranked according to their CDOCKER-Interaction energy. Validation of the docking protocol was pursued by redocking the co-crystallized ligands into their respective binding pockets. The reproduced poses aligned well with the crystallographic conformations, yielding a Root Mean Square Deviation (RMSD) of 1.88 Å (6LU7) and 1.14 Å (7BV2), confirming excellent reliability of the docking procedure (Fig. 1). The pharmacokinetic properties (ADMET, Absorption, Distribution, Metabolism, Elimination and Toxicity) of all drug candidates have been calculated using the BIOVIA, Dassault Systèmes software.

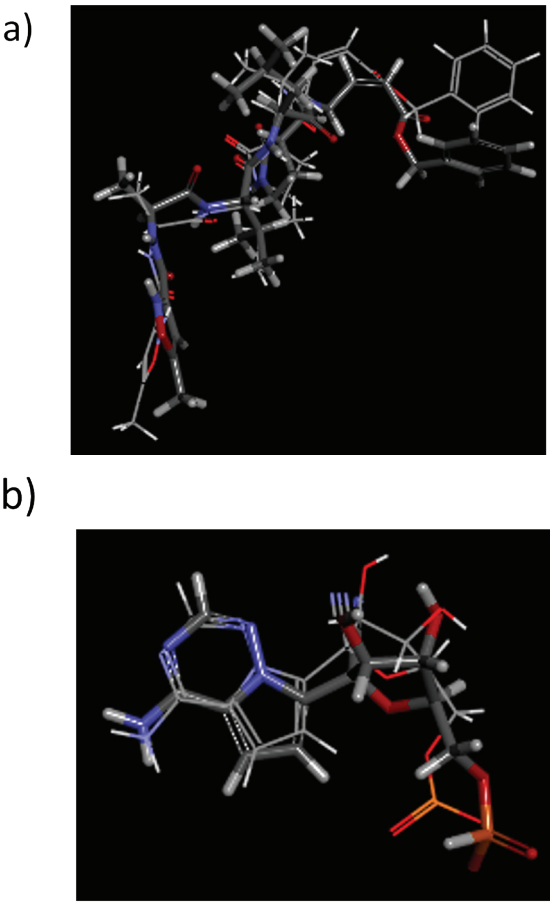

Figure 1: RMSD overlay images of the co-crystallized ligand (line) and the redocked ligand (ball and stick) in the binding sites of a) 6LU7, b) 7BV2

Results and Discussion

Remdesivir is the first approved antiviral agent, but due to its limited oral bioavailability and extensive side effects, the search for a more potent agent has been pivotal.[Molnupiravir was then developed, showing improved binding affinity to the target protein and better oral bioavailability. In this study, molecular docking simulations of both antiviral agents, Remdesivir and Molnupiravir (Fig. 2a, Fig. 3a, respectively), were performed against RNA-dependent RNA polymerase SARS-CoV-2 proteins, and their binding interactions were evaluated for ongoing comparison with other agents under repurposing investigation (Fig. 2b, Fig. 3b, respectively). The negative interaction energy of Remdesivir and Molnupiravir was calculated (50.24 and 51.29, respectively) along with their ADMET properties. From the extracted ADMET diagram, it can be concluded that both drugs have moderate pharmacokinetic properties, while Molnupiravir, as previously discussed, may have improved characteristics.

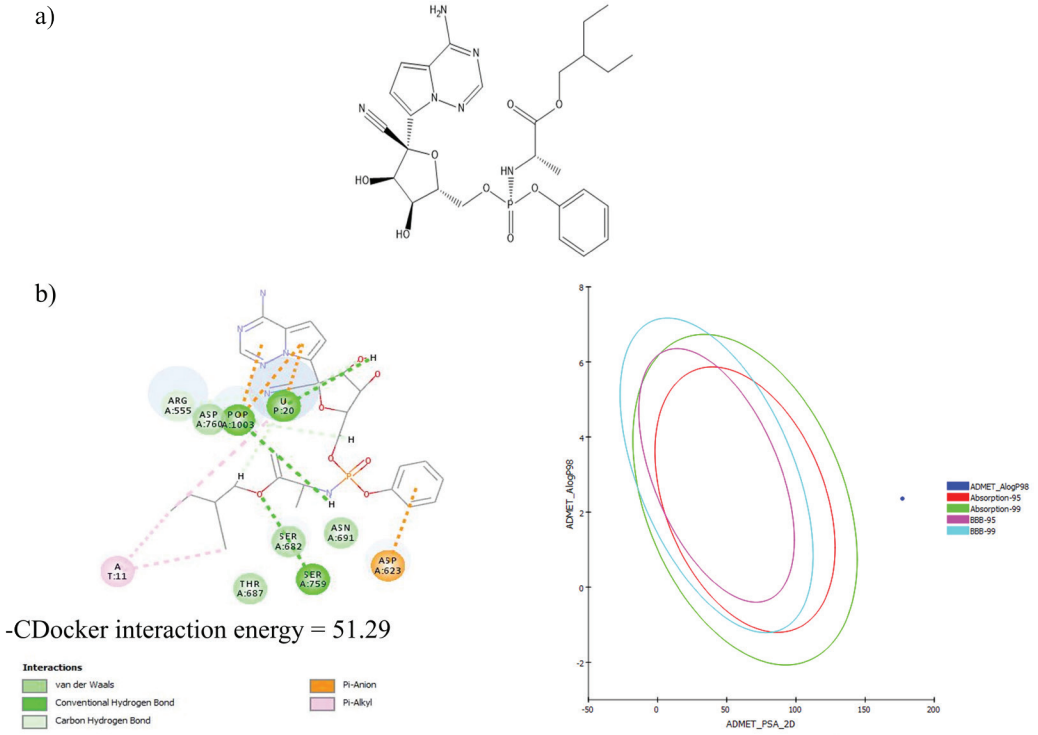

Figure 2: Remdesivir a) chemical structure, b) Molecular docking 3D interaction against 7BV2, c) ADMET calculation

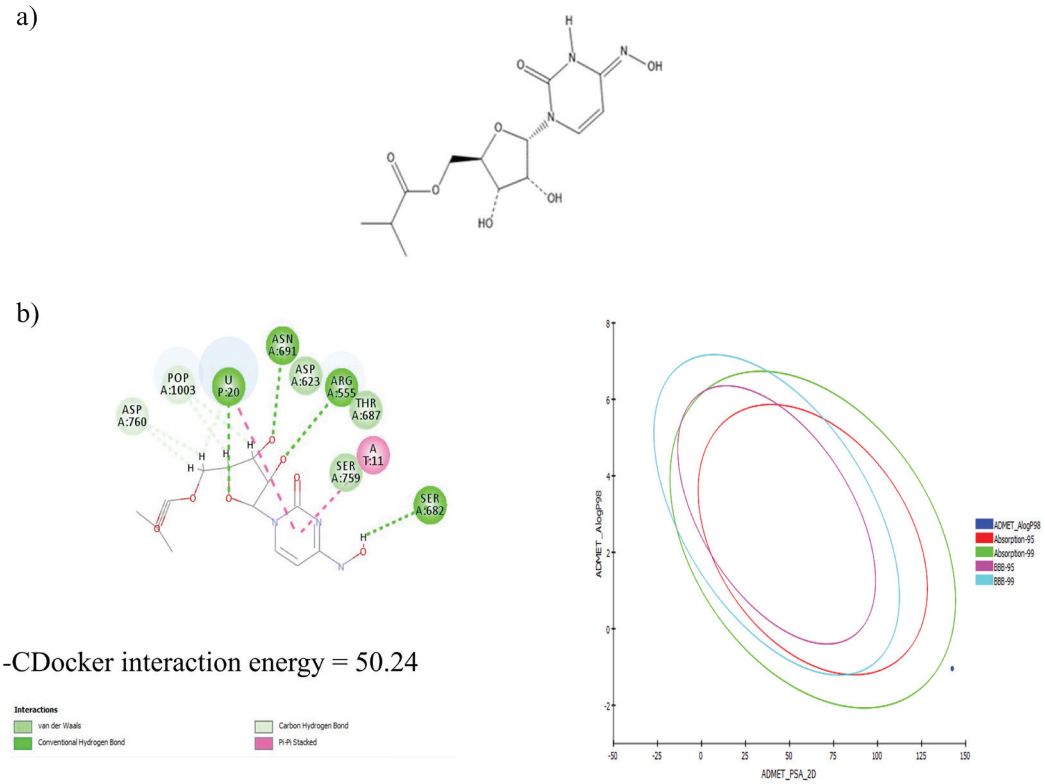

Figure 3: Malnupiravir a) chemical structure, b) Molecular docking 3D interaction against 7BV2, c) ADMET calculation

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Taking the antiviral agents' generated results into consideration, anthelmintic drug candidates, Niclosamide and Nitazoxanide, were examined to investigate their antiviral activity. The novel input of this study is the implementation of structural modifications on anthelmintic agents and comparing their binding affinity and pharmacokinetic properties to both the parent compounds and existing antiviral agents, Remdesivir and Molnupiravir. Niclosamide and Nitazoxanide (Fig. 4a, Fig. 5a, respectively) have been successfully repurposed for the eradication of coronavirus infection.[1, 2]

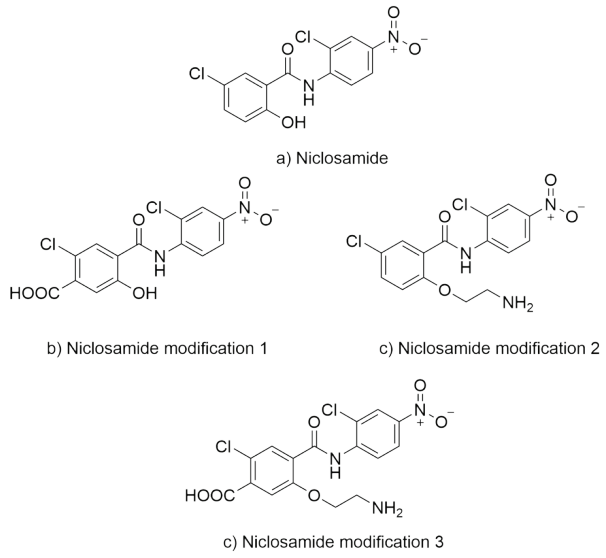

Figure 4: Niclosamide and its proposed structural modifications

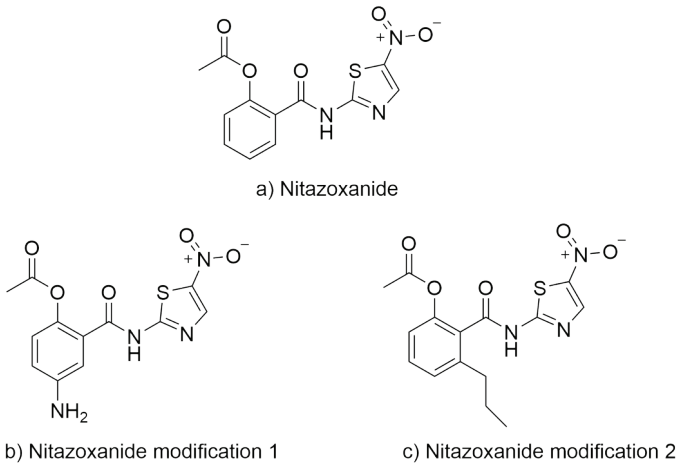

Figure 5: Nitazoxanide and its proposed structural modifications

Niclosamide has faced challenges progressing to clinical therapy due to its poor water solubility, lowering its bioavailability.[2] In this study, the main goal is to implement chemical modifications, such as the addition of ionizable groups like carboxylic acid or amino groups (Fig. 4b, c, d), on the parent compound to render it more water-soluble and examine their effect on the activity of the drug. In silico modeling of the three modified Niclosamide analogs has been established and compared to the parent compound (Fig. 6).

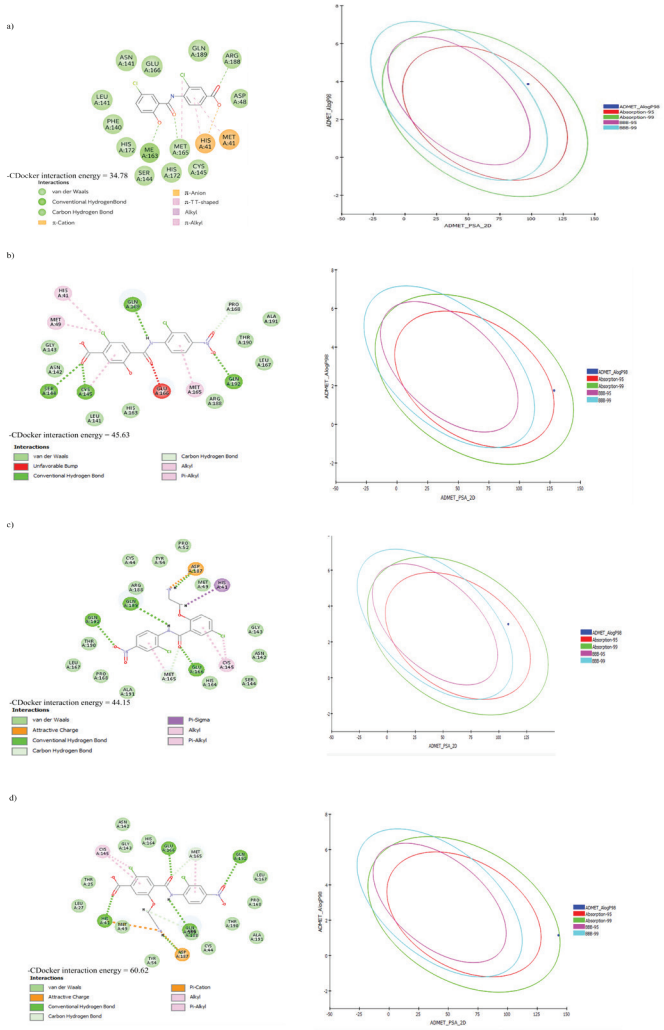

Figure 6: Molecular docking 3D interaction with 6LU7 and ADMET calculation respectively of: a) Niclosamide, b) Niclosamide modification 1, c) Niclosamide modification 2, d) Niclosamide modification 3

Analysis of docking interactions revealed that all Niclosamide analogs showed improved binding affinity compared to their parent compounds. Importantly, Niclosamide modification 3 achieved the most favorable binding profile, with a C-docker score of −60.62 kcal/mol, surpassing the parent Niclosamide and the benchmark antivirals. Interaction mapping indicated multiple hydrogen bonds and stabilizing hydrophobic contacts with key residues of the SARS-CoV-2 main protease, enhancing its binding stability. From these findings and the almost comparable pharmacokinetic calculations, it can be concluded that rendering

Niclosamide more water soluble to overcome its limited bioavailability had a positive effect on the activity of the drug.

On the other hand, Nitazoxanide has almost no water solubility, which has drawn the attention of researchers to enhance its aqueous solubility by finding a suitable organic solvent for dissolution. [] Recognizing that the drug's dissolution and solubility is an important factor for absorption and bioavailability, two structural modifications of Nitazoxanide have been suggested to enhance its hydrophilicity through the addition of an amino group (Fig. 5b) or its lipophilicity through the addition of a propyl chain (Fig. 5c). To validate the study's findings, it is crucial that the proposed modifications do not diminish the drug's effectiveness. Molecular docking simulation and ADMET calculations have been performed (Fig. 7).

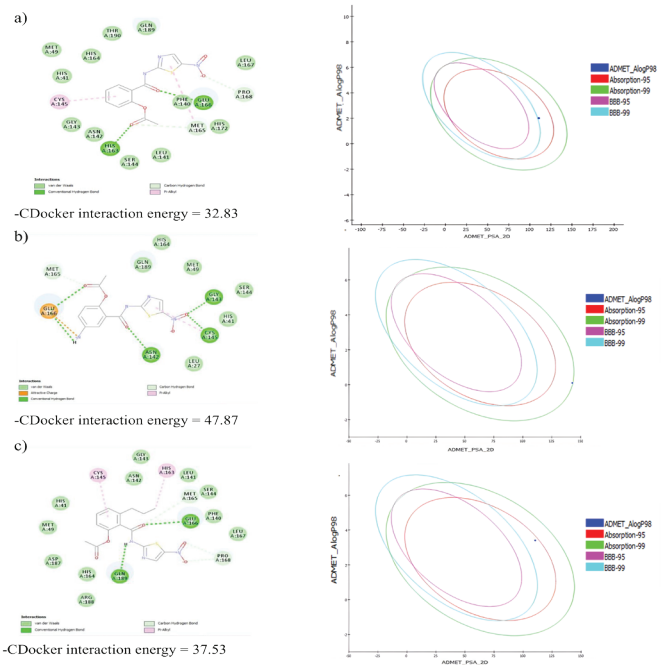

Figure 7: Molecular docking 3D interaction with 6LU7 and ADMET calculation respectively of: a) Nitazoxanide, b) Nitazoxanide modification 1, c) Nitazoxanide modification 2

Among Nitazoxanide analogs, modification 1 was the best-performing candidate with a C-docker score of −47.87 kcal/mol. It formed a favorable number of hydrophilic and hydrophobic interactions. The analogs also showed comparable pharmacokinetic properties.

These findings highlight Niclosamide modification three as the top-performing analog overall, while Nitazoxanide modification one also shows potential as a viable repurposed candidate.

Conclusion

Anthelmintic agents, Niclosamide and Nitazoxanide, have been previously repurposed as antivirals for the treatment of COVID-19. In this research study, it was aimed to prove their repurposing and propose different structural analogs with different hydro-lipophilicity to overcome the parent drugs' limited bioavailability, resulting from their poor dissolution. This study demonstrates that structural modification of Niclosamide and Nitazoxanide can significantly enhance their pharmacokinetic and binding properties for potential repurposing as COVID-19 therapeutics. Among the tested analogs, Niclosamide modification 3 emerged as the most promising candidate, with favorable hydrophilic and hydrophobic interactions and an improved ADMET profile. Its pharmacological performance was superior to that of its parent compound and comparable to benchmark antivirals. Nitazoxanide modification one also demonstrated improved binding affinity and favorable pharmacokinetic properties, making it a viable candidate. These results suggest that Niclosamide modification 3, in particular, could serve as a valuable lead for further synthesis, in vitro validation, and potential clinical development.

Declarations

Availability of Data and Materials:

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing Interests:

The authors have no relevant financial or non-financial interests to disclose.

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Authors Contributions:

All authors contributed to the study conception and design. The experimental part, data collection, analysis, and discussion, was carried out by SE. All authors read, edited, and approved the final manuscript.

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Evaluation of factors for medication non-adherence in chronic diseases and its association with the level of stress in health care providers in a tertiary care hospital- A cross sectional observational study

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ABSTRACT

Background: *Therapeutic regimen effectiveness highly depends on medication adherence. Poor medication adherence resulting in therapeutic failure is seen in healthcare providers (HCPs). Literature reviews focusing exclusively on the illness experiences of HCPs are lacking; hence, they need to be evaluated.*

Methodology: *A cross-sectional study among HCPs having one chronic disease, lasting 1year, and taking one chronic medication was conducted after ethics approval. An online questionnaire comprising an e-consent form, inclusion criteria, demographic characteristics, factors affecting adherence, Adherence to Refills and Medication-Scale (ARMS), Perceived Stress-Scale (PSS), and Brief-Illness Perception Questionnaire (BPIQ) was used. Data was analysed by applying chi-square and binary logistic regression using SPSS version 24.*

Results: *About 207 participants took up the study, and 52 (25.1%) were excluded. Of 155 (74.9%) continued, age group 46-55 (42.6%), female (81.3%), married (81.3%), having Master's degree (72%) with regular employment (71%), medium income (81.3%), non-smokers (82.6%), having disease-duration>3yrs (71%), 1-chronic-disease (77.4%), 1-chronic-medication (66.8%) and no health insurance (64.5%). Forgetfulness (49.7%), Anxiety (21.2%), busy lifestyle was observed (49.7%), mild disease (43.9%), treatment complexities (42.6%), indirect costs (15.5%), and concern at workplace (60%) were the important factors for non-adherence to medication. ARMS showed 93.4% adherence to medications. Among adherents, 62% had moderate stress and 13% showed high stress. Among non-adherent, 100% showed moderate stress. BPI reflected that 69% participants had a positive perception of the disease, and had moderate stress (76.6%). A statistically significant but weak positive correlation between stress score and perception about the disease was observed.*

Conclusions: *Study results showed forgetfulness, busy lifestyle, treatment complexities, side effects of medications, and negative perceptions of illness had led to non-compliance in HCPs. A significant association between factors for non-adherence and stress was observed. A weak positive correlation was seen between adherence, perception of illness, and stress.*

KEYWORDS

Adherence to Refills and Medication Scale"; "Brief Illness Perception Questionnaire"; "Medication Adherence"; "Perceived Stress Scale"; "Therapeutic Failure.

1. Introduction

It is a well-known quote in Western medicine that doctors should always be healthy, as their illness experiences constantly influence not only their perceptions of illness and roles, but also their medical practice. But as no one is free from disease, health care providers(HCPs) also have to behave as patients when they encounter one of the chronic diseases. It is also as important for them to adhere to their medications as it is for the patients they treat. Medication-adherence, therefore, refers to the degree to which a patient correctly takes and adheres to their prescription regimen as directed by their physician [1].

Non-adherence to medications in HCPs can be a result of improper training during their medical

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education to deal with their own illness. This may indirectly have an adverse impact on the health outcomes, cost-effectiveness of medical care, psychological, social, and physical well-being of HCPs as well [2-5]. It is important that, as patients adhere to medications, prescribers also adhere to medications when prescribed to them. It is shown in previous studies that doctors' disease prognoses are mostly better than or similar to those of patients belonging to the general population [6].

Adherence to medication is essential because it enhances quality of life by managing both transient and chronic diseases. The World Health Organization (2003) reported that the adherence rate to long-term therapies in developed countries was 50% [7]. Studies showed that half the patients are considered non-adherent to their chronic medications. As medication adherence is a dynamic process, a number of elements are identified that can cause hurdles to it. Many techniques to detect non-adherence have been proposed in the literature over the past few decades, but only a small number of them have been proven to be effective [8]. The Morisky Medication-Adherence Scale8-items(MMAS-8) [9] and the Adherence to Refills and Medications Scale 12-items(ARMS) [10] are the most widely utilized, valid, and accurate questionnaires to detect non-adherence.

Factors associated with medication non-adherence in HCPs need to be evaluated, and have not been done in the past. Factors like mental-health conditions, most commonly depression and Anxiety, as well as inadequate health-related information, are correlated to patient non-adherence to medicines [11]. Despite awareness of their illness and the importance of adherence, healthcare providers' limited time for personal health management may contribute to suboptimal adherence behaviors. Despite awareness of their illness and the importance of adherence, healthcare providers' limited time for personal health management may contribute to suboptimal adherence behaviors. It is suggested that age, race, anxiety, depression, and perceived social support influence compliance in chronic illnesses. Depression's role and how anxiety and stress alter the management of chronic disease-related parameters are still not clear [12]. Moreover, the majority of prior research has ignored the social and historical predispositions of physicians as well as their biomedical viewpoints of their own ailments. Importance of taking their medications on time without missing them, even under undue work-related pressure, is a crucial step towards the management of chronic diseases. Job-related travels and job conditions may also contribute as stress factors that can lead to non-compliance [13]. Social issues like job security, concerns, and environmental stress in the workplace could also be factors for non-adherence [14].

Perceived Stress Scale (PSS) is one of the classical tools for assessing stress [15] and is widely used to help comprehend how various circumstances impact emotions and perception of stress. This scale measures the thoughts and feelings that a person has throughout the past month.

Illness perceptions play a significant role in determining health behaviors (treatment adherence), and thus influence outcomes like quality of life, functional recovery, and clinical parameters. BIPQ is a valid and dependable metric [16] to evaluate the influence of illness on emotion and cognition

There is a scarcity of studies regarding the behavior of HCPs in relation to medication adherence when they are suffering from one or more chronic diseases. Limited studies are available that analysed whether stress due to their work affects their adherence to their prescribed medications. Hence, a study was conducted to assess factors responsible for the medication non-adherence in HCPs and to determine its relationship with job-related stress in a tertiary care hospital.

Objectives:

To evaluate factors affecting medication non-adherence and their association with the level of stress in HCPs, and secondarily to study HCPs' attitude towards adherence to medication using the "Adherence to Refills and Medication Scale 12-items" scale, analyze stress scores in HCPs using the "Perceived Stress Scale", and to assess perception of HCPs towards their Illness using the "Brief Illness Perception Questionnaire"

2. Materials and Methods

Subjects and Methods:

A cross-sectional questionnaire-based study was conducted after taking institutional ethics committee permission (ESICMC/SNR/IEC-F653/09/2024) between November 2024

and January 2025. The Declaration of Helsinki Guidelines were followed. HCPs at a tertiary-care hospital participated in the study. They were requested to fill in the questionnaire sent through Google Forms after taking informed consent. HCPs between 26 -70 years, both genders, and having at least one chronic disease (disease lasting 1year or more, limiting daily activities and taking one or more medications) were included, and HCPs having an episodic nature of disease for a duration of less than a year were excluded.

Section 1 in the questionnaire included demographic characteristics and factors affecting medication non-adherence in HCPs. Section 2 comprised the Adherence to Refills and Medication Scale-12-items (ARMS). An ARMS is a validated instrument to measure patients' adherence, especially in chronic diseases, and consists of 12 items, each including a 4-point scale. The scale ranges from "None of the times", rated as "1", to "All of the times", rated as "4", making the total score range from 12-48, and the 12th item is reverse-coded. It measures participants' adherence to taking medications (8items) and adherence to refilling prescriptions (4items). The score of 12will be considered as a cut-off point value, and all participants with a score of 12will be taken as having high-adherence, while those with a score >12 will be rated as having low-adherence to medications.

Section 3 measured the perceived work-related stress, a well-tested stress-assessment instrument. Scores for questions 4, 5, 7, and 8 are reversed like 0=4, 1=3, 2=2, 3=1, 4=0. PSS scores can range from 0 to 40. Scores from 27-40 indicate higher-stress, 0-13 low-stress, and 14-26 moderate-stress.

Section 4 included BIPQ, having 8items (cognitive illness assessing consequences, timeline, personal control, treatment control, and identity (5items); emotional representations assessing concern and emotions (2items); and illness comprehensibility (1item). An open-ended response item asking participants to list three 3most likely factors in having roles in their illness was included. Each item is scored on a 0-10 ordinal scale, making the total score from 0 to 80. Scores nearing 80 would indicate a higher negative illness perception of the disease.

Statistical Methods:

A sample size of 176 was considered sufficient to detect a 50%frequency of the outcome factor in the population of 500, with confidence limits of 90%. Considering a 20%non-response rate, a total sample of 220 has to be studied.

Sample-size $\boldsymbol{n = [DEFF*Np(1-p)]/[(d^2/Z^2_{1-\alpha/2}*(N-1)+p*(1-p)]}$

Design-effect (DEFF) for cluster surveys-1

3. Data Analysis

IBM-SPSS version 24.0, Armonk, NY: IBM Corp., was used to analyze the data. Categorical variables were presented as frequencies and percentages. The relationship between stress levels and socio-demographic characteristics, and risk factors was evaluated using the Chi-square test as the data was non-normal (Shapiro–Wilk test), the correlation among stress-scores and adherence and BIPQ was analysed using Spearman's correlation-coefficient test. $P<0.05$ was considered statistically significant.

4. Results

Out of 207participants, 155 had 1chronic disease and were on 1or more medications for a duration of 1 or more years. About 74.9% of them participated. (Figure 1) Participants were higher in age-group 46-55 years, mostly females and married, with an MD degree, regular employment, non-smokers, having a medium income, and few having health insurance. (Table 1)

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Figure 1: Study Flow Diagram

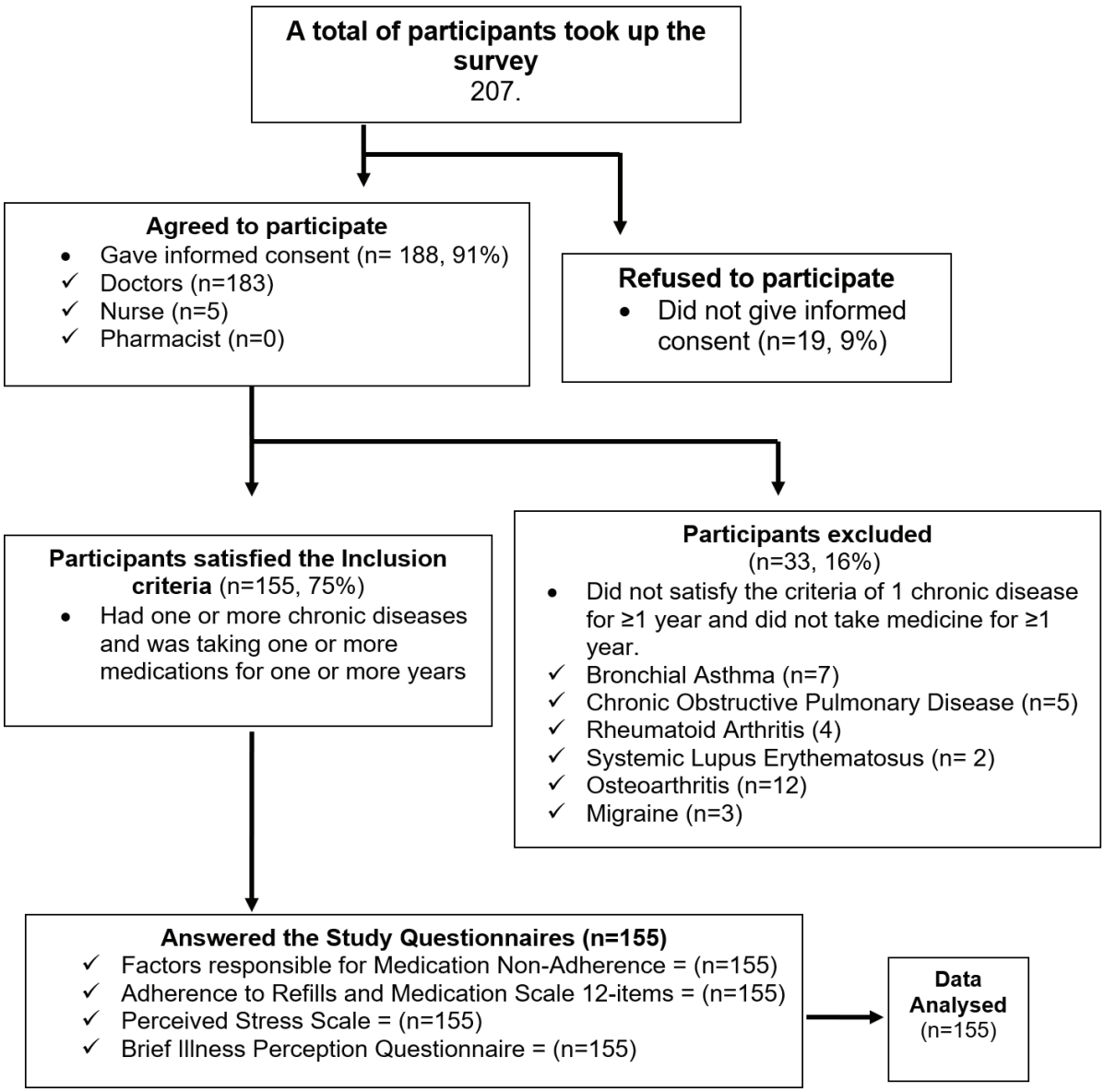

Table 1: Demographic Characteristics of Participants

Variable	Category	Frequency (n=155)	Percentage (%)
Age	26-35	22	14.2
	36-45	33	21.3
	46-55	66	42.6
	56-65	30	19.4
	66-70	4	2.6
Gender	Males	29	18.7
	Females	126	81.3
Marital status	Single	26	16.7
	Married	129	83.2
	Divorced/ widowed	---	---
Educational status	MBBS	22	14.2
	MD/MS/DNB	113	72.9
	DM/MCh/ DrNB	15	9.7
	BSc/ MSc Nursing	5	3.2
	Bpharm/ MPharm	---	---
Employment status	Regular	110	71
	Contract	45	29
Income rate	Low	---	---
	Medium	126	81.3
	High	29	18.7
Smoking status	Smoker	27	17.4
	Non-smoker	128	82.6

The majority had >3years of disease duration, one chronic disease, and were on a single chronic medication. The disease characteristics included 74.4% Type-2 DM, 48% dyslipidemia, 82.2% hypertension, and 26.4% hypothyroidism, respectively. (Table 2)

Table 2: Disease Characteristics of Participants

Variable	Category	Frequency (n=155)	Percentage (%)
Duration of disease, years	1 year	32	20.6
	2years	13	8.4
	≥3years	110	71
Number of Chronic Diseases	1	120	77.4
	2	21	13.5
	≥ 3	14	9.1
Number of Chronic Medications	1	102	65.8
	2	27	17.4
	≥ 3	26	16.8
Medication Insurance	Yes	55	35.5
	No	100	64.5
Major Types of Chronic Disease	Hyper-tension	53	82.2
	Type 2 Diabetes M	48	74.4
	Dyslipid-emia	31	48
	Hypothy-roidism	17	26.4

Association of Level of Stress (Low, Moderate, and High) with Participant Characteristics

The present study demonstrates that age and stress levels are significantly associated ($p < 0.001$*). Most participants with high stress were between the ages of 26 and 35 years, followed by the age group of 46- 55 years. Stress levels were lower among participants aged 56–70years. Gender did not show a statistically-significant correlation with stress level ($p=0.156$), even though a higher percentage of males reported lower stress than women. There was a significant association between stress levels and marital status ($p < 0.001$*). Stress levels were higher among single people than among married people. There was a substantial association ($p < 0.001$*) between stress and educational status. Participants with DM/MCh qualifications reported mild stress. However, a significantly higher proportion of those with either MBBS or MD/MS experienced severe stress, respectively. There was a significant correlation between stress levels and work status ($p=0.026$*). Contractual workers showed higher levels of stress than regular employees. Patients who had been ill for two years experienced very little stress, whereas those who had been ill for three or more years experienced more stress. Longer illness-duration could also be the reason for non-adherence and may be associated with higher stress levels ($p=<0.001$*). All participants with at least five drugs experienced moderate stress (neither low nor high). Stress levels were higher among participants who were taking four or more drugs ($p < 0.001$*), indicating that polypharmacy may play a significant role in stress. Lower stress was reported by insured individuals compared to uninsured individuals. (Table: 3)

Table 3: Association of Level of Stress with Participant Characteristics

Age range	Level of Stress			Total (n=155, %)	P value
	Low Stress (n,%)	Moderate Stress (n, %)	High Stress (n, %)		
26-35	1(4.5)	9 (40.9)	12 (54.6)	22 (14.2)	<0.001***
36-45	6 (18.2)	27 (81.8)	0	33 (21.2)	
46-55	18 (27.3)	47 (71.2)	1 (1.5)	66 (42.5)	
56-65	17 (56.7)	13 (43.3)	0	30 (19.5)	
65-70	0	4 (100)	0	4 (2.6)	
Gender					0.156
Male	10 (34.5)	19 (65.5)	0	29 (18.7)	
Female	32 (25.4)	81 (64.3)	13 (10.3)	126 (81.3)	

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Marital Status					
Single	6 (23.1)	12 (46.2)	8 (30.7)	26 (16.8)	<0.001***
Married	36 (27.9)	88 (68.3)	5 (3.8)	129 (83.2)	
Educational status					
MBBS	1(4.5)	17 (77.3)	4(18.2)	22 (14.2)	<0.001***
MD, MS	27 (23.9)	77 (68.1)	9 (8)	113 (72.9)	
DM, MCh	14 (93.3)	1 (6.7)	0	15 (9.7)	
Nursing	0	5 (100)	0	5 (3.2)	
Employment Status					
Regular	31(28.2)	74 (67.3)	5 (4.5)	110 (70.9)	0 .026*
Contract	11 (24.4)	26 (57.8)	8 (17.8)	45(29.1)	
Smoking					
Yes	5 (18.5)	22 (81.5)	0	27 (17.4)	0.079
No	37 (28.9)	78 (60.9)	13 (10.2)	128 (82.6)	
Income Rate					
Medium	38 (30.2)	76 (60.3)	12 (9.5)	126 (81.3)	0.074
High	4 (13.8)	24 (82.8)	1 (3.4)	29 (18.7)	
Duration of chronic disease					
1year	12 (37.5)	20 (62.5)	0	32 (20.6)	<0.001***
2 years	11 (84.6)	2 (15.4)	0	13 (8.4)	
≥3 years	19 (17.3)	78 (70.9)	13 (11.8)	110 (71)	
No. of chronic diseases					
1	32 (26.7)	75 (62.5)	13 (10.8)	120 (77.5)	0.310
2	5 (23.8)	16 (76.2)	0	21 (13.5)	
≥ 3	5 (35.7)	9 (64.3)	0	14 (9)	
Number of medications					
1	25 (24.5)	68 (66.7)	9 (8.8)	102 (65.8)	<0.001***
2	9 (33.3)	18 (66.7)	0	27 (17.4)	
3	5 (35.7)	9 (64.3)	0	14 (9.1)	
4	3 (42.9)	4 (57.1)	0	7 (4.5)	
≥5	0	1(20)	4(80)	5 (3.2)	
Medical Insurance					
Yes	1 (1.8)	53 (96.4)	1(1.8)	55 (35.5)	<0.001***
No	41(41)	47 (47)	12 (12)	100 (64.5)	

Data comparisons were done by the Kruskal-Wallis Test, and the association was determined by the Chi-Square test

**** Highly significant, p<0.001*

Association of the Level of Stress with Factors Responsible for Medication Non-Adherence

Numerous non-adherent factors, such as forgetfulness (*p<0.001**), psychological factors like anxiety (*p=<0.001**), behavioral habits like being too busy (*p=0.006*), disease-related factors like stage and severity (*p=0.000*), therapy-related factors like treatment complexity (*p=<0.001**), socioeconomic factors like indirect treatment costs (*p=<0.001**), and social issues like workplace stress and concerns (*p=0.006*), are statistically significantly correlated with stress-levels. (Table: 5)

Table 5: Association between Factors Responsible for Medication Non-Adherence and Level of Stress

	Level of Stress			Total (n=155, %)	P value
	Low Stress (n,%)	Moderate Stress (n,%)	High Stress (n,%)		
Cognitive factors					
Bad Previous experience	5 (35.7)	9 (64.3)	0	14 (9)	0.001***
Religious beliefs'	0	10 (100)	0	10 (6.5)	
Forgetfulness	16 (20.7)	48 (62.3)	13 (16.8)	77 (49.7)	
None of the above	21 (38.9)	33(61.1)	0	54 (34.8)	
Psychological factors					
Fear	6 (31.5)	12 (63.2)	1 (5.3)	19 (12.3)	<0.001***
Obsession	0	4 (100)	0	4 (2.6)	
Anxiety	9 (27.3)	12 (36.4)	12 (36.4)	33 (21.2)	
None of the above	27 (27.3)	72 (72.7)	0	99 (63.9)	
Behavioral factors					
Behavioural habits	9 (45)	7 (35)	4 (20)	20 (12.9)	0.006 **
Lifestyle too busy	16 (20.8)	52 (67.5)	9 (11.7)	77 (49.7)	
None of the above	17 (32.1)	36 (67.9)	0	53 (34.2)	
Anxiety	0	5 (100)	0	5 (3.2)	
Disease-related factors					
Type of disease	34 (50)	26 (38.2)	8 (11.8)	68 (43.9)	<0.001***
Disease stage, severity	1 (3.7)	26 (96.3)	0	27 (17.4)	
None of the above	7 (11.7)	48 (80)	5 (8.3)	60 (38.7)	
Therapy-related factors					
Treatment complexity	21 (31.8)	32 (48.5)	13 (19.7)	66 (42.6)	<0.001***
Treatment Duration	12 (40)	18 (60)	0	30 (19.4)	
Medication Side Effects	9 (15.3)	50 (84.7)	0	59 (38)	
Socioeconomic Factors					
Direct cost of treatment	0	8 (50)	8 (50)	16 (10.3)	<0.001***
Indirect cost of treatment	4 (16.7)	20 (83.3)	0	24 (15.5)	
None of the above	38 (33.1)	72 (62.6)	5 (4.3)	115 (74)	
Social issues					
Concerns about workplace stress	26 (28)	54 (58.1)	13 (13.9)	93 (60)	0.006**
None of the above	16 (25.8)	46 (74.2)	0	62 (40)	

Data comparisons were done by the Kruskal-Wallis test, and association by the Chi-Square test
**** Highly significant, p<0.001*

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Association of Level of Stress to Medication Adherence and Brief Illness Perception

Participants were classified as "adherent" or "non-adherent" to their medicine based on their ARMS score, and their level of stress was separated into three categories: low, moderate, and high. To evaluate the relationship between participants' stress levels and medication adherence (ARMS Score), a cross-tabulation was conducted. Out of the 155, 10participants did not take their medications as prescribed, whereas 145 did. Interestingly, none of the non-adherent participants fell into the low or high stress categories, whereas 100% reported moderate stress. Of the adherent individuals, 90 (62.1%) indicated moderate stress, 13 (9%) reported high stress, and 42 (28.9%) reported low stress. Stress-score and ARMS-score showed weak positive correlation (Spearman's rho=0.066; *p=0.414*), which was non-significant, indicating that as stress increases, ARMS-score slightly increases. (Table: 5)

Table 6 also shows the distribution of participants with positive vs. negative illness perceptions across three stress levels (low, moderate, and high), wherein 76.6% with a positive perception reported moderate stress, whereas 25% of those with a negative perception of illness reported higher stress. There is a weak but statistically significant positive correlation between stress score and Brief Illness Perception score. Individuals with more negative illness perceptions tend to experience slightly higher stress levels (Spearman's rho=0.220; *p=0.006*). (Table: 6)

Table 6: Correlation of Medication Adherence and Brief Illness Perception to Level of Stress

ARMS	Perceived Stress Scale (PSS) (n,%)			Total (n,%)	Spearman's rho	P value	Remarks
	Low Stress	Moderate Stress	High Stress				
Medication Adherence	42 (29)	90 (62)	13(8.9)	145 (93.5)	0.066	0.414	Weak positive but non-significant correlation
Medication Non-Adherence	--	10 (100)	--	10 (6.4%)			
BIPQ							
Positive Perception of the disease	24 (22.4)	82 (76.6)	1 (0.9)	107 (69)	0.220	0.006**	Weak positive but significant correlation
Negative Perception of the disease	18 (37.5)	18 (37.5)	12 (25)	48 (31)			

Data analyzed by Spearman's correlation coefficient test *** = p<0.001*

ARMS - Adherence to Refills and Medication-Scale, PSS - Perceived Stress Scale, BIPQ - Brief Illness Perception Questionnaire

A weak positive correlation between stress score and Perception of disease existed, indicating that as the negative illness-perception score increases, the stress score also increases slightly, and the association is statistically significant.

5. Discussion

Study results showed that participants' socio-demographic factors, gender, smoking status, income rate, and number of chronic diseases did not show any association with stress. Forgetfulness, busy lifestyle, severity of disease, treatment complexities, and concerns at the workplace were the most common factors responsible for non-adherence to medications ($p<0.05$). The majority of participants were adherent to medications, showed moderate stress, and had a positive perception of illness.

Stress is known to contribute to treatment non-adherence, and the socio-demographic factors also influence medication-adherence behaviour[14]. Participants experiencing higher stress had 4.2 times the likelihood of non-adherence compared to those experiencing moderate stress (AOR=4.2, 95%CI: 1.7–10.3; *p=0.002**). Furthermore, non-adherence and moderate stress were significantly correlated (*p=0.036**).

The present study showed a higher non-response rate, as most of the participants did not provide informed consent and failed to meet the predetermined inclusion criteria; thus, a comparatively high exclusion rate of 25.1% was noted. Questionnaire-based studies frequently had to exclude participants due to response fatigue, confidentiality concerns, or time constraints. This is particularly true for studies that focus on sensitive behavioral domains like medication adherence. Furthermore, because those who are less engaged or take their prescriptions less frequently may be less inclined to participate, voluntary participation inevitably carries the potential of non-response bias [17]. To guarantee data quality, dependability, and compliance with ethical research requirements, these responses have to be excluded. However, this exclusion should be taken into account when extrapolating the results because it can reduce the final sample's representativeness and may lead to selection bias.

This study highlighted the predominance of female health care participants in the middle-aged group. It is in contrast to data collected by Karan et al. (2021) [18], which show that while women exceed men in the nurse category, there is a definite numerical superiority of men in the doctor, dentist, and AYUSH categories. The majority of doctors' employment is in the private sector, where physicians are more concentrated in the 50+ age range (18%) than dentists (3%), and nurses (5.5%) are in the same age group. Due to time/access constraints, denial of vulnerability, and masculinity standards, men may be less likely to participate in health surveys and devote time to self-care, which could explain the higher proportion of female participants in the current study. Because male physicians responded less frequently, there is a chance that gender response bias will occur, making physician/HCP surveys less representative.

One significant finding was that HCPs in the younger age group showed higher stress and were negligent about their prescription of medicines. The tendency towards higher non-adherence in younger patients raises the possibility of a generational difference in health attitudes or lifestyle-related restrictions, even if age did not achieve statistical significance ($p=0.082$). According to earlier research (Jimmy & Jose,2011) [19], younger persons frequently put less importance on managing chronic illnesses because they feel invincible or have other obligations. Non-adherence was significantly predicted by being unmarried ($p=0.016$) and working under contract ($p=0.025$). These results are consistent with those of (Gast and Mathes, 2019) [20], who found that poor adherence in populations with chronic illnesses is influenced by social isolation and erratic income sources.

Higher non-adherence was also predicted by level of educational attainment (e.g., MBBS vs. DM/MCh) ($p=0.048$), indicating that medication-taking habits may be influenced by health literacy and perceived treatment value. A patient's educational background frequently influences their comprehension of prescription instructions and the long-term advantages of following those (Unni & Farris, 2011) [21]. These findings therefore imply that healthcare personnel, who are younger, unmarried, less experienced (MBBS), or employed on a contract basis, may be more vulnerable to non-adherence because of greater stress levels. Focused interventions like stress management training, job security, and counselling should be considered to support these groups.

Stress levels were considerably higher among patients without health insurance, those prescribed more than three medications, and those with chronic diseases that persisted longer than three years. Polypharmacy and lack of insurance also turned out to be important factors. These results are in line with past studies (Gast & Mathes, 2019) 20]; DiMatteo, 2004) [22] that highlight regimen complexity and financial load as the main obstacles to adherence. Direct and indirect financial stress exacerbates psychological strain, leading to a vicious cycle of non-adherence and declining health outcomes.

The present study, in addition, demonstrates a significant association between stress levels and multiple factors contributing to medication non-adherence, encompassing cognitive, psychological, behavioral, and disease-related domains. Forgetfulness among cognitive characteristics was observed as a significant factor associated with elevated stress levels. This result is consistent with earlier studies (Jimmy & Jose, 2011) [19] showing that the effect on memory and focus could be associated with stress, resulting in inadvertent non-adherence. Furthermore, religious beliefs were also substantially linked to higher levels of stress. Sometimes, especially when under psychological pressure, patients with strong spiritual or religious beliefs may choose faith-based healing over medications (Kretchy *et al*., 2013) [23].

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Poor medication adherence and higher stress were also closely associated with psychological factors, especially anxiety. This is in line with meta-analytic data (Grenarde *et al*., 2011) [24] that indicate psychological distress, such as depression and anxiety, seriously impairs a patient's capacity to stick to treatment plans. Distress like this can make people less motivated, apathetic, or less confident in their ability to manage a chronic condition (DiMatteo, 2004) [22]. Patients who reported a busy lifestyle or time restrictions were more likely to suffer stress and therefore not comply with their treatment plans, according to behavioral factors. This confirms the results of (Unni and Farris, 2011) [21], who found that the main obstacles to regular medication use were conflicting priorities and lifestyle burden.

There was a substantial correlation found between stress levels and the therapy-related factors that influence non-adherence. If HCPs had negative side effects from their drugs or thought their treatment was challenging, they were more likely to report moderate to higher stress. Side effects and treatment complexity significantly increase stress, potentially leading to non-adherence. There is no pattern of increased stress with longer treatment duration. Higher stress levels were also strongly associated with higher treatment costs, both directly and indirectly.

These findings suggest that stress-linked non-adherence is significantly influenced by financial strain and therapy-related difficulties (Gast & Mathes, 2019) [20]; DiMatteo, 2004) [20]. This is consistent with past research (Jimmy & Jose, 2011) [19] that indicates adverse drug reactions and complex prescription schedules greatly hinder adherence by increasing treatment burden and patient pain. Furthermore, a robust relationship was observed between higher stress and both direct (cost of medications) and indirect (travel and lost pay) financial burden ($p < 0.001$*). According to (Gast & Mathes, 2019 [20]; Park & Iacocca, 2014) [25], financial limitations are one of the most reliable indicators of medication non-adherence, especially for patients with chronic conditions. The protective function of financial security in medication-taking behavior was further supported by the low stress levels of patients who reported no financial difficulties.

Non-adherence was substantially correlated with stress from social and professional demands ($p=0.006$). Environmental stressors, including social and professional obligations, may lead to higher stress levels and may make it difficult to adhere to a plan. Those who reported no such stresses did not experience any high-stress situations, suggesting a strong protective function. According to Unni and Farris (2011) [21], burnout at work or a lack of social support might impair a patient's capacity to prioritize health-related behaviors. There was no correlation between ARMS score and stress score as per the study results. This gradient raises the possibility of a threshold effect, in which low stress is not disruptive, but mild to moderate stress may hinder self-care practices. Hence, the notion that stress acts as a trigger and a modulator in the adherence pathway is supported by these findings (Grenarde *et al*., 2011) [24].

The brief Illness Perception score and the stress score have a weak but statistically significant positive association. This suggests that people who perceive disease more negatively may be associated with slightly greater stress levels. The high correlation between stress and the sense of disease, as determined by the BIPQ, is a convincing conclusion. Stress levels were noticeably greater among patients who had pessimistic, fearful, or hopeless views about their disease. This supports the theory put forward by Park and Iacocca (2014) [25], according to which emotional reactions and coping mechanisms, such as adherence, are mediated by cognitive assessment of illness. Therefore, encouraging a more optimistic and controllable perspective related to disease could be a crucial intervention strategy for lowering stress and enhancing adherence.

Study limitations and strengths:

Very few qualitative studies, to the best of our knowledge, were undertaken to record compliance of HCPs with their own prescription orders or with prescriptions by other specialists. It highlighted that the medical fraternity, in spite of being aware of the consequences of medication non-adherence, shows reluctance to adhere due to various factors that can be corrected by their own expertise. The study has given an insight into how younger HCPs are experiencing a higher level of stress. It may possibly be linked to an imbalance between their disease and health status due to inadequate psychological and financial support extended by the organizations.

The present study had a few major limitations related to the generalization of the results,

considering the qualitative nature of the study. This study was designed to explore HCPs' perspectives on the factors affecting the adherence and non-adherence of medications of their self-therapeutic orders and to their fellow prescribers. One of the most important limitations in the study was the loss of sample size due to insufficient responses, lack of consent, or inability to meet the inclusion criteria. Approximately 25.1% of participants were excluded from the study. Although exclusion may have a minor impact on generalizability, it was required to maintain data integrity and ethical compliance.

Despite careful sample size estimation, the final number of participants included in the analysis (n = 155) was lower than the minimum calculated requirement of 176 to achieve adequate statistical power. This deficit was primarily due to non-consent and exclusions based on eligibility criteria. As a result, the study may have been underpowered to detect certain associations, thus increasing the risk of a Type-II error (false negative). Consequently, some correlations, such as the relationship between medication adherence (ARMS scores) and perceived stress (PSS scores), may have appeared weak or statistically non-significant ($p = 0.414$) despite the possibility of an underlying true association. Hence, future research with larger, adequately powered samples and strategies to reduce non-response rates is recommended to validate and strengthen these findings.

Another limitation was the Hamilton Anxiety Scale (HAM-A), which rates the level of anxiety based on clinical questions, and the Numerical Rating Scales (NRS), where participants can rate their fear level on a scale (e.g., 0 to 10) in response to stimuli or events, were not included. Study highlights only the subjective means of assessment for anxiety and fear, whereas the addition of these scales would have given a better understanding of these factors in a more objective manner. The assessment of psychological variables, including anxiety and fear, was mostly subjective, despite the use of established tools such as the Brief Illness Perception Questionnaire (BIPQ), Perceived Stress Scale (PSS), and Adherence to Refills and Medications Scale (ARMS). Social desirability bias, reporting bias, and inter-individual differences in emotional Perception are all inherent risks of self-reported assessments. This reduces the objectivity of psychological evaluation and could affect the correlation between medication non-adherence and perceived stress or disease. Therefore, it is essential that both subjective and objective evaluations can be done to offer a more thorough analysis of participants' subjective sentiments.

Hence, future studies with a larger sample size taken from major tertiary care centres across the local region, along with subjective and objective evaluation of factors affecting medication adherence and their correlation to stress, are recommended, as they would give a better understanding of the correlation between factors for medication non-adherence and workplace stress.

6. Conclusion

The present study, therefore, emphasizes the necessity of multifaceted therapies that focus on adherence support as well as stress reduction in HCPs. Stress-related obstacles might be lessened by addressing side effects, streamlining treatment plans, and providing financial support by means of providing health insurance coverage to HCPs. In order to promote more adaptive attitudes and behaviors, physicians should simultaneously evaluate and alter perspectives of their own condition through psycho-education and motivational therapies. Improving long-term results for HCPs with chronic illnesses requires comprehensive treatment models that incorporate socioeconomic support and psychological assessment programs to improve compliance with treatment. Adequate notification about diseases and their treatments in younger physicians can be useful to promote the individuals' health literacy and knowledge. Promoting concordance between healthcare providers and fellow physicians through shared decision-making and mutual understanding of treatment goals can enhance medication adherence and foster a more collaborative approach to disease management. These approaches can help to reduce non-adherence, especially in younger HCPs. In addition, providing insurance is one important factor that policymakers should pay attention to when they decide to improve the service provision system.

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CONFLICT OF INTEREST:
The authors have no conflicts of interest.

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TP53 Gain-of-Function Mutations and Metabolic Adaptation in Prostate Cancer: A Comprehensive Review

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ABSTRACT

The tumor suppressor TP53 is frequently mutated in advanced prostate cancer, and certain TP53 gain-of-function (GOF) variants paradoxically promote tumor growth by acquiring oncogenic activities that reprogram cellular metabolism, enhance proliferation, and drive therapeutic resistance. This review synthesizes current mechanistic and translational evidence linking TP53-GOF mutations (notably R175H, R273H, and related hotspot variants) to metabolic rewiring in prostate cancer, including altered glucose handling, lipid and cholesterol metabolism, and amino acid-dependencies such as asparagine biosynthesis. It highlights how these changes create targetable vulnerabilities. We place particular emphasis on (i) molecular routes by which mutant p53 acquires new activities (dominant-negative effects, altered DNA binding, and novel protein–protein interactions), (ii) mutation-specific versus shared GOF phenotypes in metabolic pathways, and (iii) clinical translation, from small molecules that reactivate or destabilize mutant p53 (e.g., APR-246 / eprenetapopt and aggregation-disrupting peptides) to metabolic strategies that exploit mutant p53 dependencies (for example, co-targeting asparagine biosynthesis). We critically appraise the preclinical and early clinical evidence, identify important gaps (heterogeneity of mutation effects, limited clinical validation, and the interplay between TME and metabolism), and propose prioritized experimental and clinical strategies to accelerate translation. By integrating mechanistic insight with emerging therapeutic approaches, this review aims to provide a concise roadmap for leveraging mutant-p53-driven metabolic liabilities in lethal, therapy-resistant prostate cancer.

KEYWORDS

TP53 gain-of-function mutation, prostate cancer, metabolic adaptation, asparagine synthetase (ASNS), squalene epoxidase (SQLE), androgen receptor resistance, tumor microenvironment, biomarker-driven therapy.

1. Introduction

Prostate cancer remains a leading cause of cancer morbidity and mortality among men worldwide and is responsible for a substantial fraction of cancer deaths in high-income countries. Population and registry data document rising numbers of advanced and therapy-resistant cases, and integrated genomic studies have repeatedly identified TP53 alteration as a key event associated with disease progression and poor outcome (1).

TP53 encodes the p53 protein, a multifunctional tumor suppressor that coordinates DNA damage responses, cell cycle checkpoints, apoptosis, and various aspects of cell metabolism. In cancer, non-synonymous TP53 mutations commonly produce stable, aberrant p53 proteins that not only lose their canonical tumor-suppressor activity but, in many cases, display gain-of-function (GOF) properties, acquiring novel oncogenic activities that actively promote malignancy. GOF mechanisms include (i) dominant-negative inhibition of wild-type p53 (when heterozygous), (ii) altered DNA-binding specificity that reprograms transcriptional networks, (iii) neomorphic protein–protein

interactions that engage oncogenic transcription factors and chromatin remodellers, and (iv) biochemical behaviors such as aggregation or altered isoform expression that create new cellular phenotypes. For a modern synthesis of these molecular mechanisms, see Chen et al. (2022) and related reviews (2,3).

A central and emerging theme is that many GOF p53 mutants rewire cancer cell metabolism in ways that promote survival under stress and create targetable dependencies. These metabolic effects are multifaceted: mutant p53 can shift glycolysis/mitochondrial balance, alter lipid and cholesterol synthesis, and change amino-acid handling; thereby supporting anabolic growth, redox balance, and therapy resistance. Importantly, several recent studies report mutation and context-specific outcomes rather than a single uniform metabolic program: for example, TP53-altered castration-resistant prostate cancers show upregulation of asparagine biosynthesis (ASNS) and functional dependency on asparagine in preclinical models (a therapeutic vulnerability identified in 2024), whereas other work highlights p53-dependent control of cholesterol biosynthesis via SQLE that connects p53 status to sterol metabolism and tumour growth (4–6).

Despite an expanding literature, the field faces important gaps and that this revised review aims to address: (i) many prior reviews summarize studies without critically synthesizing whether different TP53 hotspot mutations (e.g., R175H vs R273H vs R248W) cause overlapping or distinct metabolic phenotypes; (ii) mechanistic depth is often uneven; pathways (PI3K/AKT, Myc, AMPK, STAT3, etc.) are listed without clear molecular routes connecting mutant p53 to specific metabolic enzymes or transporters; and (iii) interactions with the tumour microenvironment (immune cells, stromal metabolism, nutrient competition) are underrepresented despite their likely importance to clinical translation.

In response to these gaps, this review (i) defines and exemplifies GOF mechanisms of mutant p53, (ii) organizes current data on how GOF TP53 mutants reprogram glucose, lipid/cholesterol and amino-acid metabolism in prostate cancer: distinguishing mutation-specific findings where possible, (iii) examines cross-talk with the tumour microenvironment and implications for immune and stromal compartments, and (iv) evaluates translational strategies (p53-reactivating agents, metabolic enzyme inhibitors, and combination approaches), highlighting outstanding questions and concrete experimental/clinical priorities. Subsequent sections synthesize mechanistic data, critically discuss controversies, and propose prioritized next steps for preclinical and clinical validation.

2. Methodology

2.1 Protocol and reporting

This review was conducted and reported following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) principles to ensure transparency and reproducibility. Where applicable, recommendations from the Cochrane Handbook for Systematic Reviews of Interventions were applied to study selection, data extraction, and synthesis. A PRISMA flow diagram and the full PRISMA checklist are provided in the Supplementary Materials.

2.2 Eligibility criteria

We included peer-reviewed primary research articles, preprints (clearly identified), reviews providing novel synthesis, and clinical-trial reports that addressed relationships among TP53 / mutant-p53, prostate cancer biology, metabolism, therapeutic strategies, or tumor microenvironment interactions. Studies were eligible if they provided original data on one or more of the following: (a) molecular mechanisms of TP53 gain-of-function (GOF) or loss-of-function (LOF) in prostate cancer, (b) metabolic reprogramming linked to TP53 alterations (glucose, lipid/cholesterol, amino-acid metabolism), (c) preclinical/intervention studies testing metabolic or p53-targeted agents, or (d) translational/clinical evidence (biomarker, ctDNA, trial outcomes) relevant to TP53 strata. Exclusion criteria: non-English language papers without an English abstract (unless a translation was provided), conference abstracts without accessible data, and purely computational/modeling studies without experimentally validated data (exceptions noted case-by-case). For preclinical assays, the focus was on studies in prostate cancer models (cell lines, organoids, PDXs, genetically engineered mouse models); when mechanisms were demonstrated in other tumor types, we flagged them as supportive but down-weighted them in synthesis.

2.3 Information sources and search strategy

We searched the following bibliographic databases from inception to 30 September 2025: PubMed/MEDLINE, Embase, Web of Science, Scopus, and the Cochrane Library. Trial registries (ClinicalTrials.gov, EU Clinical Trials Register)

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were checked for ongoing/terminated trials of p53-targeted or metabolic agents. Preprint servers (bioRxiv, medRxiv) were searched, and preprints were included but flagged as non-peer-reviewed. Reference lists of key reviews and included articles were hand-searched for additional studies.

A representative search string for PubMed (adapt to other databases and report full strings in Supplementary Table S1) was:

("TP53" OR "p53" OR "mutant p53" OR "p53 mutant")

AND ("prostate cancer" OR "prostatic neoplasm" OR "castration-resistant prostate cancer" OR "CRPC")

AND ("metabolism" OR "metabolic" OR "glycolysis" OR "oxidative phosphorylation" OR "OXPHOS" OR "lipid" OR "cholesterol" OR "asparagine" OR "ASNS" OR "SQLE" OR "asparaginase")

All database searches and date ranges, plus the full, reproducible search strings for each platform, are provided in Supplementary Table S1.

2.4 Study selection and screening

Search results were imported into a reference manager and deduplicated. Title/abstract screening was performed in the Covidence platform by two independent reviewers (F.J.U. & K.B) against the eligibility criteria. Full-text screening of selected records was also performed independently by two reviewers; disagreements were resolved by discussion and by adjudication with a third senior reviewer (A. J. A). Reasons for exclusion at the full-text stage are reported in the PRISMA flow diagram (Supplementary Fig. S1). For transparency, a table of excluded full texts with reasons is provided in the Supplementary Materials.

2.5 Data extraction

A standardized extraction form was developed and piloted on a sample of included studies. For each study we extracted: author, year, study type (in vitro, in vivo, clinical), model system (cell line with parental background, organoid, PDX, GEMM), TP53 status/allele (if reported), experimental interventions (drugs, genetic perturbations), key metabolic endpoints (e.g., ECAR/OCR, 13C flux results, sterol profiling), outcome measures (proliferation, apoptosis, tumor growth, PSA response), sample sizes/replicates, and main conclusions/limitations. For clinical studies, we additionally extracted patient numbers, line of therapy, biomarker methods (tumor sequencing/ctDNA), safety, and efficacy endpoints. When necessary, corresponding authors were contacted for missing or clarifying data; contact attempts and outcomes are recorded in Supplementary Table S2.

2.6 Quality assessment and risk-of-bias appraisal

Given the heterogeneous nature of the literature (preclinical mechanistic studies, animal models, and clinical reports), we applied distinct, appropriate risk-of-bias tools:

- **Clinical intervention and observational studies:** assessed using the Newcastle–Ottawa Scale (for cohort/case-control designs) or an appropriate Cochrane risk-of-bias tool for randomized trials. Where adequate, we applied the GRADE approach to judge the overall certainty of evidence for clinically relevant outcomes.

- **Animal studies:** assessed using the SYRCLE risk-of-bias tool adapted for preclinical in vivo experiments (randomization, blinding, outcome reporting, sample-size calculation).

- **In vitro / mechanistic studies:** because no single validated universal tool exists for bench studies, we used a pragmatic checklist adapted from best-practice recommendations: (i) clear reporting of cell-line provenance and authentication, (ii) use of appropriate controls (WT, null, isogenic alleles), (iii) reporting of biological vs technical replicates and sample sizes, (iv) independent validation in orthogonal models (organoid/PDX) where available, and (v) appropriate statistical testing. Each study received a qualitative rating (low/moderate/great concern) for internal validity on the adapted checklist.

Two reviewers independently assessed risk-of-bias; disagreements were resolved by consensus. Summary risk-of-bias tables and study-level ratings are provided in Supplementary Tables S3–S5.

2.7 Data synthesis

Because the included literature combined mechanistic laboratory studies and heterogeneous clinical reports, the primary synthesis was narrative and thematic: we grouped findings into metabolic axes (glucose/OXPHOS, lipid/cholesterol, amino-acid metabolism), allele-specific phenotypes (contact vs conformational mutants and common hotspot alleles), TME interactions, and translational implications.

For preclinical experimental outcomes that reported comparable quantitative metrics (for example, ECAR, OCR, tumor volume change), we considered pooled analyses where appropriate; a formal meta-analysis was performed only when ≥3 comparable, homogeneous data sets with extractable numeric outcomes were available. Heterogeneity for pooled analyses would be quantified using I^2 statistics and random-effects models (DerSimonian–Laird or REML) implemented in R (metafor) or RevMan; sensitivity and subgroup (allele, model type) analyses were pre-specified. For small-study or mechanistic datasets without comparable metrics, we summarized direction and effect size qualitatively and highlighted consistency across models.

2.8 Preclinical evidence appraisal and translational grading

For translational recommendations, we graded the preclinical evidence for each candidate vulnerability (e.g., ASNS/asparagine, SQLE/cholesterol) using a pragmatic three-tier scheme:

- **Tier 1 (High translational priority):** replicated in ≥2 model systems (isogenic lines + organoid or PDX), mechanistic causality demonstrated (genetic + pharmacologic perturbations with rescue), and at least one correlative clinical observation (tumor expression or ctDNA association).

- **Tier 2 (Moderate):** reproduced in multiple in vitro models and one in vivo model, but with limited clinical correlation.

- **Tier 3 (Exploratory):** single-model evidence or primarily observational signal without mechanistic perturbation.

This grading guided prioritization of candidate combinations and the proposed early-phase trial designs in Section 3.4.

2.9 Software and reproducibility

Data management and analyses were performed using EndNote/Zotero (reference management), Rayyan (screening), Excel/Google Sheets (data extraction templates), and R (version unspecified in text; specify exact version in final manuscript) or RevMan for any quantitative synthesis. All extraction templates, analytic scripts, and the full search strings are provided in the Supplementary Materials and will be shared publicly on request (or deposited in an open repository such as GitHub/Zenodo) to facilitate reproducibility.

2.10 Limitations of the methods

We acknowledge several methodological constraints: (i) inclusion of heterogeneous preclinical and clinical studies limits the ability to perform comprehensive quantitative meta-analysis; (ii) potential publication bias toward positive mechanistic findings in preclinical work; (iii) language restriction to English may omit relevant non-English studies; and (iv) inclusion of preprints introduces non-peer-reviewed evidence that was labelled and interpreted with caution. These limitations were considered when grading translational priority and making recommendations.

3. Discussion

3.1 TP53 mutation and metabolic alteration in prostate cancer

Mutations in TP53 commonly produce stable mutant-p53 proteins that not only lose canonical tumor-suppressor functions but also gain oncogenic activities that reprogram cellular metabolism to support survival, proliferation, and therapy resistance. These *gain-of-function* (GOF) activities occur through several mechanisms: dominant-negative inhibition of remaining wild-type p53, altered DNA-binding/transcriptional programs, novel protein–protein interactions (for example, with transcription factors or metabolic regulators), and non-transcriptional interactions with metabolic enzymes and sensors. The literature shows that GOF mutant p53 rewires multiple metabolic axes in a context-dependent and mutation-specific manner(3).

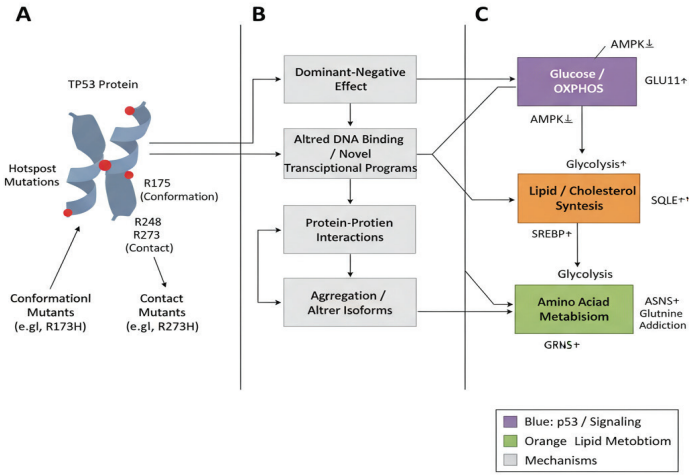

Figure 1: Allele-aware mechanisms by which mutant p53 reprograms tumor metabolism.

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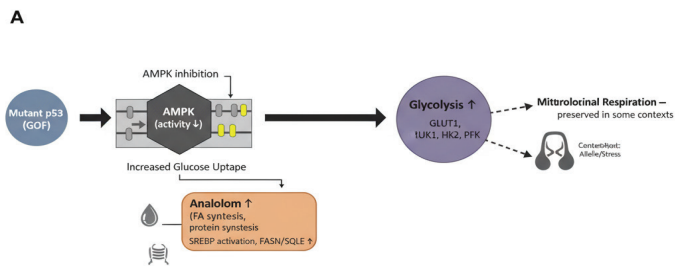
Schematic overview of TP53 gain-of-function mechanisms and their impact on tumor metabolic pathways, including glucose/OXPHOS balance, lipid/cholesterol synthesis, and amino acid metabolism.

3.1.1 Glucose handling, glycolysis, and mitochondrial metabolism

GOF mutant p53 promotes glycolytic reprogramming (the "Warburg effect") while also enabling metabolic plasticity that preserves mitochondrial fitness under stress. Mechanistically, mutant p53 has been shown to (i) increase glucose uptake by promoting GLUT1 translocation via RhoA/ROCK signalling, thereby enhancing aerobic glycolysis; (ii) interact with and activate transcriptional programs (directly or via partners) that upregulateupregulate glycolytic enzymes and regulators; and (iii) paradoxically enhance mitochondrial oxidative phosphorylation (OXPHOS) in certain contexts by stabilizing PGC-1α or other mitochondrial effectors: a dual program that permits adaptation to fluctuating nutrient/oxygen supply and supports metastasis. These basic points are supported by genetic and functional studies in cell lines and mouse models showing that mutp53 both stimulates glycolysis and, depending on the allele and context, preserves mitochondrial capacity to favor invasion/metastasis(7).

At the signaling level, one clear route is mutant-p53 inhibition of the energy sensor AMPK: several GOF p53 variants bind AMPKα and prevent its activation under energy stress, removing a brake on anabolic metabolism (fatty-acid synthesis, protein synthesis) and blunting autophagy/mitophagy responses that would otherwise constrain tumor growth. This transcription-independent interaction explains how mutp53 can acutely shift metabolic setpoints in energy stress conditions (8).



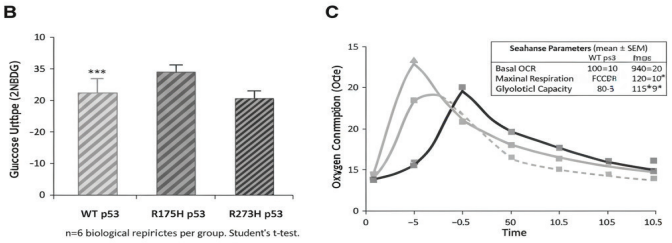


Figure 2: GOF p53 promotes glucose uptake and glycolysis through AMPK inhibition while preserving mitochondrial respiration in specific contexts.

(A) Schematic representation showing how gain-of-function (GOF) mutant p53 inhibits AMPK activity, leading to increased glucose uptake, enhanced glycolysis, and anabolic lipid synthesis via SREBP-mediated activation of FASN and SQLE. (B) Representative 2-NBDG assay illustrating elevated glucose uptake in R175H and R273H mutants compared with wild-type p53 (n = 6 biological replicates per group; Student's t-test). (C) Seahorse extracellular flux analysis demonstrating altered oxygen consumption and glycolytic capacity in mutant-p53–expressing cells. Data are presented as mean ± SEM. P < 0.05, P < 0.01, P < 0.001 vs. WT p53.

Translational note. Because mutant p53 enables both high glycolytic flux and preserved mitochondrial function, single-agent metabolic inhibitors (glycolysis *or* OXPHOS alone) may be insufficient; preclinical data support rational combination strategies (for example, glycolysis inhibitors with mitochondrial poisons or with modulators of AMPK signaling) (9).

3.1.2 Lipid and cholesterol metabolism

Mutant p53 often moves lipid metabolism toward anabolic lipid synthesis and cholesterol accumulation; metabolic programs that supply membranes for proliferation and generate signaling lipids that promote survival. A mechanistic exemplar is p53 regulation of squalene epoxidase (SQLE): wild-type p53 represses SQLE transcriptionally, decreasing cholesterol synthesis, whereas p53 loss or certain GOF contexts lead to increased SQLE activity and higher sterol production that supports tumor growth. Pharmacologic SQLE inhibition (e.g., terbinafine) reduces proliferation in p53-deficient models, indicating a therapeutically actionable axis (5).

Mutant p53 also cooperates with oncogenic drivers (MYC, PI3K/AKT) to enhance lipogenesis (acetyl-CoA and NADPH supply) and fatty-acid desaturation, producing membranes for rapid proliferation and lipid signaling that promotes invasion. Because prostate tumors are frequently lipogenic, these p53-driven shifts have particular

relevance to prostate cancer biology (9).

Translational note. SQLE and downstream cholesterol esterification enzymes represent candidate metabolic targets in TP53-altered prostate tumors, but patient-level validation (correlating TP53 alleles with SQLE expression/activity and response to inhibitors) is currently limited and a priority for clinical translation (5).

3.1.3 Amino-acid metabolism: asparagine, aspartate, and others

Recent work has defined a compelling amino-acid vulnerability in TP53-altered castration-resistant prostate cancer (CRPC): increased *asparagine synthetase* (ASNS) expression creates dependence on de novo asparagine biosynthesis and flux through asparagine–aspartate homeostasis. Yoo et al. (2024) combined transcriptomics, metabolomics, and functional perturbations to show that TP53-altered CRPCs upregulate ASNS and are sensitive to strategies that reduce intracellular and extracellular asparagine (genetic ASNS suppression, asparaginase, or combination approaches). This represents a concrete metabolic vulnerability tied to TP53 alterations and validates earlier mechanistic links between p53, ASNS transcriptional control, and stress responses (4).

Broader amino-acid control is also implicated: mutant p53 affects serine/glycine and glutamine pathways (through transcriptional and indirect regulatory networks), and p53 status alters aspartate/asparagine availability that feeds nucleotide synthesis and redox balance. However, the degree to which these dependencies are allele-specific (different for R175H vs R273H, etc.) remains incompletely resolved (3).

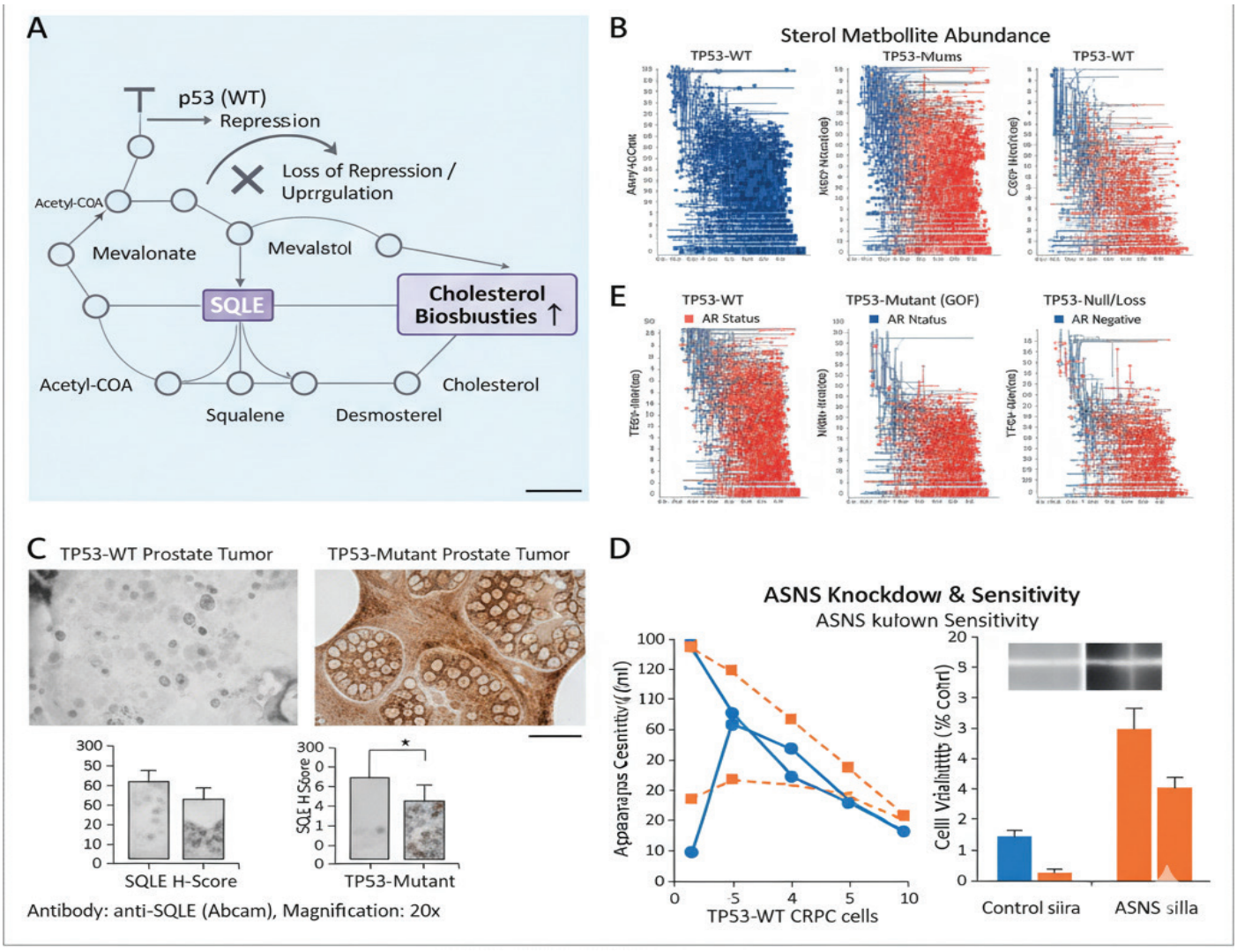


Figure 3: Lipid/cholesterol and amino acid rewiring in TP53-altered prostate cancer.
(A) Schematic illustrating how the loss of wild-type p53 (WT) or the presence of a gain-of-function (GOF) mutant p53 represses the enzyme SQLE, leading to a downstream cholesterol enrichment in TP53-altered prostate tumor cells.

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Translational note. The ASNS/asparagine axis is a high-priority target for translation in TP53-altered CRPC; clinical strategies could combine asparaginase or ASNS inhibitors with ARSIs or p53-reactivating agents, but toxicity and tumor heterogeneity must be addressed (4).

3.1.4 Mutation specificity, context dependence, and the tumor microenvironment (TME)

A recurring theme and a major reviewer point is that GOF effects are not uniform: different hotspot mutations (conformational vs contact mutants; e.g., R175H vs R273H) can produce overlapping but also distinct transcriptional and non-transcriptional outputs that alter metabolic phenotypes. Some alleles preferentially engage transcriptional reprogramming (e.g., forming aberrant complexes with NF-Y or SREBP family factors), while others act via cytosolic interactions (for example, with AMPK) or by forming aggregation-prone oligomers that sequester partners. The literature, therefore, argues for allele-aware analyses (cell models and patient cohorts stratified by specific TP53 alleles) rather than collapsing all TP53 alterations into a single group (10).

The TME (immune cells, fibroblasts, adipocytes, vascular supply) further modifies metabolic dependencies: mutant p53 can alter tumor–stromal signaling and cytokine profiles that change nutrient availability and immune cell metabolism, while nutrient competition in the TME can amplify tumor-intrinsic dependencies (for example, asparagine exported/imported between compartments). These bidirectional interactions make ex vivo and in vivo models (co-culture, organoids, PDXs) essential for translational validation beyond cell-line mechanistic work.

3.2 Proliferation and drug resistance driven by TP53 mutation in prostate cancer

3.2.1 Mutant-p53 mechanisms that directly increase proliferation

Mutant p53 proteins promote tumor cell proliferation by acquiring new biochemical activities that reprogram transcriptional networks, alter cell-cycle control, and blunt apoptotic responses. Mechanisms include: (i) transcriptional activation of pro-growth programs via novel interactions with transcription factors and chromatin remodelers; (ii) dominant-negative inhibition of any residual wild-type p53, removing cell-cycle checkpoints; (iii) stabilization of oncogenic signaling cascades such as PI3K/AKT and Myc; and (iv) direct non-transcriptional modulation of cell-cycle kinases and checkpoint proteins (for example, by interfering with AMPK and DNA-damage signaling). Together, these actions shorten G1/S control, increase S-phase entry, and reduce programmed cell death: molecular outcomes that accelerate tumor growth and increase clonogenic survival (3).

Practically, these activities manifest as increased proliferation indices, higher Ki-67, and enhanced clonogenicity in cell lines and xenografts expressing hotspot GOF alleles (for instance, R175H, R273H), supporting the concept that mutant p53 is an active driver rather than a passive marker of aggressive disease (11).

3.2.2 Mutant p53 and resistance to androgen receptor-targeted therapies (ARSIs)

Clinical and translational data increasingly link TP53 alteration to poor response and earlier progression on androgen receptor signaling inhibitors (enzalutamide, abiraterone) and castration-resistant evolution. TP53 mutation correlates with worse outcomes and shorter progression-free intervals following ARSI therapy, although the effect size is modulated by co-occurring alterations and tumor stage. Mechanistically, mutant p53 contributes to ARSI resistance through several, not mutually exclusive routes: (i) promoting lineage plasticity and neuroendocrine trans-differentiation that bypasses AR dependency; (ii) cooperating with transcriptional and epigenetic regulators to maintain alternative growth programs; and (iii) enabling survival under androgen deprivation by enhancing metabolic plasticity and DNA-repair adaptations. These mechanisms help explain why TP53 alterations are over-represented in more therapy-resistant, advanced prostate cancers (12).

Because ARSI resistance is multifactorial, TP53 status alone is not a perfect predictor, but in combination with RB1 and PTEN loss (the AVPC signature), it defines a high-risk group more likely to exhibit lineage plasticity and rapid progression. This highlights the need for multi-gene biomarker panels (including ctDNA) to stratify patients in trials (12).

3.2.3 Mutant p53 and resistance to cytotoxic and targeted agents

Mutant p53 promotes resistance to DNA-damaging chemotherapies and to certain targeted agents by diminishing apoptosis and altering DNA-damage response (DDR) pathways. GOF alleles can (i) down-regulate pro-apoptotic mediators and upregulate survival factors, (ii)

rewire DDR signaling to tolerate replication stress, and (iii) engage antioxidant and metabolic programs that blunt therapy-induced stress. Preclinical studies also show that mutant p53 can reduce sensitivity to mitotic kinase inhibitors and other targeted compounds, sometimes via allele-specific interactions, meaning p53 status can condition not only chemosensitivity but also responses to newer targeted agents (13).

The complete loss of TP53 and specific gain-of-function (GOF) missense mutations can lead to different treatment responses. For instance, some mutations mainly enhance resistance to cell death, while others activate gene programs that strengthen DNA repair. Therefore, grouping all 'TP53-mutant' tumors can hide important allele-specific differences in their clinical behavior (3).

3.2.4 Co-occurring tumor-suppressor losses, lineage plasticity, and aggressive variant prostate cancer (AVPC)

TP53 mutation frequently co-occurs with loss of RB1 and PTEN in aggressive prostate cancer phenotypes. The combined loss of these tumor suppressors promotes lineage plasticity, a shift from AR-dependent luminal epithelial programs to AR-indifferent or neuroendocrine-like states, which confers intrinsic resistance to ARSIs and often to conventional cytotoxics. Clinically, AVPC, defined by defects in RB1/PTEN/TP53, is associated with rapid progression and poor prognosis; mechanistically, concurrent pathway losses amplify chromatin and transcriptional reprogramming that enables adaptive survival programs. Therefore, therapeutic strategies for TP53-altered tumors must consider co-alterations and the resulting plasticity phenotype (12).

3.2.5 Therapeutic implications and candidate strategies

Given the central role of mutant p53 in proliferation and resistance, several therapeutic approaches merit priority testing:

1. **Direct reactivation/destabilization of mutant p53:** Small molecules that covalently modify mutant p53 (e.g., APR-246/eprenetapopt) or peptides that disrupt mutant-p53 aggregation (ReACp53) can restore tumor-suppressor function or reduce oncogenic activity in preclinical models and early-phase trials. APR-246 has shown clinical activity in hematologic malignancies and is in multiple combination trials; ReACp53 showed promising preclinical activity in prostate cancer models by reversing aggregation and restoring p53 function. Translating these agents to prostate cancer (especially allele-stratified cohorts) is a rational next step (14).
2. **Synthetic-lethal and pathway-targeting approaches:** Exploiting vulnerabilities that arise when p53 function is compromised: for example, targeting cell-cycle kinases (PLK1, WEE1), checkpoint kinases (CHK1), or altered metabolic dependencies (asparagine/ASNS, SQLE), can selectively kill TP53-altered cells. Preclinical data support PLK1/WEE1/CHK1 inhibition in TP53-compromised contexts, but the efficacy can depend on the specific allele and co-mutations, so preclinical validation in allele-aware models is essential (15).
3. **Combination strategies with ARSIs and DNA-damage agents:** Combining mutant-p53 reactivators or synthetic-lethal drugs with ARSIs, PARP inhibitors (in DNA-repair-deficient contexts), or chemotherapy may overcome resistance phenotypes. Rational combos should be guided by molecular biomarkers (TP53 allele, RB1/PTEN status, DDR markers) and validated in organoids/PDXs with preserved TME interactions (12).
4. **Biomarker-driven trial design and ctDNA monitoring:** Because allele specificity and co-alterations critically affect phenotype, clinical trials should use ctDNA or tumor sequencing to enroll and stratify patients by TP53 allele and co-occurring alterations. Window-of-opportunity trials with pre-/post-biopsies and metabolic/flux readouts will accelerate translation (12).

3.2.6 Prioritized experimental needs

1. **Allele-aware preclinical pipelines.** Use isogenic panels with major hotspot alleles (R175H, R248W, R273H) and patient-derived organoids/PDXs to test therapy responses and identify allele-specific synthetic lethals (11).
2. **Modeling co-alterations.** Explicitly test TP53 alterations together with RB1/PTEN loss to model AVPC biology and therapy resistance (12).
3. **Integrated biomarker strategies.** Develop ctDNA assays for TP53 allele tracking, combine with functional biomarkers (IHC for p53, Ki-67, SQLE/ASNS; metabolic flux/

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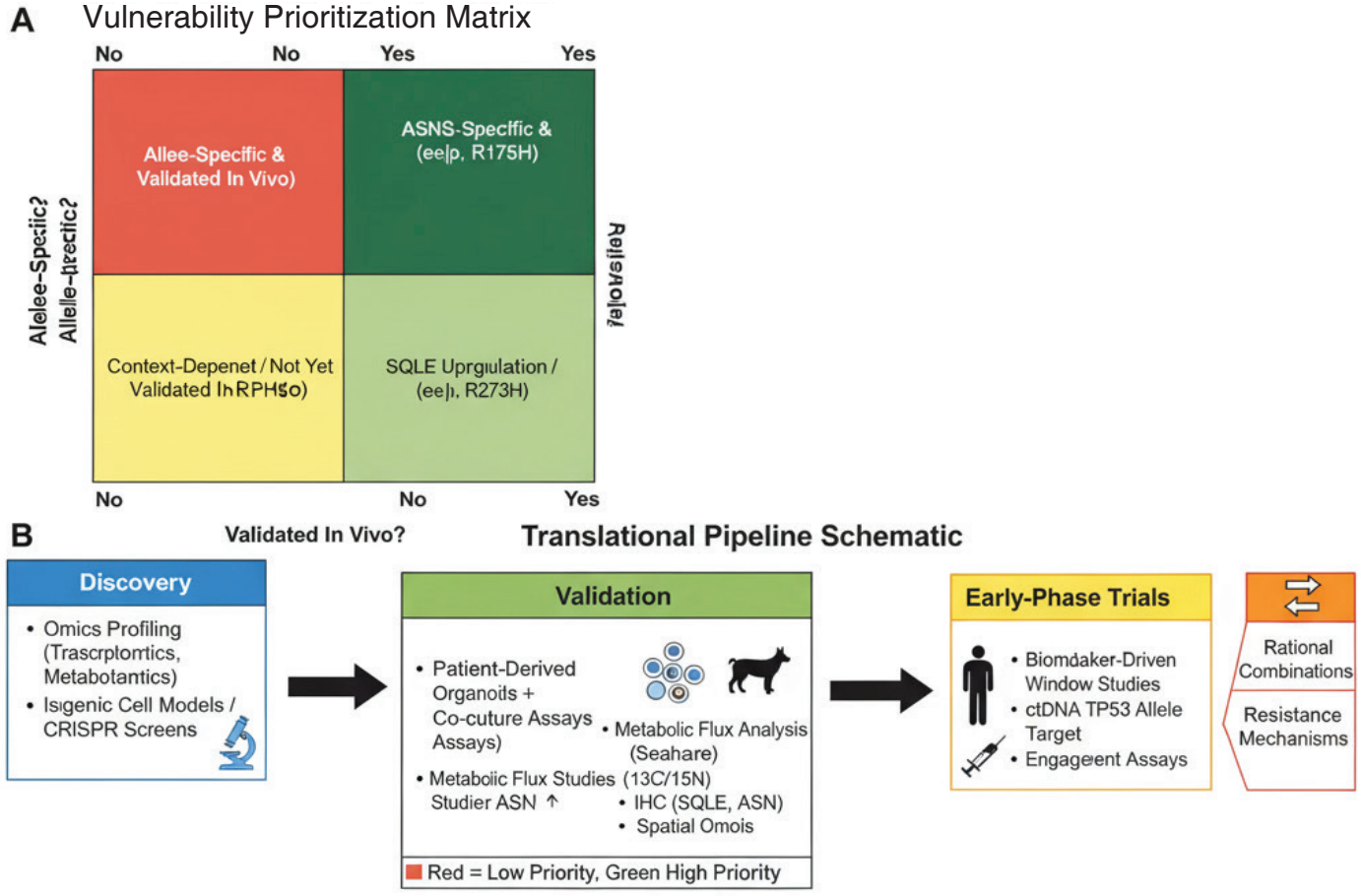
tracer studies) in early-phase trials to link mechanism to clinical signal (4–6,16).



C Candidate Therpauetics & Combinations

Drug Candidate	Mechanism	Clinical Status	Potential Combinations	Biomamer / Context
Asparaginasse	ASNS Inhibition	Phase 1/2 Solid Turnos)	ASNS High, Chemo	APHS High, Chemo
SQLE Inhbitor (ee.þ. **Lovastatin**		Repurossing / Pre-crinical	Phase 2/3 (MDS, Ov Ca)	SQLE High, Azi-CIR.
SQLE Inhibitor (Cholse Synth. Blockade		Asparagianited Axis Inh.	SQLE High (MDS, Ov Ca)	APR-A-tjitat ?
Azi-CDR (Azacicltine+Dectabialion (Hecitaibhated)		FDA-Approved (MB{, AML	FDA-Approved (all)	DDA-Mut, DNA Hyperphhated
Venetoloax		BCL-2 Inhibition	F53-High, (CLL, AML	BCL-Mutt, BCL2 High

Figure 4: Translational strategies and prioritized experimental pipeline

3.3 Tumor microenvironment (TME), immune–metabolic crosstalk, and mutant-p53

Mutant p53 reshapes the tumor microenvironment through both cell-intrinsic and secreted factors that together create an immunosuppressive, metabolically altered niche favoring tumor survival and therapy resistance. Mechanisms include (i) altered tumor secretome and exosome content that reprogram neighboring stromal and immune cells, (ii) transcriptional induction of chemokines and cytokines that favor suppressive myeloid populations, (iii) metabolic competition for nutrients (glucose, amino acids such as asparagine) and accumulation of immunosuppressive metabolites (lactate), and (iv) modulation of antigen presentation and immune-checkpoint pathways. These TME effects magnify the clinical impact of TP53 alterations and are therefore critical for translational strategies (17).

3.3.1 Mutant-p53, the secretome, and stromal remodeling

Mutant p53 alters the tumor secretome (cytokines, chemokines, growth factors, and extracellular vesicle cargo) in ways that promote cancer-associated fibroblast (CAF) activation, extracellular matrix remodeling, and pro-

tumorigenic inflammation. Recent literature shows that mutant-p53 can drive the secretion of factors that convert stromal fibroblasts to a more supportive phenotype and may alter ECM stiffness and collagen deposition, changes that favor invasion and therapeutic resistance. These secretome changes also modify local nutrient handling (for example, CAFs can supply metabolites to tumor cells), creating a reciprocal metabolic crosstalk that strengthens mutant-p53–driven metabolic programs (17).

3.3.2 Immune suppression: myeloid cells, T cells, and checkpoint biology

Multiple studies indicate that TP53 mutations promote an immunosuppressive microenvironment. Mechanisms include upregulation of immunosuppressive chemokines and PD-L1 in some contexts, reduced antigen presentation, and recruitment/education of suppressive tumor-associated macrophages (TAMs) and myeloid populations. Exosome-mediated delivery of mutant-p53 or related factors can directly impair T cell function and promote regulatory programs in macrophages, reducing antitumor immunity and limiting checkpoint inhibitor efficacy in preclinical models. These effects help explain clinical observations linking TP53 alterations to poor immunotherapy responsiveness in some tumor types and argue for combining metabolic or p53-targeted therapy with immune modulation in TP53-altered tumors (18).

3.3.3 Metabolic competition and immunometabolism

Metabolic reprogramming by mutant p53 affects available nutrients in the TME and thereby directly influences immune cell function. Two clinically relevant examples are: (a) asparagine metabolism: TP53-altered prostate cancers show ASNS upregulation and increased asparagine utilization, which may alter local asparagine availability and affect lymphocyte function or stromal support; and (b) lactate accumulation: enhanced glycolysis and poor lactate clearance polarize TAMs to an immunosuppressive phenotype and impair cytotoxic T-cell effector function. Thus, tumor-intrinsic metabolic shifts driven by mutant p53 have non-cell autonomous consequences that can be targeted to restore immune competence (4).

3.3.4 Therapeutic implications: combining metabolic, p53-directed, and immune strategies

Because mutant p53 both creates metabolic dependencies (e.g., ASNS/asparagine) and suppresses antitumor immunity, rational combinations should be prioritized. Examples include asparagine-depleting strategies (asparaginase or ASNS inhibition) combined with p53-reactivating agents (to re-sensitize tumor cells) and myeloid-modulating therapies (CSF1R inhibitors or lactate-targeting approaches) to re-enable T-cell function. Preclinical work also suggests that reversing lactate-mediated macrophage suppression enhances responses to PD-1 blockade in PTEN/TP53-deficient prostate cancer models, a paradigm readily adaptable to TP53-altered cohorts. Careful staging (window PD studies with paired biopsies) is required to show target engagement and immune reprogramming before larger efficacy trials (19).

3.3.5 Prioritized experimental approaches to validate TME effects

To translate TME-centric hypotheses into the clinic, we recommend allele-aware, TME-inclusive preclinical workflows:

• Use co-culture systems and organoids with matched CAFs, macrophages, and autologous T cells to measure how specific TP53 alleles alter stromal/immune phenotypes and metabolite exchange (e.g., conditioned media experiments, isotope tracing across compartments)(17).

• Employ extracellular vesicle (EV) profiling and proteomics to define mutant-p53-dependent secretome changes and test EV depletion or secretion inhibitors as modulators of the TME (18).

• Apply spatial transcriptomics and multiplexed IHC/IF on paired pre-/post treatment biopsies to map immune cell states (T cells, TAMs) and metabolic enzyme expression (ASNS, SQLE) relative to TP53 allele status (4)

• Use metabolic tracer research (13C/15N tracers in organoids/PDXs and PET tracers where available) to quantify intercellular nutrient flux and to confirm on-target metabolic effects of interventions (e.g., reduction in intra-tumoral asparagine) (4).

3.4 Therapeutic strategies & clinical evidence

Delivering clinically useful therapies for TP53-altered prostate cancer requires both agents that act on mutant p53 itself and strategies that exploit the metabolic and synthetic-lethal vulnerabilities created by TP53 loss or GOF. Below, we review the principal classes of therapies, summarize available clinical and preclinical evidence, and

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lay out concrete recommendations for allele-aware translation.

3.4.1 Direct p53-targeting approaches

Covalent reactivators / small molecules (eprenetapopt / APR-246): APR-246 (eprenetapopt) is a small molecule that modifies thiol groups in mutant-p53 proteins and can restore wild-type-like folding and transcriptional activity in some alleles; it has advanced furthest in clinical development. In haematologic malignancies, APR-246 combined with azacitidine produced high response rates and molecular remissions in TP53-mutant MDS/oligoblastic AML, although a large phase-3 readout for frontline MDS did not meet its primary endpoint, underscoring the need for careful patient selection and combination strategies. Early phase studies and a Phase I/IB program have shown on-target biological effects and tolerability across tumor types, including limited prostate cancer cohorts, supporting further exploration in allele-stratified CRPC cohorts. Clinical biomarkers such as SLC7A11 expression have been proposed as predictors of APR-246 sensitivity and may refine patient selection (6).

Aggregation-disrupting peptides (ReACp53) and protein-destabilizers: Peptide agents designed to disrupt mutant-p53 amyloid/aggregation (for example, ReACp53) restore nuclear localization and p53 transcriptional function in preclinical prostate cancer models and sensitize cells to apoptosis and to standard agents. ReACp53 has demonstrated tumor growth inhibition in xenografts and represents an orthogonal approach to small-molecule reactivation that may be particularly valuable for conformational/aggregation-prone alleles (e.g., R175H). These agents remain at preclinical/early-development stages in prostate cancer, but the available data support advancing allele-matched evaluation (20).

Clinical-development lessons: Clinical experience with APR-246 highlights two translational lessons: (i) single-agent activity in solid tumors has been limited, arguing for rational combinations (epigenetic agents, chemotherapies, metabolic drugs) and (ii) tumor-intrinsic determinants (for example, SLC7A11) and tumor heterogeneity materially affect activity; therefore, biomarker-driven, allele-aware trial designs are essential. Early phase prostate cancer programs should prioritize short window PD endpoints and molecularly stratified expansion cohorts rather than broad unselected populations (6).

3.4.2 Metabolic targeting: exploiting mutant-p53 dependencies

Asparagine/ASNS axis. The most concrete, recently validated metabolic vulnerability in TP53-altered CRPC is increased dependence on asparagine biosynthesis driven by ASNS upregulation. Yoo and colleagues used transcriptomics, metabolomics, and functional perturbation to show TP53-altered CRPCs rely on ASNS and are sensitive to asparagine depletion strategies (genetic ASNS suppression, asparaginase), making the ASNS/asparagine axis a high-priority translational target in allele-selected CRPC. Clinical translation will require attention to toxicity (asparaginase side-effects) and to patient selection via tumor ASNS expression or TP53 allele (4).

Cholesterol biosynthesis / SQLE. Wild-type p53 directly represses SQLE (squalene epoxidase), a rate-limiting enzyme of cholesterol biosynthesis; loss or GOF activity of p53 derepresses this pathway, creating an actionable dependency in several tumor types. SQLE inhibition (terbinafine, NB-598 in preclinical work) reduces growth in p53-deficient models, and retrospective clinical observations and small case series have reported PSA declines or survival signals associated with incidental terbinafine use in prostate cancer cohorts. While these data are encouraging, prospective, biomarker-guided trials are lacking and should be pursued in TP53-altered, SQLE-high tumors (5).

Glycolysis / OXPHOS and metabolic plasticity. Mutant p53 frequently promotes metabolic plasticity (heightening glycolysis while preserving mitochondrial function), which reduces the likelihood that single-pathway metabolic inhibition will be effective. Preclinical models, therefore, support combination metabolic approaches (e.g., glycolysis inhibitor + OXPHOS inhibitor, or metabolic inhibitor + p53-reactivator) and integration of metabolic flux readouts in early trials. Comprehensive flux (13C tracer) studies accompanied by paired biopsies are recommended to confirm target engagement (9).

3.4.3 Synthetic-lethal and cell-cycle targets

TP53 loss impairs canonical checkpoint responses and creates reliance on alternative regulators of cell-cycle progression and replication stress responses. In preclinical prostate and other cancer models, inhibition of WEE1, PLK1, CHK1, and related effectors produces selective toxicity in TP53-defective contexts; these agents therefore represent attractive partners for combination

studies with p53-reactivators or metabolic agents. As with other strategies, allele specificity and co-occurring alterations (for example, RB1/PTEN) modulate responses and require testing in isogenic and organoid/PDX platforms before clinical translation (21).

3.4.4 Immune strategies and combinations

Mutant p53 fosters an immunosuppressive TME and metabolic reprogramming that can limit the efficacy of immune checkpoint inhibitors. Preclinical studies indicate that reversing tumor metabolic suppression (for example, reducing lactate accumulation or depleting tumor asparagine) can recondition myeloid populations and enhance checkpoint efficacy. Therefore, combining p53-directed or metabolic agents with immune modulators (CSF1R inhibitors, PD-1/PD-L1 blockade) is a rational translational path, but early trials must include paired immune and metabolic PD markers to demonstrate reprogramming before claiming synergy (21).

3.4.5 Biomarkers, patient selection, and trial design recommendations

Biomarker priorities: Trials should be allele-aware and biomarker-rich. Minimum candidate biomarker assays for inclusion/stratification and PD readouts are:

- Baseline tumor or ctDNA sequencing to define TP53 allele(s) and co-alterations (RB1, PTEN, DDR genes) (22).
- Tumor IHC (p53 pattern, ASNS, SQLE, Ki-67) and targeted transcriptomic signatures (ASNS high/low) (4).
- Predictive molecular markers for specific agents (e.g., SLC7A11 expression for APR-246 sensitivity) (23).
- Metabolic PD: tumor metabolomics (targeted LC-MS for asparagine, sterol intermediates) and 13C tracer studies in a subset of patients (4).

Trial design: For proof-of-mechanism and early efficacy testing, we recommend a staged approach:

1. Window PD cohorts (12–20 patients per allele cohort): Short (7–21-day) pre-operative or pre-treatment window studies that administer the investigational combination (e.g., APR-246 + asparaginase; or p53-reactivator + SQLE inhibitor for SQLE-high tumors) with mandatory paired biopsies for PD (IHC, metabolomics, ctDNA). Primary endpoint: predefined PD effect (e.g., ≥X% reduction in intratumoral asparagine / ASNS expression, or ctDNA TP53 VAF decline). Secondary endpoints: safety, PSA50, radiographic signal. Use paired analyses for improved power (4).

2. Signal-seeking expansions (30–40 patients) or randomized phase II biomarker-enriched designs. If PD signals and tolerability are acceptable, expand to exploratory efficacy cohorts powered to generate estimates of PSA response, ORR, and short PFS for go/no-go decisions. Consider randomized signal-seeking arms versus best-available control if feasible (6).

3. Correlative framework: Include ctDNA TP53 VAF dynamics, targeted transcriptomics, tumor metabolomics, and spatial immune profiling as mandatory correlative endpoints. Pre-specify statistical PD thresholds and stopping rules for toxicity and futility (4).

Limitations and proposed early-phase design.

A limitation of the current preclinical literature is that many studies collapse all TP53 alterations into a single "mutant" group and rely heavily on 2-D cell lines; this practice obscures allele-specific biology and the modifying influence of co-occurring lesions (RB1, PTEN) and the tumor microenvironment. Translationally, we therefore propose a staged, biomarker-driven early-phase program: begin with a window-of-opportunity (proof-of-mechanism) cohort in metastatic castration-resistant prostate cancer (mCRPC) enriched for validated TP53 hotspot alleles (separate cohorts for common hotspots where feasible, e.g., R175H and R273H) and with pre-specified stratification by RB1/PTEN status. Eligibility: mCRPC after ≥1 ARSI, measurable disease, and willingness for paired biopsies. Interventions should test a *mechanism-matched* combination (example: APR-246 or other p53-reactivator plus metabolic perturbation where preclinical data support the vulnerability, e.g., asparaginase or ASNS suppression in TP53/ASNS-high tumors). Primary endpoints should be pharmacodynamic (PD) proof-of-mechanism (paired baseline and on-treatment tumor biopsies assayed for ASNS expression, intratumoral asparagine by metabolomics, SQLE/IHC when applicable, and ctDNA TP53 VAF dynamics) and safety; secondary endpoints should include PSA50, objective response rate, and short-term radiographic PFS. Correlative assays should include targeted ctDNA for TP53

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allele tracking, tumor IHC for p53/SQLE/ASNS/Ki-67, bulk and single-cell RNAseq on paired biopsies, and tracer-based flux (where feasible) to confirm metabolic engagement. For sample size, a window PD cohort of approximately 12–20 patients per allele cohort is typically sufficient to detect large paired PD effects (paired analyses comparing baseline vs on-treatment, which improve power); if a robust PD signal is observed, expand to an exploratory efficacy cohort of approximately 30–40 patients (or use a randomized signal-seeking expansion) to obtain preliminary estimates of clinical activity. Statistical analyses should pre-specify paired tests for PD markers (paired t/ t/Wilcoxon) and clearly defined criteria for "go/no-go" to larger trials (e.g., pre-specified magnitude of ASNS reduction or ctDNA VAF decline plus acceptable safety). This allele-aware, biomarker-heavy design will maximize the chance of detecting true, targetable mutant-p53 metabolic vulnerabilities while minimizing heterogeneity that has confounded prior translational efforts.

4. Conclusion

TP53 Mutations, particularly gain-of-function (GOF) hotspot alleles, fundamentally reshape prostate cancer biology by reprogramming energy metabolism, altering lipid and amino-acid pathways, and modifying the tumor microenvironment. These changes sustain proliferation, drive therapeutic resistance, and create distinct metabolic vulnerabilities. This review highlights that TP53 alterations are not uniform: conformational and contact mutants differ in their molecular interactions and downstream metabolic effects. Recognizing these allele-specific differences is essential for developing effective, targeted therapies. Among the emerging opportunities, the ASNS/asparagine and SQLE/cholesterol represented promising metabolic targets for translation. The future progress depends on integrating mechanistic discoveries with clinical validation. Allele-aware preclinical models, biomarker-driven patient selection, and early-phase trials incorporating pharmacodynamic and metabolic readouts will be crucial. Therefore, combining p53-reactivating, metabolic, and immune-modulating strategies offers a rational path to improve outcomes in patients with therapy-resistant prostate cancer.

In summary, a precision approach that links TP53 allele type, metabolic dependencies, and microenvironmental context can transform our understanding of prostate cancer and accelerate the development of personalized therapies.

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Competing interest:
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Assessment of Dental Anxiety Scale and Oral Health Literacy Among a Sample of the New Alamein Population: A Cross-Sectional Study.

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ABSTRACT

Background: *Oral health awareness is a crucial component of dental public health, as it provides opportunities for disease prevention, early diagnosis, and treatment of dental problems. Oral health literacy (OHL) has an influential impact on the physical, mental, social, and economic health of both individuals and communities. Inadequate oral health knowledge and low oral health levels endanger communication between patients and dentists in different communities. Consequently, such ignorance may lead to postponing or avoiding dental appointments, ultimately contributing to poor oral health care.*

Purpose: *The purpose of this study was to assess dental anxiety (DA) and oral health literacy (OHL) among Alamein's population.*

Material and Methods: *A cross-sectional convenience study was carried out to assess the dental anxiety scale (DA) and oral health literacy (OHL) of 80 patients selected from the outpatient clinic of the Arab Academy for Science, Technology, and Maritime Transport. Any patient between 20 and 50 was considered for the study. Data were collected through a structured, interview-administered questionnaire based on the Modified Dental Anxiety Scale (MDAS). The questions were developed and modified from the previous studies. This questionnaire was designed to assess the dental anxiety levels of patients and analyze the knowledge, attitude, and behavior (KAP) toward oral health literacy. The assessment of the patient's anxiety levels took into account several variables that can influence their mental health. The survey covered their age, gender, level of education, and the number of dental visits.*

Results: *This study involved 80 participants; the majority were aged 35–50 years (57.5%) and male (57.5%). Regarding employment, a slightly higher proportion were employed (53.8%). Overall, dental anxiety scores indicated moderate levels of anxiety, particularly fear of invasive procedures. Females had significantly higher anxiety scores than males (p-value = 0.004). Correspondingly, unemployed individuals reported higher anxiety compared to employed individuals (p-value = 0.006). There were no significant differences in dental anxiety scores based on age, marital status, and education level ($p > 0.05$).*

Conclusion: *This current study found that dental anxiety was significantly higher among females than among males. However, gender and employment status showed statistically significant associations with dental anxiety. Preventive measures should be promoted among dentally anxious patients to increase awareness and education about comprehensive oral care practices.*

KEYWORDS

Dental Anxiety, Oral Health Literacy, Dental Care Utilization, Patient Education, Fear of Dental Visits, Oral Health Outcomes, Psychological Impact..

1. Review of Literature

Dental anxiety (DA) is still a psychological problem that affects a significant portion of the population. It includes a wide range of emotional reactions, from mild nervousness to intense fear when faced with dental visits or procedures. A fear of pain, embarrassment about oral hygiene, traumatic dental experiences in the past, or even

the sounds and smells of the dental office can all contribute to this anxiety. [1] Between 2 and 30 percent of people worldwide have DA, which is highly prevalent. [2] [3] It is ranked ninth among severe fears and fourth among common fears. [4] According to other studies, poor compliance, low utilization, and treatment discontinuation are associated with dissatisfaction with the standard of dental care and costs. [5]

According to Pado et al. (2020) [6], 10–20% of people reported having moderate to severe dental anxiety, and another 30–40% reported having mild anxiety related to dental visits. Consequently, DA often leads to the avoidance of dental care, causing delayed visits and worsening oral health conditions over time.

The impact of dental anxiety (DA) extends beyond oral health, as it can lead to systemic health problems. Untreated dental conditions such as dental caries, gingival disease, and tooth loss may occur in those who neglect their dental care. Furthermore, there is growing evidence that respiratory infections, diabetes, cardiovascular disease, and other more general systemic health issues are associated with poor oral health. However, people who suffer from high dental anxiety frequently attribute their anxiety to past dental experiences, which can result in decreased cooperation, missed appointments, and the possibility of misdiagnosis and mistreatment. [7] By examining these factors and exploring their consequences on oral health care, dental professionals can implement customized strategies to ameliorate the negative effects and improve oral health outcomes. [8]

Oral health literacy (OHL) has made remarkable advancements, in addition to being crucial in lowering oral health disparities and significantly improving dental care, oral hygiene habits, and preventative measures. Studies have consistently demonstrated that increased levels of oral health literacy correlate with improved oral health outcomes, including adherence to preventive measures and being more comfortable while receiving dental treatment. [9] However, a substantial segment of the population experiences limited oral literacy due to factors such as low educational level, language barriers, and cultural influences. This lack of knowledge can not only prevent patients from seeking appropriate care but also contribute to increased dental anxiety, as individuals may feel overwhelmed about treatments. [10]

There is a growing connection between dental anxiety (DA) and oral health literacy (OHL). Those with low oral health literacy are more liable to experience heightened anxiety about dental visits. [11] However, individuals with high dental anxiety may experience considerable challenges in maintaining their regular dental care routines. A harmful cycle occurs between dental anxiety and oral literacy, which results in postponed dental care and the worsening of oral conditions. Those caught in this detrimental loop may experience heightened dental anxiety and may need more extensive dental treatment for a current oral issue compared to individuals who regularly attend dental follow-ups. [12] These factors, which hinder individuals from seeking appropriate dental care, affect people on an individual and community level and pose a significant dental public health challenge. [8]

Prior studies on dental care in relation to dental anxiety have mostly concentrated on individual factors like oral hygiene awareness, brushing frequency, and dental visit avoidance. Most studies consistently show that poor oral hygiene practices are associated with higher levels of dental anxiety. [13] [14] [15] By enhancing dental education, improving communication between patients and dental providers, and enhancing the bond of trust between the patient and the dentist, dental professionals are in a better position to reduce anxiety and encourage individuals to engage more proactively with their dental care. [12]

The purpose of this study was to assess dental anxiety and oral health literacy among the New Alamein population.

2. Material and Methods

2.1. Study Design

This study was designed as a cross-sectional study, "descriptive type," aimed at assessing dental anxiety (DA) and oral health literacy (OHL) among a sample of the New Alamein population. The approach of this study allowed for data collection at a single point, providing a snapshot of the variables of interest within the study sample. All data were gathered through structured, interview-administered questionnaires during routine clinical workflow.

2.2. Study setting

This study was conducted at the outpatient clinic of the "Arab Academy for Science, Technology, and Maritime Transport Hospital" (AASTMT). This outpatient clinic serves a wide range of dental services for Alamein's population, making it an appropriate setting for evaluating the

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dental anxiety scale, knowledge, attitude, and behavior toward oral health literacy among a convenience sample. The environment allowed for proper interaction with patients, facilitating data collection through a structured interview.

2.3. Pilot study

Before implementing the main study, a pilot study with a convenience sample of 15 participants was conducted to assess the feasibility, clarity, and reliability of the questionnaire. Based on the trial, minor adjustments were made to ensure a precise and smoothly structured questionnaire.

2.4. Study participants

A convenience sample of 80 patients, both male and female, was enrolled in the outpatient clinic. To establish the prevalence of dental anxiety, power analysis was used to calculate the sample size (n = 80), assuming a moderate effect size (Cohen's d = 0.5), a power of 0.80, and α = 0.05. The calculated minimum sample size was 64, and to compensate for possible nonresponses, 80 participants were included. Although a convenience sample was used, participants were recruited from the Arab Academy Hospital, serving diverse socioeconomic groups across New Alamein. Any patient between 20 and 50 was included in this study. Inclusion criteria encompassed patients who were able to participate in the survey and had dental problems. The exclusion criteria comprised patients not willing to participate in the study, those with medically compromised diseases, and those with physical and mental disabilities. A structured, interview-administered questionnaire based on MDAS, originally developed by **Humphris et al. (1995)** [17]. The questions were developed and modified from the previous studies. [18] [19] This questionnaire, composed of 5 questions, was designed to assess the dental anxiety scale of patients.

2.5. Ethical Considerations

The following research proposal was submitted on July 1, 2025, and was registered and exempted by **the Institutional Review Board Organization, Arab Academy for Science and Technology, School of Dentistry**, IRB #1. Research Number: IORG0012504.

Verbal consent was obtained from each patient with an agreement to participate in this questionnaire. Prior to data collection, all participants were informed about the plan of the study and its purpose. All gathered information was safely stored and used exclusively for research objectives.

2.6. Study variables

Dental anxiety, knowledge, attitude, and behavior about oral health literacy were crucial dependent variables, which were assessed in this study. Other independent variables included age, gender, educational level, and employment status. All data were systematically analyzed to identify the dental anxiety scale in the study sample.

2.7. Study instrument

The study used a structured, interview-administered questionnaire based on MDAS, originally developed by **Humphris et al. (1995)** [17], as the primary data collection tool. MDAS is a widely recognized tool that measures a patient's level of dental anxiety (DA). The survey included three parts: a section on demographics, the Modified Dental Anxiety Scale, and an evaluation of knowledge, attitudes, and behaviors related to oral health literacy.

The first section discussed the demographic data from the sample of Alamein's population, depending on variables of the participants like age, gender, marital status, educational level, and employment status. The second section consisted of 5 questions to measure the anxiety level of the participants using MDAS, a brief, self-completed questionnaire composed of 5 questions summed together to produce a total score ranging from 5 to 25. It has good psychometric properties and low instrumental effects, is relatively quick to complete, and scoring is easy. It can be incorporated into routine dental practices. The third section was concerned about the oral health literacy of a sample of the new Alamein population. Oral Health Literacy (OHL) was assessed indirectly through a structured Knowledge, Attitude, and Practice (KAP) questionnaire. This approach provides a practical measure of functional literacy through the comprehension of dental terminology, self-care behaviors, and preventive awareness. Future studies should consider cross-cultural adaptation and validation of the standardized (OHL) instrument for the Egyptian population. The KAP survey is a useful diagnostic tool for assessing knowledge, attitudes, and practices related to particular subjects, especially oral health literacy. This survey is based on the concept that knowledge positively influences attitude, which subsequently influences actions. [19] Questions about KAP in the questionnaire discuss the knowledge of the Alamein sample towards oral health literacy (OHL), such as knowledge of some

definitions, such as "dental caries" or "plaque", and hearing about fluoride toothpaste. Other questions were about the patient's attitude about visiting the dentist regularly, and the last section was about behavior regarding oral health care, such as tooth brushing.

2.8. Questions coding

Using the questionnaire, dental anxiety was assessed. The MDAS is a validated survey consisting of five questions; each question in the MDAS consists of five options, scored from 1 (not anxious) to 5 (extremely anxious). The total score ranges from 5 to 25, with higher scores indicating greater levels of dental anxiety. [3]

All items will be rated on a 5-point scale: 1 = not anxious, 2 = slightly anxious, 3 = fairly anxious, 4 = very anxious, and 5 = extremely anxious.

2.9. Statistical Analysis

Data was analyzed using **IBM SPSS Statistics software version 23 for Windows, Armonk, NY, USA**. Qualitative data were described using frequency and percentage, while quantitative data, "anxiety scores," were summarized using mean, standard deviation, median, and interquartile range (IQR) according to the normality of distribution of the data by using the Kolmogorov-Smirnov test, so the Mann-Whitney U test was employed to assess differences in anxiety scores in relation to demographic variables. All tests were two-tailed, and the significance level was set at p-value < 0.05.

3. Results

This study was conducted to assess dental anxiety (DA) and oral health literacy (OHL) among Alamein's population and to analyze the relationship between dental anxiety and sociodemographic variables. Among the 80 study participants, the majority were aged 35–50 years (57.5%) and male (57.5%). Most participants were married (77.5%) and had received some form of education (58.8%). Regarding employment, a slightly higher proportion were employed (53.8%) compared to unemployed (46.3%). **[Table 1]**

The Modified Dental Anxiety Scale results among Alamein's population revealed varying anxiety levels across different dental situations. Most participants reported low anxiety before visiting the dentist (Q1: 48.8% not anxious) and in response to the dental clinic environment (Q2: 55% not anxious). Similarly, 68.8% were not anxious about the smell of dental materials (Q3), showing minimal sensory-related anxiety. In contrast, anxiety increased significantly when participants were asked about dental anesthesia (Q4), with 21.3% feeling "extremely anxious" and a higher mean score of 2.69 ± 1.61. The highest anxiety was observed in response to surgical procedures like tooth extractions (Q5), where 27.5% were "extremely anxious," and the mean score peaked at 3.21 ± 1.67. The overall dental anxiety score averaged 11.28 ± 4.64, indicating moderate anxiety, particularly driven by fear of invasive procedures. According to the dental anxiety questionnaire's results, the fifth question (which asked about the patients' feelings about surgical procedures like extractions) had the major impact on the overall anxiety score. **[Table 2]**

Dental anxiety levels were recorded among the patients in New Alamein, showing that 21.3% experienced low anxiety, 28.8% had moderate anxiety, 17.5% faced high anxiety, and 13.8% dealt with extreme anxiety. **[Figure 2]**

Most participants demonstrated good basic oral health knowledge, with 85% correctly recognizing the term "plaque" and 100% identifying "caries" as a known term. However, awareness declined for "cavities," with only 70% responding correctly. Knowledge about preventive measures was limited: only 31.3% had heard of fluoride toothpaste, and 33.8% were aware of dental floss as part of good oral hygiene. Concerning the replacement of toothbrushes, only 13.8% accurately recognized "every 3 months" as the recommended time frame. Encouragingly, 72.5% correctly recognized that both sugar and poor oral hygiene contribute to tooth decay. Only 17.5% correctly identified brushing twice daily as the standard. These results highlight strong recognition of disease-related terms but indicate gaps in awareness of preventive practices. **[Table 3]**

A majority of participants (56.3%) felt comfortable with dental treatments, indicating a generally positive perception toward oral health care, yet 16.3% reported extreme fear. While 83.8% believed oral health is important compared to overall health, only 48.8% considered regular dental visits crucial, and over a quarter (27.5%) viewed them as not important. This reflects a potential gap in awareness of the prevention of dental care within the community. Most participants (67.5%) were willing to invest in dental care, but 66.3% incorrectly believed that brushing alone is sufficient for maintaining oral hygiene, while 33.8% disagreed. This reveals a misconception that may undermine the importance of oral hygiene practices such as regular tooth brushing, flossing, and regular dental checkups. Overall,

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these findings reflect generally positive attitudes toward oral health. **[Table 4]**

Most participants (73.8%) had visited a dentist within the past six months, indicating a positive trend in dental attendance. However, 13.8% had not visited a dentist in over a year. Daily oral hygiene habits were suboptimal; only 5% used dental floss, and 12.5% used mouthwash. Sugar consumption was frequent, with 30% consuming it multiple times daily and 27.5% once per day. Additionally, tongue cleaning was not widely practiced, with just 17.5% including it in their routine, while 82.5% did not include it in their hygiene routine. This suggests that most individuals may not be receiving adequate guidance on complete oral care techniques. Overall, while dental visit frequency was high, preventive oral hygiene behaviors were limited, suggesting a need for increased awareness and dental public health education on comprehensive oral care practices. **[Table 5]**

Analysis of the relationship between dental anxiety and sociodemographic variables revealed no significant differences in dental anxiety scores based on age, marital status, or education level ($p > 0.05$). However, gender and employment status showed statistically significant associations with dental anxiety. Females exhibited notably higher levels of anxiety (mean = 12.79 ± 3.82) compared to men (mean = 10.15 ± 4.91; $p = 0.004$). Similarly, unemployed individuals reported higher anxiety (mean = 12.57 ± 3.74) compared to employed individuals (mean = 10.16 ± 5.08; $p = 0.006$). Overall, most demographic variables did not show a significant relationship with dental anxiety in this study; the key factors were gender and employment status. Suggesting that social and economic factors may influence psychological responses to dental care, particularly among women and unemployed individuals. **[Table 6]**

Table 1: Demographic variables of the study participants

Variables		Total sample = 80 n (%)
Age in years	20 – 35	34 (42.5%)
	35 – 50	46 (57.5%)
Gender	Male	46 (57.5%)
	Female	34 (42.5%)
Marital status	Single	18 (22.5%)
	Married	62 (77.5%)
Educational level	Not Educated	33 (41.3%)
	Educated	47 (58.8%)
Employment status	Unemployed	37 (46.3%)
	Employed	43 (53.8%)

Table 2: Dental anxiety levels using the Modified Dental Anxiety Scale among Alamein's population

	Q1	Q2	Q3	Q4	Q5
	n (%)				
Not anxious (1)	39 (48.8%)	44 (55%)	55 (68.8%)	29 (36.3%)	21 (26.3%)
Slightly anxious (2)	10 (12.5%)	20 (25%)	17 (21.3%)	15 (18.8%)	11 (13.8%)
Fairly anxious (3)	19 (23.8%)	6 (7.5%)	7 (8.8%)	5 (6.3%)	8 (10%)
Very anxious (4)	7 (8.8%)	6 (7.5%)	0 (0%)	14 (17.5%)	10 (12.5%)
Extremely anxious (5)	5 (6.3%)	4 (5%)	1 (1.3%)	17 (21.3%)	30 (27.5%)
Mean ±SD	2.11 ±1.28	1.83 ±1.17	1.44 ±0.76	2.69 ±1.61	3.21 ±1.67
Median (IQR)	2.00 (2.000	1.00 (1.00)	1.00 (1.0)	2.00 (3.00)	3.50 (4.00)
Overall score Mean ±SD Median (IQR)	11.28 ±4.64 11.00 (7.50)				

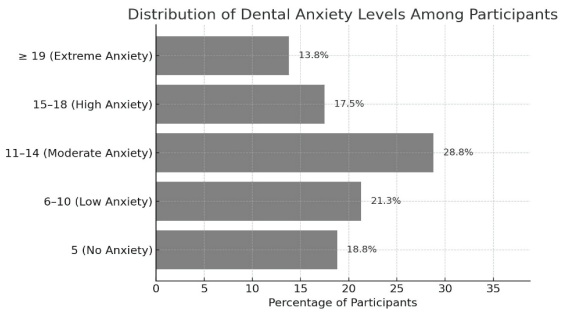

Figure 1: Distribution of dental anxiety levels among participants – (Horizontal Bar Chart)

Table 3: Oral health knowledge among Alamein's population

Items		Total sample = 80
Do you know terms like "plaque"?	Yes	68 (85%)
	No	12 (15%)
Do you know terms like "Caries"?	Yes	80 (100%)
	No	0 (0%)
Do you know terms like "Cavities"?	Yes	56 (70%)
	No	24 (30%)
Have you ever heard about toothpaste containing fluoride?	Yes	25 (31.3%)
	No	55 (68.8%)
Have you ever heard about dental floss for good oral hygiene?	Yes	27 (33.8%)
	No	53 (66.3%)
How often should you replace your toothbrush?	Every 1 month	30 (37.5%)
	Every 2 months	9 (11.3%)
	Every 3 months	11 (13.8%)
	Don't know	30 (37.5%)
Which of the following can cause tooth decay?	Sugar	4 (5%)
	Poor oral hygiene	18 (22.5%)
	Both together	58 (72.5%)
What is the recommended tooth brushing frequency?	Once	33 (41.3%)
	Twice	14 (17.5%)
	More than twice	3 (3.8%)
	No brushing	30 (37.5%)

Table 4: Oral health attitude among Alamein's population

Items		Total sample = 80
How do you feel about dental treatments?	Comfortable	45 (56.3%)
	Neutral	22 (27.5%)
	Extremely fear	13 (16.3%)
What do you think about visiting the dentist regularly?	Important	39 (48.8%)
	Neutral	19 (23.8%)
	Not important	22 (27.5%)
What do you think about the importance of dental health compared to general health?	Important	67 (83.8%)
	Sometimes important	9 (11.3%)
	Not important	4 (5%)
Are you willing to invest money in a dental procedure to maintain good oral hygiene?	Yes	54 (67.5%)
	No	26 (32.5%)
Do you believe brushing alone can be sufficient to maintain good oral hygiene?	Yes	53 (66.3%)
	No	27 (33.8%)

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Table 5: Oral health behavior among Alamein's population

Items		Total sample = 80
When was your last dental visit?	<6 months	59 (73.8%)
	6 months – 1 year	6 (7.5%)
	>1 year	11 (13.8%)
	Never	4 (5%)
Do you use additional oral hygiene aids like dental floss?	Yes	4 (5%)
	No	76 (95%)
Do you use additional oral hygiene aids like mouthwash?	Yes	10 (12.5%)
	No	70 (87.5%)
How often do you consume sugar?	Multiple times/day	24 (30%)
	Once/day	22 (27.5%)
	Twice/day	11 (13.8%)
	Don't know	23 (28.7%)
Do you clean your tongue as part of oral hygiene?	Yes	14 (17.5%)
	No	66 (82.5%)

Table 6: Relation between demographic variables and dental anxiety scores

Variables		Mean ±SD	Median (IQR)	*p* value[1]
Age	20 – 35	11.26 ±4.81	11.00 (8.00)	0.973
	35 – 50	11.28 ±4.57	11.00 (6.00)	
Gender	Male	10.15 ±4.91	9.00 (9.00)	***0.004****
	Female	12.79 ±3.82	14.00 (4.00)	
Marital status	Single	9.94 ±3.67	9.00 (7.25)	0.235
	Married	11.66 ±4.85	11.00 (7.25)	
Education	Not Educated	11.09 ±4.72	11.00 (9.00)	0.910
	Educated	11.40 ±4.64	11.00 (6.00)	
Employment	Unemployed	12.57 ±3.74	12.00 (4.00)	***0.006****
	Employed	10.16 ±5.08	9.00 (9.00)	

***Statistically significant difference at p value<0.05, p value1: Mann Whitney U test**

4. Discussion

Dental anxiety (DA) poses a significant challenge that negatively affects both patients and dentists, greatly contributing to the development of dental problems by preventing patients from seeking proper oral health care. This study highlights dental anxiety (DA) as a prevalent obstacle among the New Alamein population. In terms of sociodemographic factors, females or individuals with lower socioeconomic status may experience greater anxiety due to stress-inducing factors in daily life, impaired stress response, or limited access to dental health education messages. [20] Furthermore, low awareness of fluoride or flossing may be derived from poor exposure to preventive health campaigns of routine dental care. The MDAS is a popular, effective, and reliable method for evaluating dental anxiety in both clinical and research settings. [21] In the present study, high dental anxiety was found more in females than in male patients (*p-value* = 0.004) **(Table 6)**, with documented evidence in the literature **(Silveira et al., 2020; Bashiru & Omotola, 2016).** [22,23].

Correspondingly, a study by **Armfield (2010)** [15] found that lack of self-confidence and unemployment can lead to avoidance behavior, with unemployed people reporting higher levels of anxiety than employed people. Furthermore, in this study, unemployed individuals showed higher dental anxiety (*p-value* = 0.006) **(Table 6).** The prevalence of dental anxiety was low in response to routine clinical settings and sensory triggers such as dental smells or waiting room environments. However, noticeable spikes in dental anxiety were observed when participants were asked about dental anesthesia; the highest anxiety was observed in response to surgical procedures like tooth extractions. The overall percentage of DA items was 17.5% for moderate anxiety, which was the highest percentage, followed by 27.5% for severe anxiety and 25% and 23.8% for low and high anxiety **(Table 2).**

The development of oral health promotion strategies that can impact individual and community health outcomes can be facilitated by oral health literacy, which is influenced by social determinants of health. [24] Despite most participants demonstrating good oral health knowledge, with 85% correctly recognizing the term "plaque" and 100% identifying "caries" as a known term, significant gaps were noted in their awareness of preventive practices related to the use of fluoride toothpaste, with only 31.3% **(Table 3)** and 5% of participants using dental floss **(Table 5).** Although many participants recognize the importance of dental health, this awareness does not consistently translate into preventive behaviors such as regular brushing, tongue cleaning, or using dental aids. For instance, only 17.5% of participants reported cleansing their tongue as a part of their oral hygiene practice **(Table 5)**, indicating a lack of adherence to comprehensive self-care practices.

A review indicates that fear and anxiety related to dental treatments often arise from a past traumatic experience at the dentist. [25] Cooperation of both the dentists and the patients has a crucial role in making the dental treatment easier. It has to make the people more aware of the necessity of dental treatment. The impact of dental anxiety and fear seems to be multifactorial, resulting in an individual's tendency to have worse oral health in addition to postponing dental appointments. [25]

Recent international studies have reinforced the link between oral health literacy and dental anxiety. For instance, **Gudipaneni et al. (2024)** [26] demonstrated that low OHL in parents increased both parental and child anxiety levels, while **Yu et al. (2024)** [27] emphasized that OHL is a determinant of preventive behavior in the elderly. These findings align with the present results, emphasizing that community-based oral health education programs can reduce fear and enhance preventive care uptake.

Considering the findings of this study, demographic variables such as age (*p-value* = 0.973), marital status (*p-value* = 0.235), and educational level (*p-value* = 0.910) did not show a significant relationship with dental anxiety **(Table 6)**. It's vital to emphasize more oral health education for patients to encourage them to seek dental treatment. For women, previous research had indicated a greater tendency to report health-related fears and seek healthcare more frequently, which may increase awareness of oral healthcare and, therefore, anxiety. [22, 23]. Integrating oral health education into primary healthcare and workplace wellness programs may help address both psychological and behavioral barriers to care. Unemployed individuals, on the other hand, may face financial barriers to consistent dental care, contributing to irregular visits and increased fear due to the anticipation of more extensive or painful treatments. [15]

5. Conclusion

This study revealed that females exhibited notably greater anxiety levels compared to males. However, gender and employment status showed statistically significant associations with dental anxiety. A high risk of oral disease, poor oral health outcomes, and inappropriate oral health behaviors is associated with dental anxiety. As a strategy to prevent oral diseases, it is important to measure and identify individuals with dental anxiety to help prevent oral diseases. Dental public health initiatives should focus on improving oral health education and providing supportive environments to encourage regular dental visits and preventive care.

6. Limitations

The findings of this study should be interpreted with the following limitations in mind. Firstly, the cross-sectional design of this study prevents it from identifying causality. An association was noted only between gender and employment status. Accordingly, this study reflects only the DA and OHL of patients attending a university-based dental clinic and does not necessarily reflect that of the greater population. Secondly, the collected data relied on self-perceived outcomes, which could be biased, as the patients may over- or

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underestimate their response. Another limitation is the absence of a standardized, validated OHL tailored for the Egyptian population. Even with these restrictions, the study provides valuable baseline data for guiding future longitudinal and interventional studies focused on reducing anxiety and improving oral health literacy.

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Conflicts of interest
There are no conflicts of interest.

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Dissemination of carbapenem and colistin resistance in Gram-negative bacteria: The emerging role of novel β-lactam/β-lactamase inhibitors for managing a global dilemma

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ABSTRACT

Antibiotic resistance is imposing an increasing burden on global health. In 2021, an estimated 4.71 million deaths were associated with bacterial antimicrobial resistance, of which 1.14 million were directly attributable to resistant infections; projections estimate nearly 39 million deaths from antimicrobial-resistant diseases between 2025 and 2050. Of particular concern is the rise of carbapenem-resistant Gram-negative bacteria, which has decreased the effectiveness of carbapenems once used against ESBL producers.colistin, previously discontinued because of severe toxicity, was reintroduced as a last-resort therapy, but its usefulness is now threatened by rising resistance driven in part by unregulated veterinary use and the spread of plasmid-mediated mcr genes. Several new β-lactam/β-lactamase-inhibitor combinations and novel agents demonstrate improved efficacy and safety compared with revived older drugs; however, their high cost and limited availability constrain their impact in low- and middle-income countries such as Egypt. This review summarizes the epidemiology and molecular mechanisms of carbapenem andcolistin resistance and evaluates the clinical evidence and mechanisms of action for last-line and novel β-lactam/β-lactamase-inhibitor therapies.

KEYWORDS

Antibiotic resistance, Carbapenem resistance, Gram-negative bacteria,colistin resistance,β-lactam/β-lactamase inhibitors, Egypt

1. Introduction

Since their discovery, antibiotics have been extensively utilized across all domains to combat bacterial diseases. The unregulated and ongoing use of antibiotics exerted selective pressure on microorganisms, resulting in a global rise in antibiotic resistance (1, 2). The continuous rise in antibiotic resistance, combined with the diminishing discovery of novel treatments to combat emerging antibiotic-resistant bacteria, may propel the globe toward a pre-antibiotic period (2).

Antibiotic resistance exerts considerable strain on global healthcare systems. In 2021, around 4.71 million deaths were linked to bacterial antibiotic resistance, with 1.14 million deaths directly attributed to it. It is projected that approximately 39 million individuals will die from antimicrobial-resistant infections between 2025 and 2050 (3). Moreover, resistance rates are elevated in low-income countries compared to high-income countries, suggesting a correlation between the high prevalence of antibiotic resistance and a nation's level of development. Additionally, significant data deficiencies exist in numerous low-income contexts, implying that the actual resistance situation in these countries may be more severe than previously estimated (4, 5).

Furthermore, the Global Antimicrobial Resistance and Use Surveillance System (GLASS) report published by the WHO in 2025 revealed that one in six laboratory-confirmed bacterial infections, which are frequent among humans worldwide in 2023, showed resistance to antibiotic

treatments. Between 2018 and 2023, antibiotic resistance escalated in almost 40% of the examined pathogen-antibiotic combinations, with an average annual increase of 5–15%. The WHO estimated that antibiotic resistance is most prevalent in the South-East Asian and Eastern Mediterranean Regions, where one in three reported cases exhibited resistance. In the African Region, 20% of infections displayed resistance. Resistance is more prevalent and deteriorating in regions where health systems lack the capacity to diagnose or manage bacterial infections. The recent analysis indicates that drug-resistant Gram-negative bacteria are increasingly becoming a global concern. The impact is most significant in nations least prepared to address the issue. *Escherichia coli* (*E. coli*) and *Klebsiella pneumoniae* (*K. pneumoniae*) are the predominant drug-resistant Gram-negative bacteria identified in bloodstream infections. These pathogens pose the most critical bacterial infections that frequently lead to sepsis, organ failure, and mortality. Over 40% of *E. coli* and more than 55% of *K. pneumoniae* worldwide are now resistant to third-generation cephalosporins, the preferred therapy for these infections. In the African Region, resistance surpasses 70%. Other critical life-saving antibiotics, such as carbapenems and fluoroquinolones, are becoming less effective against *E. coli, K. pneumoniae, Salmonella* spp., and *Acinetobacter* spp. Carbapenem resistance, once uncommon, is increasingly prevalent, limiting treatment alternatives and necessitating dependence on last-resort medicines. Such antibiotics are expensive, challenging to obtain, and frequently inaccessible in low-and middle-income countries (LMICs) (5).

The most concerning pathogens include multidrug-resistant (MDR), extensively drug-resistant (XDR), and pan-drug-resistant (PDR) bacteria. MDR, XDR, and PDR bacteria are defined, respectively, as nonsusceptibility to ≥1 agent in ≥3 antimicrobial classes, susceptibility limited to ≤2 classes, and nonsusceptibility to all antimicrobial classes (6). Classical resistant pathogens are part of the ESKAPE group, comprising *Enterococcus faecium*, *Staphylococcus aureus* (*S. aureus*), *K. pneumoniae*, *Acinetobacter baumannii* (*A. baumannii*), *Pseudomonas aeruginosa* (*P. aeruginosa*), and *Enterobacter spp.* The most alarming pathogens presently include carbapenem-resistant *Enterobacterales* (CRE), particularly carbapenem-resistant *K. pneumoniae* (CRKP), methicillin-resistant *S. aureus*, extended-spectrum-β-lactamase (ESBL)-producing *Enterobacterales*, vancomycin-resistant *Enterococci*, multidrug-resistant *P. aeruginosa*, and multidrug-resistant *A. baumannii* (7, 8).

In 2024, the WHO recognized CRE as one of the top four drug-resistant bacteria necessitating urgent antibiotic discovery (Critical group), alongside carbapenem-resistant *A. baumannii,* third-generation cephalosporins-resistant *Enterobacterales*, and rifampicin-resistant *Mycobacterium tuberculosis.* Conversely, carbapenem-resistant *P. aeruginosa* was categorized as a high-priority group, thus deemed a lesser threat than the previously mentioned species. The WHO indicated that although *P. aeruginosa* is challenging to treat, emerging evidence suggests a global decline in its resistance profile. Additionally, its low transmissibility relative to other carbapenem-resistant species influenced the WHO's decision to prioritize the issue of carbapenem-resistant *P. aeruginosa* as less critical (9).

Although emerging medications effective against Gram-positive bacteria offer a temporary reprieve (10), the 2020 global antibiotic clinical pipeline included merely 23 candidates exhibiting activity against Gram-negative bacteria, none of which were from a novel class. In fact, the last antibiotic approved by the United States (US) Food and Drug Administration (FDA) with a new mechanism of action targeting Gram-negative bacteria was identified nearly 60 years ago, resulting in infections caused by antibiotic-resistant Gram-negative bacteria emerging as a significantly greater threat (11-13).

Antibiotic-resistant microorganisms and their resistance genes are increasingly recognized as environmental pollutants. Once largely confined to point sources such as hospitals, sewage systems, and agricultural sites, they now contaminate relatively pristine rivers, lakes, and soils (1,14). LMICs are particularly vulnerable because of weak surveillance and diagnostics, poorly regulated antibiotic use in humans and animals, overcrowded hospitals, inadequate hygiene, rapidly expanding meat and fish production, higher infectious-disease burdens, and limited access to costly second- and third-line drugs. These vulnerabilities are amplified by insufficient waste- and wastewater management, which releases resistant fecal bacteria and antibiotic residues into the environment; excessive manufacturing emissions have also been reported from major producers such as China and India. Because resistance crosses borders, addressing the problem in LMICs is a global imperative; therefore, cost-effective measures that overlap with water, sanitation, and hygiene improvements should be prioritized, and sewage surveillance offers a promising, less-resource-demanding complement to conventional clinical monitoring. The One Health Concept emphasizes that successfully managing this global health

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challenge is critical, as it requires understanding the connections between the human, animal, and environmental microbiota due to the common crossing of species and environmental boundaries by bacteria and genes (14).

The unprecedented rise and prevalence of XDR and MDR bacteria have necessitated the reintroduction of last-resort antibiotics, includingcolistin, which had previously been discontinued due to their toxic side effects, primarily nephrotoxicity and neurotoxicity. However, considering these significant circumstances, they have resurfaced to combat these formidable bacterial infections (1, 15). Alternative antimicrobials, such as the novel β-lactams and β-lactam/β-lactamase inhibitors, meropenem/vaborbactam, ceftazidime/avibactam, imipenem/cilastatin/relebactam, and the siderophore cephalosporin cefiderocol, have been deemed superior and have largely supplantedcolistin in the treatment of carbapenem-resistant Gram-negative infections. (16).colistin may be necessary for treating carbapenem-resistant *A. baumannii* infections, and in cases when the novel β-lactams and β-lactam/β-lactamase inhibitors have limited accessibility (16, 17).

This review provides a comprehensive overview of the epidemiological data, mechanisms of action, and resistance associated with last-resort antibiotics, particularly carbapenems andcolistin, and highlights the emerging role of novel β-lactams and β-lactam/β-lactamase inhibitors in combating these vicious pathogens.

2. Burden and Impact of Antimicrobial-Resistant Bloodstream Infections

Bacteremia, defined as the presence of bacteria in the bloodstream, constitutes an important public health threat (18) that can lead to devastating diseases (19, 20) and incur annual costs in the billions of dollars to the world economy (21, 22). Clinical bacteremia is linked to sepsis, a critical organ failure resulting from an aberrant host response to infection (23). The WHO has identified sepsis as a global health issue (18), with data from 2017 indicating 48.9 million cases and 11 million sepsis-related deaths worldwide, accounting for nearly 20% of all global deaths (24). Sepsis is associated with a variable but incredibly high mortality rate (25-27) and can cause permanent dysfunction, including cognitive impairment or organ failure (26, 28).

Bloodstream infections (BSIs) by themselves are associated with substantial morbidity and mortality (29, 30). In 2019, BSIs accounted for 2.91 million deaths worldwide. Nearly half of these fatalities were attributed to Gram-negative bacteria, which are known to be linked to elevated mortality rates (31, 32). Carbapenem-resistant isolates accounted for 26.3% of these fatalities. *A. baumannii*, *K. pneumoniae*, and *P. aeruginosa* were the predominant carbapenem-resistant infections associated with mortality (32). A study conducted between 2019 and 2021 on patients suffering from hospital-acquired BSIs concluded almost the same results (33). This highlights the significance of carbapenem resistance and its global impact on the mortality associated with BSIs. Furthermore, the GLASS report published by the WHO in 2025 identified significantly elevated levels of antibiotic resistance in bacteria responsible for BSIs (5).

Moreover, antibiotic resistance correlates with inferior outcomes compared to typical cases. A study of 131 US hospitals demonstrated a significant correlation between antimicrobial resistance in BSIs and *in vitro* susceptibility-discordant empiric antibiotic therapy, leading to increased crude mortality, extended total hospital stay, and heightened intensive care unit admissions (34). Previous research from Turkey indicated that carbapenem resistance in bloodstream pathogens was associated with a 30-day fatality rate reaching 66% (35). Furthermore, CRE are associated with increased length of hospital stay and mortality compared to carbapenem-susceptible *Enterobacterales* in LMICs (36).

Antimicrobial resistance substantially increases both the mortality and economic burden associated with BSIs in LMICs. A recent systematic review and meta-analysis conducted across diverse LMIC settings found that BSIs caused by antibiotic-resistant bacteria were associated with significantly higher mortality rates than infections due to susceptible strains, with CRKP yielding the highest mortality risk among the pathogens studied. Furthermore, the direct medical costs for antimicrobial-resistant BSIs were estimated to be approximately USD 12,442 higher per patient compared to infections with susceptible organisms. The economic impact was further compounded by premature mortality, contributing an additional average cost of USD 41,103 per patient (37).

3. Carbapenems as a Last Resort for ESBL-Producing Gram-Negative Bacteria

Carbapenems, a broad-spectrum β-lactam antibiotic, are structurally related to penicillin (38). Carbapenems have a carbon instead of a sulfone at the fourth position of the β-lactam ring, differing from other β-lactams. The unique structure plays a major role in their stability against β-lactamases (39). Carbapenems enter bacteria through outer-membrane porins and bind penicillin-binding proteins (PBPs). By acylating PBPs via their β-lactam ring, they inhibit peptidoglycan cross-linking, trigger autolytic enzymes, and cause osmotic cell lysis. (38, 39). A key advantage of carbapenems is their ability to bind to several PBPs (40).

ESBLs are enzymes that deactivate most β-lactam antibiotics, including penicillins, cephalosporins, and Aztreonam. Nonetheless, ESBL-producing *Enterobacterales* typically retain susceptibility to carbapenems. Carbapenems were regarded as the preferred treatment for these resistant pathogens (41), as ESBLs do not deactivate non-β-lactam drugs. Organisms possessing ESBL genes frequently have supplementary genes or mutations that enhance their resistance to a wide array of antibiotics. Globally, the majority of ESBLs are classified into various categories of sulfhydryl reagent variable (SHV) β-lactamases, Temoniera (TEM) β-lactamases, and cefotaxime-M (CTX-M) β-lactamases (16, 42). Recent outbreaks of ESBL have primarily been linked to the CTX-M type rather than the TEM or SHV types, with CTX-M-15 being the most widespread ESBL globally (16, 43, 44).

Reduced treatment options, complex infections, increased mortality, and pricey treatments are some of the key concerns for individuals infected with ESBL-producing pathogens (45). Consequently, in critical illnesses such as BSIs, carbapenems are established as the preferred therapeutic option. However, this has led to the rise of carbapenem resistance and the spread of CRE (5).

4. The Global Spread of Carbapenem-Resistant Gram-Negative Bacteria

The Centers for Disease Control and Prevention (CDC) defines CRE as *Enterobacterales* exhibiting resistance to at least one carbapenem *in vitro* (46). In recent years, the global prevalence of CRE transmission has escalated, and the spread of COVID-19 has exacerbated the situation to some degree through enhanced bacterial colonization and patient-staff contact, leading to higher prevalence, longer hospital stays, and worse outcomes in co-infected patients as well as antibiotic misuse during the pandemic (47). Epidemiological investigations of CRE have predominantly focused on the dominant strains of CRKP and carbapenem-resistant *E. coli* (CREco), which together account for over 90% of CRE isolates and are widely disseminated worldwide through several transmission routes (48). Furthermore, imipenem resistance has shown a significant upward trend among Gram-negative bacteria, with *K. pneumoniae* causing BSIs exhibiting the most pronounced increase, rising by approximately 15.3% annually (5).

According to the GLASS report, the WHO published in 2025, carbapenem resistance exhibits marked regional variability. In Africa, imipenem resistance was highest among *K. pneumoniae* bloodstream isolates (20.2%). The Eastern Mediterranean Region showed the largest proportion of imipenem-resistant *Acinetobacter* spp., causing BSIs at 66.5%. Egypt has reported notably high imipenem resistance among *K. pneumoniae*, *E. coli*, and *Acinetobacter* spp. Isolated from bloodstream and urinary tract infections. In Europe, overall imipenem resistance remains relatively low, but several countries in Eastern Europe have experienced sharp increases; for example, Greece reported imipenem resistance of 71.8% in *K. pneumoniae*, 2.1% in *E. coli*, and 93.7% in *Acinetobacter* spp. Causing BSIs. South-East Asia recorded the highest imipenem resistance for bloodstream isolates of *E. coli* and *K. pneumoniae*, at 17.5% and 41.2%, respectively. The Western Pacific Region reported lower overall rates, although the Republic of Korea had a high imipenem resistance proportion for *Acinetobacter* spp. Causing BSIs (72.1%) (5).

Ultimately, these significant regional differences show that while resistance hotspots vary by location, CRE remains a serious global threat that requires specific local monitoring combined with international cooperation.

Figures 1 and 2 illustrate the global percentages of imipenem resistance across WHO regions for BSIs and urinary tract infections, respectively.

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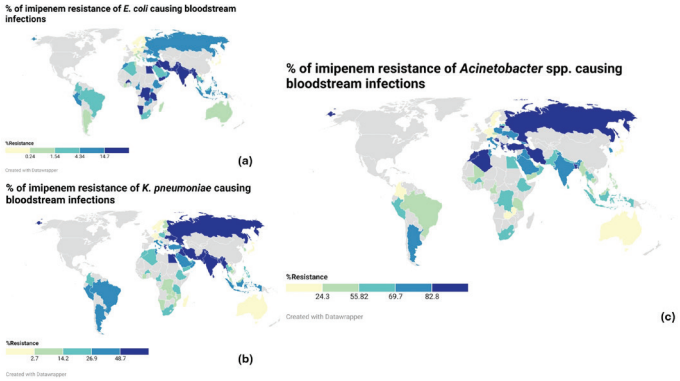

Figure 1: Global distribution of imipenem resistance percentages among different bloodstream pathogens (a) *E. coli*, (b) *K. pneumoniae*, and (c) *Acinetobacter spp.* (5).

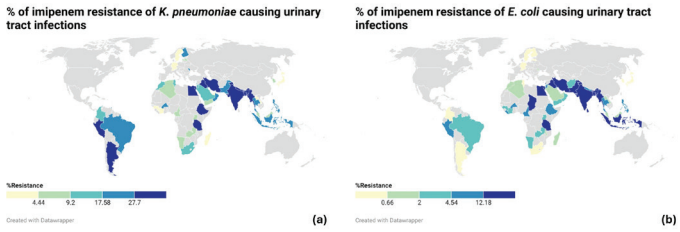

Figure 2: Global distribution of imipenem resistance percentages among different urinary tract pathogens: (a) *K. pneumoniae*, (b) *E. coli* (5).

5. Insights into the Different Mechanisms of Resistance to Carbapenems

The mechanisms of resistance to carbapenems among CRE and some non-*Enterobacterales* including carbapenem-resistant *A. baumannii* and carbapenem-resistant *P. aeruginosa* involve (i) antibiotic degradation, (ii) obstruction of antibiotic entrance into bacterial cells, (iii) alteration of antibiotic binding sites, (iv) deletion or mutation of pore proteins, (v) hyperactivation of efflux pumps, (vi) modifications in PBP and (vii) biofilm formation (47).

5.1. Production of carbapenemases

Carbapenemase production is a significant resistance mechanism in Gram-negative bacteria, especially within *Enterobacterales*, which hydrolyzes carbapenems and other β-lactam antibiotics. Carbapenem-resistant pathogens can be categorized into carbapenemase-producers and non-carbapenemase-producers, where carbapenem resistance in the latter results from alternative resistance mechanisms, including the overexpression of other β-lactamases like ESBL (16).

β-lactamases are commonly grouped by the Ambler classification into four classes: A, B, C, and D (49). Carbapenemases fall within classes A, B, and D, whereas class C enzymes are not considered true carbapenemases. However, class C β-lactamases have a low but measurable ability to hydrolyze carbapenems, and their overproduction can contribute to carbapenem resistance when combined with reduced outer-membrane permeability and/or efflux pump overexpression (50). **Figure 3** illustrates the Ambler molecular classification of the major carbapenemase classes, providing a brief description of each, including the antibiotics they affect and their structural differences.

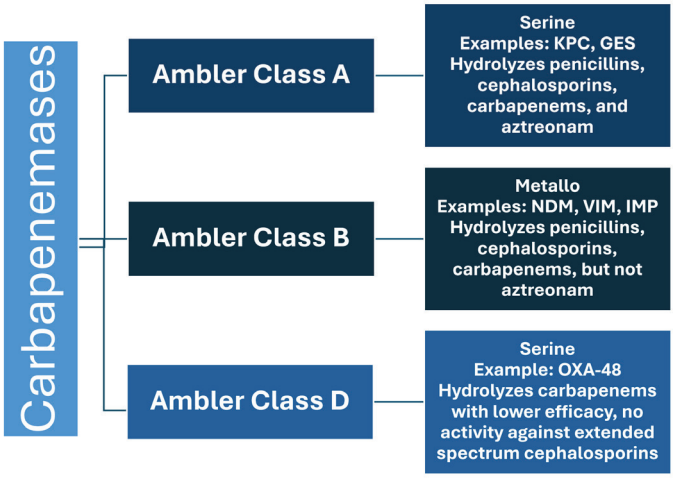

Figure 3: Ambler classification of carbapenemases (51).

5.1.1. Class A carbapenemases

The predominant class A carbapenemases comprise *K. pneumoniae* carbapenemase (KPC), imipenem-hydrolyzing β-lactamase (IMI), non-metallo carbapenemase of class A (NMC-A), Guiana extended-spectrum β-lactamase (GES), and *Serratia marcescens* enzyme (SME) (52). Class A carbapenemases include a serine residue at their active sites and are distinguished by their capacity to hydrolyze penicillins, cephalosporins, carbapenems, and Aztreonam (52). bla_{KPC} is the most prevalent carbapenemase-encoding gene in CRE and is the most frequently identified in the US (47, 52).

5.1.2. Class B carbapenemases

Class B carbapenemases are characterized by metallo-β-lactamase (MBL) structures (52). This class contains amino acids at the binding site that interact with zinc (53). Class B enzymes include NDM (New Delhi metallo-β-lactamase), IMP (Imipenemase), and VIM (Verona integron-encoded metallo-β-lactamase) (52). Most class B carbapenemases

degrade all β-lactams, excluding Aztreonam (53). Class B carbapenemases-encoding genes are typically located on plasmid vectors and other transposable elements, facilitating their dissemination across bacteria (53).

5.1.3. Class D carbapenemases

Oxacillinase (OXA) enzymes constitute class D carbapenemases (52). Similar to class A carbapenemases, class D carbapenemases include a serine amino acid at their binding sites (52). They differ from class A carbapenemases due to their diminished hydrolytic activity against carbapenems and penicillins, lack of activity against extended-spectrum cephalosporins, and resistance to earlier β-lactamase inhibitors (e.g., clavulanic acid, tazobactam, or sulbactam) (52, 54). Nonetheless, the majority is hindered by avibactam (54). OXA-48 is the predominant carbapenemase enzyme in this category and is usually identified in *K. pneumoniae* (53).

5.2. Outer membrane protein deletion or alteration

Bacteria can restrict the penetration of carbapenems into the periplasmic region, where PBPs reside. This process entails modifications in porin expression or variations in the porin encoding gene, resulting in either a total loss or deficiencies in the corresponding porin (55). The primary mechanism of resistance to carbapenems in *P. aeruginosa* isolates is the downregulation of the gene encoding the OprD porin (56). Additionally, the modified expression of OmpK35 and OmpK36 in *K. pneumoniae* was found to confer significant resistance to ertapenem (57).

5.3. Overexpression of efflux pumps

Efflux pumps typically recognize several substrates, as their affinity is determined by physicochemical qualities (e.g., electric charge, aromatic or hydrophobic characteristics) rather than by chemical structures. This elucidates the existence of MDR efflux pumps capable of expelling numerous structurally diverse antimicrobials (58). Gram-negative bacteria, including *P. aeruginosa* and *Acinetobacter spp.*, are recognized for their efflux-mediated β-lactam resistance (59). The overexpression of efflux pumps active on carbapenems may lead to carbapenem resistance (60, 61).

5.4. Penicillin-binding protein alterations

PBPs are essential proteins for the synthesis of peptidoglycans in bacterial cell walls. Carbapenems exert their antibacterial activity by covalently binding to PBPs, leading to stable acylated complexes that obstruct cell wall production (62). Drug resistance is largely caused by structural changes, increased PBP production, decreased antibiotic affinity, and the emergence of new PBPs. In 2019, Ranjitkar et al. (63) found that mutations in the *mrdA* gene, responsible for encoding the PBP2 protein, were found to reduce *E. coli*'s sensitivity to carbapenems. Moreover, the co-existence of *mrdA* mutations with modifications in the *ftsI* gene, which encodes PBP3, intensified the decline in antibiotic susceptibility. It has been suggested that although PBP mutations lead to elevated minimum inhibitory concentration (MIC) values, these mutations alone do not significantly correlate with clinical carbapenem resistance. Instead, they may contribute to clinical drug resistance in combination with reduced porin production or increased carbapenemase production (64).

5.5. Altered biofilm components

For bacteria, biofilm is a technique to protect themselves and fight against hostile circumstances. Its components include LPS, flagella, and type I and III fimbria (65). Modulating biofilm components can govern biofilm synthesis and enable bacteria to endure antibiotic stress, thereby demonstrating antibiotic resistance. The principal surface structures implicated in the biofilm formation of *K. pneumoniae* are type III bacterial fimbriae and capsular polysaccharide; the former facilitates bacterial adhesion, while the latter affects biofilm architecture and intercellular communication (66). Sharma et al. (67) indicated that CRKP can downregulate flagella and bacterial pili proteins under meropenem stress to complete biofilm remodeling and promote bacterial survival under meropenem stress. However, Cusumano et al discovered that CRKP had a 91% reduced likelihood of developing robust biofilm-forming capabilities, indicating a negative link between biofilm development and antibiotic resistance (68). In 2021, the experimental findings of Fang et al. demonstrated that, unlike carbapenem-susceptible *K. pneumoniae*, CRKP exhibited diminished biofilm-forming ability due to the absence of the *mrkH* gene, which governs biofilm formation (65).

6. Polymyxins: Last–Resort for Carbapenem-Resistant

Polymyxins are non-ribosomal, cyclic oligopeptide antimicrobials that are structurally comprised of a cyclic heptapeptide with five

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major chemical compounds: polymyxins A, B, C, D, and E. Polymyxin B and polymyxin E (Colistin) are used extensively in clinical practice (69).

Colistin is a polypeptide antibiotic discovered from the bacterium *Paenibacillus polymyxa* subspecies*colistinus* in 1947 (70). In the 1950s, it was introduced as an intravenous formulation. In 1959, the FDA authorized the use ofcolistin as a therapeutic option for various forms of diarrhea and UTIs, deeming it a "miracle" antibiotic due to its potent bactericidal efficacy against Gram-negative bacteria while maintaining a low resistance profile (71). Nonetheless, owing to its deleterious side effects, especially nephrotoxicity and neurotoxicity, its use was ultimately discontinued in the 1980s in favor of less hazardous alternatives. Despite this, it continued to be utilized as a viable clinical alternative for individuals with cystic fibrosis suffering from pseudomonal lung infections, as well as in topical formulations combined with other antimicrobials for the treatment of ocular or aural infections. Moreover, it kept being used as a viable option in veterinary medicine for decades (72, 73).

In response to the persistent and unprecedented rise in antibiotic resistance, especially to carbapenems, regarded as a last-resort antibiotic for numerous MDR pathogens,colistin was reintroduced in the 2000s to address MDR bacteria. This resurgence has subsequently resulted in the emergence ofcolistin-resistant strains that currently afflict the global population. In 2015, the discovery ofcolistin resistance mechanisms, mediated by plasmids and referred to as mobilecolistin resistance genes (*mcr* genes), was particularly alarming since it indicated the potential for horizontal transfer of this resistance (1, 74). **Figure 4** illustrates the history ofcolistin, from its discovery to the identification of *mcr* genes.

In Egypt, the situation is particularly concerning. A recent systematic review reported that approximately 9% of Gram-negative isolates exhibitcolistin resistance, increasing to nearly 31% among carbapenem-resistant strains (75).

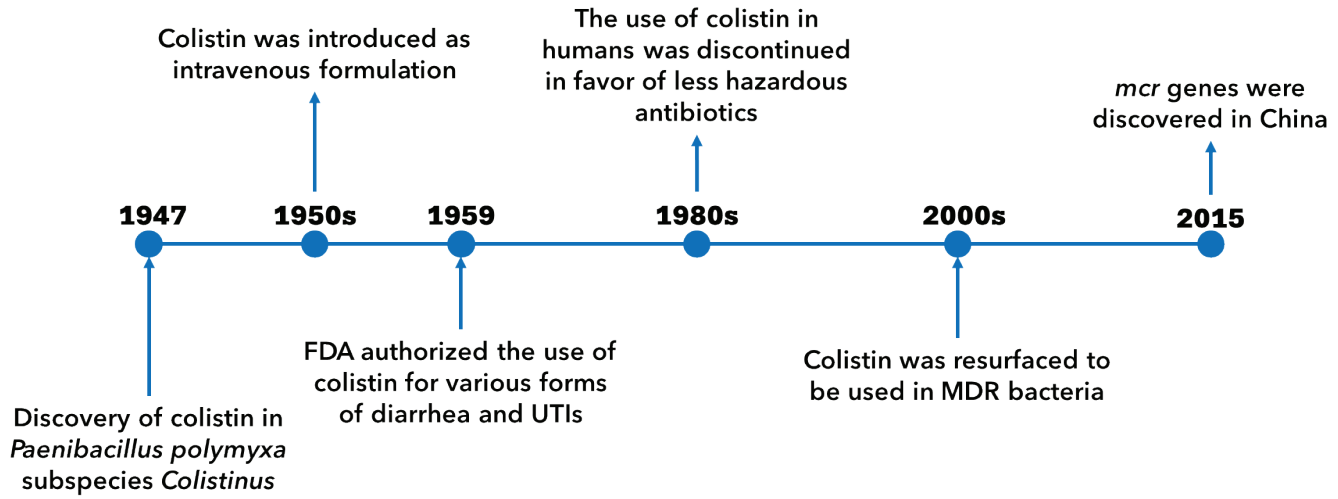

Figure 4: The historical development ofcolistin, from its discovery through to the identification of plasmid-mediated *mcr* genes.

7. Colistin Use in Veterinary Medicine

Colistin has been utilized in veterinary medicine for decades (73), primarily for medicinal and preventive use in food animals, in addition to metaphylactic and growth enhancement purposes (76, 77).

Colistin was historically regarded as an uncommon antibiotic for humans, as its use in human medicine was infrequent due to its neurotoxicity and nephrotoxicity, coupled with poor gastrointestinal absorption. Consequently, the incidence of resistance tocolistin remained low, primarily attributed to chromosomal resistance (73, 78, 79). In 2015, this perspective shifted markedly following the discovery of the plasmid-borne *mcr* gene and its globally disseminated variants, which have been largely attributed to the use ofcolistin in agriculture in China, particularly for prophylaxis and as a feed additive. Since then, the use ofcolistin in veterinary medicine has come under sustained scrutiny (74, 80). Moreover, there is a growing dependence oncolistin for the treatment of multidrug-resistant Gram-negative bacterial infections, particularly in LMICs, where alternative treatments (e.g., tigecycline) are sometimes prohibitively costly when available (81).

Due to the rising utilization ofcolistin for severe infections in various regions globally, the identification of *mcr* genes that impart transmissible resistance tocolistin, and the dissemination ofcolistin-resistant bacteria through the food chain, the WHO has determined that polymyxins, includingcolistin, should be classified as a "Highest Priority Critically Important Antimicrobial", necessitating the implementation of multiple strategies to address antimicrobial resistance (82).

The improper use ofcolistin and other antibiotics in veterinary medicine intensifies the issue of antibiotic resistance. A significant factor is the accessibility of veterinary medications without a prescription, facilitating unregulated usage (83). However, numerous initiatives undertaken in several countries have resulted in significant reductions in the sales and utilization ofcolistin in livestock production (77). The production and commerce ofcolistin, including pharmaceutical raw materials, completed pharmaceutical products, and veterinary feed additives or growth boosters, remained unchanged in several LMICs despite their consensus on the antibiotic resistance crisis (76).

8. Insights Intocolistin's Antibacterial Activity and Its Possible Mechanisms of Action

Colistin predominantly targets the outer membrane of Gram-negative bacteria, particularly the LPS layer. The LPS comprises three domains: the innermost lipid A, the central core oligosaccharide area, and the outermost O-antigen chain (84). Lipid A is crucial for preserving the overall outer membrane structure by firmly binding to the fatty acyl chains. Cations such as calcium (Ca^{2+}) and magnesium (Mg^{2+}) promote interactions between neighboring LPS molecules, thus enhancing outer membrane stability (84). The antibacterial efficacy ofcolistin arises from electrostatic interactions between the phosphates of lipid A on the bacterial outer membrane and the cationic diaminobutyric acid (Dab) residue incolistin (85).colistin demonstrates antimicrobial effectiveness against Gram-negative bacteria via five unique methods.

8.1. The classical membrane lysis pathway

The classical mechanism of action entails the membrane lysis pathway, triggered by the electrostatic contact between negatively charged phosphate head groups on lipid A and positively charged Dab residues oncolistin within the LPS component of the outer membrane of Gram-negative bacteria. This interaction causes the displacement of divalent cations, including Ca^{2+} and Mg^{2+}, from the anionic phosphate groups of membrane lipids, thus destabilizing the LPS.colistin subsequently expands the outer membrane by infiltrating it and incorporating either the D-Leu^6-l-Leu^7 segment or its hydrophobic terminal fatty acyl chain, which increases membrane permeability and facilitates the "self-promoted uptake" ofcolistin through destabilized areas in the outer membrane created during its interaction with LPS. Ultimately, the inner membrane of the phospholipid bilayer is undermined due to membrane thinning, which weakens the bilayer's structural integrity, culminating in cell lysis (85, 86).

8.2. Vesicle–vesicle interaction pathway

An alternate mechanism that augments the antibacterial activity ofcolistin entails vesicle–vesicle contact. In this process,colistin promotes interaction between the outer leaflet of the cytoplasmic membrane and the inner leaflet of the outer membrane by binding to anionic phospholipid vesicles (87). This interaction facilitates the transfer of phospholipids between vesicles, leading to a reduction in the specificity of phospholipid composition. This ultimately disrupts osmotic equilibrium within the cell, resulting in cell lysis (85).

8.3. Hydroxyl radical-induced cell death pathway

The alternative mechanism ofcolistin action involves the stimulation of rapid cell death by the formation of hydroxyl radicals resulting fromcolistin's attachment to the lipid membrane. Free radicals are produced whencolistin traverses the outer membrane and inner membrane of lipopolysaccharides. The creation of hydroxyl radicals happens through the production of reactive oxygen species, including hydroxyl radicals ($^{\cdot}OH$), superoxide (O_2^-), and hydrogen peroxide (H_2O_2), which induce oxidative stress. Superoxide is produced whencolistin penetrates and traverses the outer membrane and inner membrane, subsequently transforming O_2^- into H_2O_2 via superoxide dismutase. Subsequently, H_2O_2 oxidizes ferrous iron (Fe^{2+}) to ferric iron (Fe^{3+}) while generating $^{\cdot}OH$; this process is referred to as the Fenton reaction. This process can cause oxidative damage to bacterial DNA, proteins, and lipids, resulting in cell death. This killing mechanism has been demonstrated incolistin-susceptible and MDR isolates of *A. baumannii,* but it does not occur in polymyxin-resistant bacteria (88).

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8.4. Respiratory enzyme inhibition pathway

Colistin has recently been recognized to possess a new mechanism of action: the suppression of essential respiratory enzymes situated in the inner membrane of Gram-negative bacteria (89). The Type II NADH oxidoreductase respiratory enzyme serves as the secondary target ofcolistin, situated in the bacterial electron transport system within the inner membrane (89). Unlike Type I NADH oxidoreductase, Type II NADH oxidoreductase, known as "alternate NADH oxidoreductase," does not facilitate the active translocation of protons across the inner membrane (90).colistin inhibits Type II NADH oxidoreductase by augmenting the respiratory chain, therefore improving its utilization of it. This inhibition impairs the bacterial electron transport chain, compromising respiratory function and threatening bacterial survival (91).

8.5. Antiendotoxin activity ofcolistin

Colistin exhibits an additional antibacterial mechanism through its significant antiendotoxin action. It targets the lipid A component of lipopolysaccharides, which serves as an endotoxin in Gram-negative bacteria, thereby inhibiting the initiation of shock via the release of cytokines such as tumor necrosis factor-alpha and interleukin 8 (72).

9. Mechanisms of Colistin Resistance

9.1. Mechanisms of intrinsic resistance in Serratia marcescens and Proteus mirabilis

Colistin resistance is inherently present in *Serratia marcescens* and *Proteus mirabilis* due to the expression of the *arnBCADTEF* and/or *eptB* genes, leading to the incorporation of phosphoethanolamine (pEtN) and 4-amino-4-deoxy-L-arabinose (L-Ara4N) cationic groups onto LPS, respectively. This modulation enhances the cationic charge on the LPS membrane, which is the primary target ofcolistin. As a result, this reducescolistin antibiotic binding, leading to inherent resistance in these bacterial species (92-94).

9.2. Mechanisms of acquired resistance in Enterobacterales

Resistance to polymyxins has been observed in various genera of the *Enterobacterales*, including *Klebsiella*, *Escherichia*, *Enterobacter*, and *Salmonella*. While for certain bacterial species the mechanisms ofcolistin resistance remain unidentified, multiple molecular processes have been elucidated. The predominant mechanism involves modification of LPS through cationic substitution, similar to that observed in bacteria with intrinsic resistance to polymyxins. To date, only a single transferable resistance mechanism, the plasmid-mediated *mcr* gene, has been identified, whereas most resistance determinants are chromosomally encoded (95).

As seen in strains that exhibit natural resistance tocolistin, the incorporation of cationic groups (L-Ara4N and pEtN) into the LPS facilitates the development ofcolistin resistance in *Enterobacterales*. A comprehensive array of genes and operons participates in the qualitative modification of LPS. These include genes and operons that encode enzymes directly involved in LPS alterations, such as those responsible for synthesizing cationic groups and/or incorporating them into LPS, for example, the *pmrC* gene, the *pmrE* gene, and the *pmrHFIJKLM* operon. Additionally, several regulatory genes play key roles, including those encoding the PmrAB and PhoPQ two-component systems, as well as regulators of these systems, such as the *mgrB* gene, which negatively modulates PhoPQ, and the *crrAB* two-component system that controls the PmrAB system. (95).

9.2.1. Genes encoding LPS-modifying enzymes

a. The *pmrC* gene:

The *pmrCAB* operon encodes three proteins: the pEtN phosphotransferase PmrC, the response regulator PmrA (also known as BasR), and the sensor kinase protein PmrB (often referred to as BasS) (96). The pEtN phosphotransferase PmrC attaches a pEtN group to the LPS (96).

b. The *pmrHFIJKLM* operon and the *pmrE* gene:

The *pmrHFIJKLM* operon (also called the *arnBCADTEF* or *pbgPE* operon) codes for a total of seven proteins (97). The *pmrE* gene and the *pmrHFIJKLM* operon are important for the biosynthesis of L-Ara4N and its attachment to lipid A (97).

c. The *pmrA* and *pmrB* genes encoding the PmrAB two-component system:

Environmental triggers, including macrophage phagosomes, Fe^{3+} iron, aluminum (Al^{3+}), and low pH (e.g., pH 5.5), facilitate the activation of PmrB via its periplasmic domain. The PmrAB and PhoPQ two-component systems are often active when bacteria are engulfed by macrophages,

facilitating bacterial survival (96).

PmrB is a protein exhibiting tyrosine kinase activity that phosphorylates and activates PmrA. PmrA subsequently promotes the transcription of the *pmrCAB* operon, the *pmrHFIJKLM* operon, and the *pmrE* gene, which are implicated in LPS modification (pEtN and L-Ara4N addition) (96).

Mutations in the *pmrA* and *pmrB* genes have been identified as responsible for acquiredcolistin resistance in *K. pneumoniae* (98, 99). These mutations cause the persistent activation of the PmrAB two-component system, resulting in the overexpression of the *pmrCAB* operon, the *pmrHFIJKLM* operon, and the *pmrE* gene, thereby facilitating the production of pEtN and L-Ara4N and their incorporation into lipid A (95). Specifically, the PmrC protein encoded by the *pmrCAB* operon catalyzes the addition of pEtN to the lipid A moiety, while ArnT, an integral membrane protein encoded by *pmrHFIJKLM*, transfers L-Ara4N to lipid A (100, 101).

d. The *phoP* and *phoQ* genes encoding the PhoPQ two-component system:

The *phoPQ* operon encodes two proteins: the regulatory protein PhoP and the sensor protein kinase PhoQ. Environmental cues, including macrophage phagosomes, low magnesium, and low pH (e.g., pH 5.5), facilitate the activation of PhoQ via its periplasmic domain (96). The PhoPQ two-component system facilitates the expression of genes responsible for magnesium transport, enzymes that alter LPS to confer resistance to cationic antimicrobial peptides, and enzymes that mitigate cellular stress induced by acidic pH or certain virulence factors. The PhoPQ two-component system enables bacterial survival in environments characterized by low magnesium levels, acidic pH, or the presence of cationic antimicrobial peptides (102, 103).

PhoQ is a protein possessing tyrosine kinase activity that stimulates PhoP by phosphorylation. PhoP subsequently promotes the transcription of the *pmrHFIJKLM* operon, which is implicated in the incorporation of L-Ara4N into the LPS. PhoP can activate the PmrA protein, either directly or indirectly through the PmrD connector protein, resulting in the addition of pEtN to the LPS (102, 103).

Polymyxin heteroresistance in *E. cloacae* has been linked to the *dedA* and *ecr* genes. Specifically, *dedA* encodes a membrane protein believed to be involved in proton motive force-dependent drug efflux, and its disruption was found to increase susceptibility towards polymyxins. While its complementation results in increased MIC (104). Moreover, the DedA protein was found to play a role in establishingcolistin resistance in many Gram-negative bacteria, including *K. pneumoniae* (105, 106). The *ecr* gene encodes a small transmembrane protein that activates the PhoPQ system, leading to the upregulation of the *pmrHFIJKLM* operon, promoting LPS modification, leading to high-levelcolistin resistance. The *ecr* gene was also found to upregulate *dedA* and *tolC*, the latter of which encodes a key component of the AcrAB-TolC efflux pump (104, 107).

Multiple mutations in the *phoP* and *phoQ* genes contribute to the development of acquired resistance to polymyxins in *K. pneumoniae* (108). These mutations cause the constitutive activation of the PhoPQ two-component system, resulting in the overexpression of the *pmrHFIJKLM* operon and consequently the production of L-Ara4N and its transfer to lipid A (95).

9.2.2. Regulators of the PmrAB and PhoPQ two-component systems

a. The *mgrB* gene:

MgrB, also known as YobG, is a diminutive transmembrane protein comprising 47 amino acids (109). Activation of PhoP results in the upregulation of the *mgrB* gene. The MgrB protein subsequently inhibits the expression of the PhoQ-encoding gene, resulting in negative regulation of the PhoPQ two-component system (110). The inactivation of the *mgrB* gene, which negatively regulates the PhoPQ two-component system, results in the overexpression of the *phoPQ* operon, subsequently activating the *pmrHFIJKLM* operon and facilitating the formation of L-Ara4N, responsible forcolistin resistance acquisition (95).

Research indicates that transcript interruption and amino acid mutations in *mgrB* are significant factors behindcolistin resistance (111). A recent study highlighted the significance of *mgrB*-related mutations in *K. pneumoniae*, reporting that these mutations account for more than 80% of the resistance-associated genetic alterations detected globally in *K. pneumoniae* isolates (98). Alarmingly, recent reports indicate that the transposition of genes encoding ESBLs or carbapenemases, resulting in the disruption of the chromosomal *mgrB* gene, serves as a source of resistance tocolistin (112, 113).

b. The *crrAB* operon:

The *crrAB* operon encodes two proteins: the regulatory protein CrrA and the sensor kinase

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CrrB. Induced mutations of the *crrB* gene result in the overexpression of the *pmrCAB* operon, which activates the *pmrHFIJKLM* operon, *pmrC* and *pmrE* genes, ultimately leading to the synthesis of L-Ara4N and pEtN, both of which confercolistin resistance. Inactivation of the CrrB protein may alter lipid A via the activation of a glycosyltransferase-like protein (95). Additionally, the CrrAB and PmrAB two-component systems are indirectly linked through the modulator protein, CrrC. Mutations in the *crrB* gene lead to elevated expression of *crrC*. Specific amino acid substitutions in the CrrB protein enhance its autophosphorylation activity, which contributes to increased resistance tocolistin (101, 114). While the *crrAB* operon was found to regulate polymyxin resistance and affect virulence, its physiological function in the absence of antibiotic pressure remains incompletely understood (115).

9.2.3. The intrinsic regulator RamA

RamA, the intrinsic regulator of *K. pneumoniae*, is recognized for its substantial role in the comprehensive response to antimicrobials. It modulates genes associated with permeability barriers and may thus contribute to diminished susceptibility to antibiotics. Findings indicate that elevated levels of this regulator resulted in modifications to LPS, hence diminishing vulnerability to polymyxins (116).

9.3. Mechanisms of acquired resistance in Pseudomonas aeruginosa and Acinetobacter baumannii

In *P. aeruginosa*,colistin resistance is primarily mediated by five two-component regulatory systems: PmrAB, PhoPQ, ParRS, ColRS, and CprRS (95). Mutations in PmrAB and PhoPQ lead to constitutive activation of the *pmrHFIJKLM* operon, resulting in the addition of L-Ara4N to lipid A, which reducescolistin binding and confers resistance (117). Unlike what is observed in *K. pneumoniae*, PhoPQ-mediated resistance in *P. aeruginosa* does not depend on the PmrAB system (95). The ParRS system contributes to adaptive resistance, also activating the *pmrHFIJKLM* operon (118). In contrast, the roles of ColRS and CprRS are less clearly defined; although their mutations have been associated with high-level polymyxin resistance, particularly when occurring alongside *phoQ* mutations (119).

In *A. baumannii*, resistance occurs via two major mechanisms: (1) qualitative modification of LPS through PmrAB mutations that activate the *pmrCAB* operon, leading to pEtN addition, and (2) quantitative loss of LPS production due to inactivating mutations in lipid A biosynthesis genes (*lpxA*, *lpxC*, and *lpxD*) (95).

9.4. Emergence of plasmid-mediated mcr genes

Resistance acquired from plasmid DNA, encoded by transposable genetic elements on plasmids containing *mcr-1* and its variants, was initially reported in *E. coli* from China. Subsequently, plasmid-mediated *mcr-1* and its variants have been identified in other Gram-negative bacterial isolates (120, 121). The resistance pattern involves encoding the *mcr-1* protein pEtN transferase. It was suggested that *mcr* genes originated from inherently resistant environmental bacteria, such as *Paenibacillus species*, yet *mcr* genes spread globally via a highly transmissible plasmid. Epidemiological and molecular studies have identified the presence of *mcr-1* within the diverse *Enterobacterales* family, which includes *K. pneumoniae, E. aerogenes, Shigella sonnei, E. cloacae, Salmonella, Kluyvera species, Cronobacter sakazakii, Citrobacter species, and Raoultella ornithinolytica.* Furthermore, bacterial isolates containing *mcr-1* demonstrated intricate reservoirs encompassing human-associated settings and natural ecosystems' food supplies (1). The LPS is altered by *mcr-1* expression through the addition of cationic pEtN transferase (71). Nonetheless, novel variations of *mcr-1* (*mcr-1.0* to *mcr-1.30*) have been documented, exhibiting expression through modifications of the LPS membrane.

Further *mcr* variations have been documented, including *mcr-2* (*mcr-2.1* to *mcr-2.7*) (122). Phylogenetic analyses revealed a novel variation of *mcr-1* exhibiting 80% identity. Subsequently, three more plasmid-mediated *mcr*-like gene variations were identified in *E. coli* and *Salmonella*: *mcr-3* (*mcr-3.1* to *mcr-3.41*), *mcr-4* (*mcr-4.1* to *mcr-4.6*), and *mcr-5* (*mcr-5.1* to *mcr-5.4*). Phylogenetic research indicated that *mcr-3*, *mcr-4*, and *mcr-5* are derived genes of *mcr-1*/*mcr-2*. In 2018, novel *mcr* gene variants, *mcr-6* (*mcr-6.1*), *mcr-7* (*mcr-7.1*), and *mcr-8* (*mcr-8.1-mcr-8.5*), were discovered, resulting in an expanded range ofcolistin resistance (123-125). Carrol et al. identified a new *mcr* homolog, designated *mcr-9* (*mcr-9.1* to *mcr-9.3*), in multidrug-resistantcolistin-susceptible *Salmonella enterica* (*S. enterica*) *serovar Typhimurium* isolates (126). Unexpectedly, the *S. enterica serovar Typhimurium* strain exhibited phenotypic sensitivity tocolistin, with an MIC of 2 µg/ml, in accordance with European Committee on Antimicrobial Susceptibility Testing (EUCAST) recommendations. Comparative research indicated that the protein structures of all nine *mcr* homologs (*mcr-1* to *mcr-9*) demonstrated

significant structural similarity among *mcr-3, mcr-4, mcr-7,* and *mcr-9* genes (126).

The *mcr-10* (*mcr-10.1*) variation has recently been found on an *Inc*FIA plasmid in a clinical strain of *Enterobacter roggenkampii*. This *mcr* variation has the highest nucleotide identity (79.69%) with *mcr-9* and yields Mcr-10, which shares 82.93% amino acid identity with Mcr-9 (127).

Given the rapid, global emergence of plasmid-mediatedcolistin resistance genes (*mcr-1* through *mcr-10*) across diverse bacterial species and environments, including human, animal, and environmental samples, the potential for widespread horizontal dissemination is a significant public health concern (128). Identifying *mcr* genes is crucial for monitoring plasmid-mediated resistance and tracking the global dissemination ofcolistin resistance among Gram-negative pathogens (129-131). Notably, *mcr* genes are often located on mobile plasmids (e.g., IncI2, IncX4, IncHI2) that frequently co-harbor other resistance determinants, such as ESBLs (bla_{CTX-M}) and carbapenemases (bla_{KPC}, bla_{OXA-48}, bla_{NDM}). This co-localization facilitates the horizontal transfer of resistance to multiple antimicrobial classes, accelerating the emergence and spread of multidrug- and extensively drug-resistant strains across bacterial species and ecological settings (80).

10. The Role of β-Lactam/β-Lactamase Inhibitor Combinations and Cefiderocol in Antibiotic Resistance

The continuous rise of carbapenem-resistant Gram-negative bacteria has driven the development of new β-lactam/β-lactamase inhibitor combinations to combat these formidable pathogens (132). These new agents offer advantages over revived agents such ascolistin, primarily due to the latter's unfavorable toxicity profile, suboptimal pharmacokinetics, and increasing resistance rates, all of which complicate therapeutic decision-making and adversely affect patient outcomes (133). These agents include carbapenem-based combinations, such as meropenem/vaborbactam and imipenem/cilastatin/relebactam; cephalosporin-based combinations, including ceftazidime/avibactam and the siderophore cephalosporin cefiderocol. Furthermore, two β-lactam/β-lactamase inhibitor combinations approved in the past two years, namely aztreonam/avibactam, a monobactam paired with a β-lactamase inhibitor, and sulbactam/durlobactam, a combination of β-lactamase inhibitors (134).

While some agents, such as ceftazidime/avibactam, are now commercially available in Egypt (135), others have yet to receive local regulatory approval, though ongoing studies are evaluating their potential clinical role.

Table 1 summarizes the core features of new agents against carbapenem-resistant Gram-negative bacteria, including their approved indications, the Ambler β-lactamase classes they inhibit, and their key mechanisms and target organisms.

Table 1: Summary of clinical use, β-Lactamase coverage, and mechanism of action of novel β- Lactam/ β-Lactamase inhibitor combinations and cefiderocol

Agent	Key Approvals & Indications	Target β-Lactamase	Mechanism of action & target organism	References
Carbapenem-based combinations				
Meropenem/ Vaborbactam	**FDA:** cUTI. **EMA:** cUTI, cIAI, HAP, VAP.	Serine carbapenemases, specifically KPC enzymes.	**MOA:** Vaborbactam (a cyclic boronic-acid inhibitor) inhibits serine carbapenemases, mainly KPC, preventing meropenem degradation. **Target organism:** Strong first-line option for severe KPC-CRE infections.	(132, 136, 137, 138)
Imipenem/ Cilastatin/ Relebactam	**United States & European Union:** Nosocomial pneumonia, cUTIs, cIAIs, and other infections by CRE and carbapenem-resistant *Pseudomonas*	Class A (including KPC) and Class C β-lactamases.	**MOA:** Relebactam (a DBO derivative) inhibits class A (KPC) and class C β-lactamases. **Target organism:** Effective against CRE strains. Retains some activity against non-MBL-producing carbapenem-resistant *P. aeruginosa* strains.	(136, 139)

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Cephalosporin-based combinations and siderophore cephalosporin				
Ceftazidime/ Avibactam	**FDA & EMA:** cIAI, cUTI, HAP, VAP.	Class A (e.g., KPC), Class C (AmpC), and selected Class D (OXA-48-like) β-lactamases	**MOA:** Avibactam (a non-β-lactam DBO inhibitor) exhibits potent inhibition of class A, C, and selected class D, protecting ceftazidime (a third-generation cephalosporin) from degradation **Target organism:** Active against CRE and carbapenem-resistant *P. aeruginosa* (non-MBL producers). **Note:** Co-administration with Aztreonam overcomes MBL limitation (viable for MBL-producing *Enterobacterales*).	(16, 136, 139, 140)
Cefiderocol	**FDA:** cUTI, HAP, VAP.	Active against all Ambler classes: Class A (KPC) and ESBLs (CTX-type, SHV-type, TEM-type), Class B (NDM, IMP, VIM), Class C (AmpC), Class D (OXA, OXA-24, OXA-48, OXA-48-like).	**MOA:** "Trojan horse" mechanism: Catechol-type siderophore cephalosporin that binds to iron, entering bacterial cell via active iron transporters. Circumvents resistance mechanisms like decreased permeability, efflux pump upregulation, and carbapenemase inactivation. **Target organism:** Effective against aerobic fermentative and non-fermentative MDR Gram-negative bacilli Reserved for patients with few or no alternative therapeutic options.	(136, 141, 142, 143, 144, 145)
Other β-lactam/β-lactamase inhibitor combinations				
Aztreonam/ Avibactam	**EMA & FDA:** cIAI, **EMA:** HAP, VAP, cUTI, and infections by aerobic Gram-negative bacteria (hindered/scarce therapeutic alternatives).	The combination covers Class A (including KPC, ESBLs), Class C (AmpC), Class D (OXA-48), and Class B MBLs (specifically VIM or NDM-type).	**MOA:** The combination works because Avibactam protects Aztreonam (a monobactam) from being destroyed by the concurrent serine β-lactamases (Classes A, C, and D) often co-expressed alongside MBLs in resistant bacteria. This safeguard ensures that the intact Aztreonam is free to act, as it is already safe from the MBLs themselves. **Target organism:** Robust efficacy against MBL-producing carbapenem-resistant Gram-negative bacteria, especially those that produce a diverse set of β-lactamases (Serine β-lactamases and MBLs together)	(132, 137, 139, 146)
Sulbactam/ Durlobactam	**FDA:** HAP, VAP.	Serine β-lactamases, predominantly OXAs (produced by the *A. baumannii–calcoaceticus* complex).	**MOA:** Durlobactam (a non-β-lactam β-lactamase inhibitor) protects sulbactam, which has inherent mild antibacterial activity. **Target organism:** Specifically approved for *A. baumannii-calcoaceticus* complex infections.	(134, 147, 148)

Abbreviations: *cIAI: Complicated intra-abdominal infections, CRE: Carbapenem-resistant Enterobacterales, cUTI: Complicated urinary tract infections, DBO: Diazabicyclooctane, EMA: European medicines agency, FDA: Food and Drug Administration, HAP: Hospital-acquired pneumonia, MBLs: Metallo-beta-lactamases, MOA: Mechanism of action, VAP: Ventilator-associated pneumonia*

10.1. Carbapenem-based combinations

10.1.1. Meropenem/vaborbactam

Meropenem/vaborbactam is the combination of meropenem with the new-generation beta-lactamase inhibitor vaborbactam. This combination was initially approved by the FDA in 2017 for the treatment of complicated urinary tract infections (cUTI), including pyelonephritis. In 2018, meropenem/vaborbactam was also approved by the European Medicines Agency (EMA) for treatment of cUTI, complicated intra-abdominal infections (cIAI), and hospital-acquired pneumonia (HAP) and ventilator-associated pneumonia (VAP) (136).

Vaborbactam, a cyclic boronic-acid inhibitor, is specifically designed for potent inhibition of serine carbapenemases, especially KPC enzymes, but lacks activity against MBLs and shows no efficacy against *A. baumannii* or *P. aeruginosa* (132, 137).

Given its activity against ceftazidime/avibactam-resistant KPC variants and its favorable pharmacokinetics/pharmacodynamics properties, meropenem/vaborbactam emerges as a strong first-line option for treating severe KPC-CRE infections (138).

10.1.2. Imipenem/cilastatin/relebactam

Imipenem/cilastatin/relebactam is approved in the US as well as in the European Union, in adults for the treatment of nosocomial pneumonia, cUTIs, cIAIs, and other infections by CRE and carbapenem-resistant *Pseudomonas* strains in the case of limited or no alternative treatment options (136).

Relebactam, a diazabicyclooctane (DBO) derivative structurally related to avibactam, effectively inhibits class A (including KPC) and class C β-lactamases but demonstrates no activity against class D carbapenemases and no efficacy against MBLs. Similar to vaborbactam, relebactam is ineffective against *A. baumannii*, although it retains some activity against non-MBL-producing carbapenem-resistant *P. aeruginosa* strains (139).

10.2. Cephalosporin-based combinations and siderophore cephalosporin

10.2.1. Ceftazidime/avibactam

Ceftazidime/avibactam is a ß-lactam/ß-lactamase inhibitor combination authorized by the FDA and the EMA for the management of cIAI, cUTI, HAP, and VAP. Ceftazidime is a third-generation cephalosporin exhibiting a wide range of efficacy against Gram-negative bacilli, including *P. aeruginosa* (136).

Avibactam, a non-β-lactam DBO inhibitor, exhibits potent inhibition of class A (e.g., KPC), class C (AmpC), and selected class D (OXA-48-like) β-lactamases, particularly those associated with *K. pneumoniae*, protecting ceftazidime against hydrolysis. However, it has no effect on MBL producers. It also has no activity against *A. baumannii* but has demonstrated activity against *P. aeruginosa* isolates that are resistant to carbapenems but do not produce MBLs (139).

Although the ceftazidime/avibactam combination is ineffective against MBL producers, co-administration with Aztreonam has been shown to overcome this limitation (16). The combination of ceftazidime/avibactam and Aztreonam has emerged as a viable therapeutic option for BSIs caused by MBL-producing *Enterobacterales*, particularly NDM and VIM producers. In a prospective study involving 343 patients from 2019 to 2022, the combination of ceftazidime/avibactam and Aztreonam was the predominant regimen for treating infections caused by MBL-producing *Enterobacterales*, utilized in 62.7% of patients. In comparison tocolistin-based regimens, the combination of ceftazidime/avibactam and Aztreonam was independently linked to a notable decrease in 30-day mortality, with synergy between ceftazidime/avibactam and Aztreonam observed in 99.7% of evaluated isolates. Patients administeredcolistin experienced significantly elevated incidence of adverse events, notably acute renal injury, in comparison to those treated with ceftazidime/avibactam combined with Aztreonam (140).

10.2.2. Cefiderocol

Cefiderocol is a siderophore-cephalosporin that was approved by the FDA for the treatment of urinary tract infections and nosocomial pneumonia, including both HAP and VAP. It exhibits *in vitro* activity against aerobic fermentative and non-fermentative MDR Gram-negative bacilli (136).

Cefiderocol is a synthetic compound consisting of a cephalosporin moiety and a catechol-type siderophore, which binds to iron and enables bacterial cell entrance through active iron transporters, employing a "Trojan horse" mechanism (141). Upon entering the periplasmic region, it dissociates from iron, and the cephalosporin moiety mostly binds to PBP3, thereby inhibiting bacterial cell wall

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formation (142). Cefiderocol's capacity for active transport within the cell enables it to circumvent resistance mechanisms caused by diminished bacterial membrane permeability, which arises from decreased expression or mutation of porin channels, upregulation of efflux pumps, and inactivation by carbapenemases (141, 143, 144). Cefiderocol exhibits efficacy against ESBLs in CRE, including CTX-type, SHV-type, and TEM-type, as well as all Ambler classes of β-lactamases: class A (KPC), class B (NDM, IMP, and VIM), class C (AmpC), and class D (OXA, OXA-24, OXA-48, and OXA-48-like) (145). Cefiderocol's broad spectrum of activity against all carbapenemases renders it reserved for patients with few or no alternative therapeutic alternatives, hence mitigating the risk of widespread resistance or serving as empirical treatment in high-resistance environments (141).

10.3. Other β-lactam/β-lactamase inhibitor combinations

10.3.1. Aztreonam/avibactam

Aztreonam/avibactam is an antibiotic authorized by the EMA for the management of cIAI, HAP, including VAP, cUTI, including pyelonephritis, and infections attributable to aerobic Gram-negative bacteria in patients with hindered therapeutic alternatives. The FDA has similarly authorized aztreonam/avibactam for the treatment of individuals with cIAI when therapeutic alternatives are scarce or nonexistent (139).

Avibactam proficiently inhibits class A and C β-lactamases, along with certain class D enzymes. Nevertheless, it does not impede MBLs. The combination of avibactam with the monobactam aztreonam exhibits robust efficacy against MBL-producing carbapenem-resistant Gram-negative bacteria. This effectiveness arises from the inability of MBLs to hydrolyze Aztreonam, which stays structurally unaltered. Moreover, avibactam augments the efficacy of Aztreonam by safeguarding it from degradation by concurrent serine β-lactamases, commonly found in carbapenem-resistant Gram-negative bacteria. Therefore, for *Enterobacterales* strains demonstrating diverse resistance mechanisms that include VIM or NDM-type MBL alongside co-expression of ESBLs, KPC, OXA-48, or AmpC β-lactamases, the combination therapy of aztreonam/avibactam is a successful treatment strategy (132, 137, 146).

10.3.2. Sulbactam/durlobactam

Sulbactam is a β-lactamase inhibitor that contains a β-lactam ring (147). The β-lactam ring imparts sulbactam with inherent mild antibacterial activity, unlike other β-lactamase inhibitors that require binding to the β-lactam to demonstrate their antibacterial efficacy (147).

Durlobactam is a non-β-lactam β-lactamase inhibitor that, when paired with sulbactam, safeguards the latter from degradation by certain serine β-lactamases, predominantly OXAs, produced by the *A. baumannii–calcoaceticus* complex (134). Durlobactam is a chemical derivative of avibactam (148).

Sulbactam/durlobactam was approved by the FDA in May 2023 for use in adult patients with HAP and VAP due to *A. baumannii–calcoaceticus* complex (134).

11. Conclusion and Future Perspectives in the Egyptian Context

The emergence of carbapenem-resistant Gram-negative bacteria is a major global public-health threat, and Egypt is no exception. This rise has created a critical gap in effective therapy, prompting the re-use ofcolistin, an older polymyxin largely abandoned because of nephrotoxicity and neurotoxicity, as a last-resort option. Alarmingly,colistin resistance is increasing, driven in large part by uncontrolled veterinary use and the dissemination of mobilizedcolistin resistance (*mcr*) genes; a recent systematic review reportedcolistin resistance in roughly 9% of Gram-negative isolates and nearly 31% of carbapenem-resistant strains in Egypt. These findings highlight the urgent need for robust antibiotic stewardship and strengthened national surveillance to curb antimicrobial resistance. Novel β-lactam and β-lactam/β-lactamase-inhibitor combinations offer promising, safer alternatives, but access and affordability remain major barriers in LMICs; although ceftazidime-avibactam is now commercially available in Egypt, several other agents lack local approval and continue to be evaluated in clinical studies.

Data Availability
This study did not generate or analyze any new data.

Conflicts of Interest
The authors declare that they have no conflicts of interest.

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Hepatoprotective Effect of Combretum bauchiense Leaves Hutch & Dalziel (Combretaceae) Against Paracetamol-Induced Hepatotoxicity in Rats.

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ABSTRACT

Medicinal plants have been traditionally used to treat liver diseases associated with impaired liver function caused by exposure to toxicants. Ethnomedicinal reports on hepatoprotective potential of some Combretum species have been documented, though there is no such information on Combretum bauchinese. This study was designed to evaluate the hepatoprotective effects of extract and fractions of Combretum bauchinese (Hutch.) leaves against Paracetamol-induced liver injury. The methanolic extract and different fractions were obtained by cold maceration and solvent partitioning methods to afford the methanol extract, hexane fraction, ethyl acetate fraction, and butanol fraction. Hepatoprotective activity was evaluated using paracetamol-induced liver injury; thirty-five (35) rats were divided into seven (7) groups of five (5) animals each and subjected to an 8-day study. Group A received distilled water, while groups B-F received plant samples: Group B received 200mg/kg of the methanolic extract, Group C received 400mg/kg of the extract, and Groups D, E, and F received 400mg/kg of fractions for 7 days. Group G received 100mg/kg standard drug. Paracetamol (3g/kg) was administered orally to induce hepatotoxicity on the eighth day. Blood was collected from each rat through ocular puncture, allowed to coagulate, and centrifuged at 3000 rpm for 15 min to obtain a clear supernatant. This supernatant was used to assay Aspartate Aminotransferase (AST), Alanine Aminotransferase (ALT), Alkaline Phosphate (ALP), and Bilirubin levels using Randox kits according to the manufacturer's protocols. The hepatoprotective activity of the methanolic extract showed a dose-dependent effect. A 400mg/kg of methanolic extract gave a significant reduction of ALT (21.75 U/L), AST (32 U/L), and ALP (46 U/L), exhibiting better hepatoprotection than the fractions at 400mg/kg, which showed similarity in activity, although not as significant as the high dose of the methanolic extract. In conclusion, Combretum bauchiense leaves were found to possess hepatoprotective activity by decreasing the level of liver enzymes due to paracetamol-induced hepatotoxicity. Our finding gives credence to Combretum bauchiense, just like other Combretum species, as used in traditional medicine to treat liver injury.

KEYWORDS

Combretum genus, liver function, medicinal plant, phytochemicals, extraction, hepatotoxins

1. Introduction

The liver is a key organ that performs vital functions in the body, such as metabolism, secretion, storage, and detoxification of a

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variety of drugs and xenobiotics. Liver injury is among the most serious human diseases as it's considered the engine house, and if left unchecked, such injury will result in loss of life. The liver consists mainly of hepatocytes, Kupffer Cells, and endothelial cells, which are targets of liver diseases caused by hepatotoxins: certain antibiotics, paracetamol overdose, long exposure to carbon-tetrachloride and other chlorinated hydrocarbons, excess consumption of alcohol, infections, and autoimmune/disorders (1). This effect is indicated by increased serum liver marker enzyme levels: Alanine Aminotransferase (ALT) and Aspartate Aminotransferase (AST), and total bilirubin levels (2). Liver detoxification of chemicals occurs via the conversion of reactive oxygen species into non-toxic compounds by antioxidant enzymes. This prevents oxidative injury by minimizing the oxidation of substrates either by inhibition of free radical formation or the propagation step or by chelation of metal ions, thereby decreasing the level of liver enzymes (3). Medicinal plants have been used to manage liver injury, and the effective hepatoprotective agent silymarin, derived from *Silyum marianum,* is available in the pharmaceutical stores (4). This highlights the need for us to investigate other medicinal plants used in traditional medicine to treat various disease conditions to uncover their potential as hepatoprotective agents.

Combretum bauchiense Hutch. & Dalziel is an underexplored species of Combretum belonging to the family of Combretaceae. *C. bauchiense* is a suffrutex known for its herbaceous, erect stems and woody rootstock, bearing leaves that are simple, opposite, whorled, sub-opposite, or alternate (5). The genus Combretum comprises about 250 different species of plants found around the tropical and subtropical regions of Africa. They are widely used in African traditional medicine for the treatment of several disease conditions, including liver injury, due to their ethnomedicinal properties (6).

Ethnomedicinal report revealed that *Combretum adenogonium*, *Combretum crotonoids*, and *Combretum micranthum* are used to treat liver diseases, as reported by (7). Some species of Combretum have been scientifically evaluated for hepatoprotective properties using different bioassay models with promising results, highlighting the plant's potential as a source of hepatoprotective agents. According to a recent report, *C. platypterum* leaf methanol extract showed hepatoprotective potential by enhancing the hepatic antioxidant defences involving SOD, CAT enzymes, and GSH on paracetamol-induced liver injury in rats (8). In another report, aqueous extract of the roots of *C. sericeum* restored the elevated level of biochemical parameters due to paracetamol-induced hepatic damage (9). The ethanol extract of *the whole plant of Combretum albidum* decreased the rise in ALT, AST, ALP, TB, and TBRAS levels caused by carbon tetrachloride intoxication (10). Ethanol extract of *Combretum hypopilium* root bark significantly decreased levels of hepatic enzymes and restored the decreased levels of hepatic antioxidants in carbon tetrachloride-induced liver injury (11).

Previous reports showed that the aqueous extract of *Combretum dolichopentalum* leaves reduced the elevated levels of liver enzymes against CCl4-induced liver damage in rats (12). Similarly, *Combretum micranthus* aqueous leaf extract significantly decreased liver marker enzyme levels in paracetamol-induced liver damage in rats (13). These findings suggested that some species of Combretum possess hepatoprotection against hepatotoxic agents due to the presence of the bioactive metabolites in the aqueous and ethanol extracts of the different plant parts. This suggested that polar compounds were responsible for the observed hepatoprotective activity of Combretum species, though contrary to this claim, research isolated three triterpenes: lupane type, 2R,6â-dihydroxybetulinic acid, 6â-hydroxyhovenic acid, and an oleanane type, 6â-hydroxyarjunic acid from *Combretum quadrangulare* seeds with potent hepatoprotective property against D-GalN/TNF-R-induced cell death in primary cultured mouse hepatocytes (14). The Combretum genus could be an invaluable resource in the discovery of therapeutic agents for the management of liver injury, either as a phytomedicine or a lead in the development of pharmaceuticals. To the best of our knowledge at the time of this research, there were no scientific reports on the hepatoprotective activity of *Combretum bauchiense*; hence, this study seeks to evaluate the hepatoprotective activity of *C. bauchiense* leaf extract and fractions against paracetamol-induced liver injury in rats.

2. Experimental

Collection of plant materials

The plant was collected from Ezeani in Nsukka Local Government Area (L.G.A) and authenticated by a Taxonomist, Mr. Felix Nwafor, in the Department of Pharmacognosy and Environmental Medicine, University of Nigeria, Nsukka, where the prepared voucher specimen was deposited (herbarium number: PCG-019/02).

Preparation and extraction of material

The leaves of *C. bauchiense* were air-dried at room temperature for about 14 days in a confined area. After drying, the leaves were pulverized into a coarse powder and made ready for extraction. The pulverized plant sample (200g) was transferred into a bottle, and 2 L of methanol was added. The container was made air-tight and allowed to extract for 72 hours with intermittent agitation. The mixture was then filtered using cotton wool clogged in a funnel and Whatman No. 1 filter paper to obtain clear filtration. The filtrate was concentrated under reduced pressure using a rotary evaporator to obtain the methanol extract.

Liquid-liquid partitioning of the methanol extract

Liquid-liquid extraction was used to partition the extract in order of increasing polarity (n-hexane, ethyl acetate, and n-butanol). The dry extract (10g) was reconstituted in 10% aqueous methanol and poured into a separatory funnel for partitioning using different solvents in succession with n-hexane (5 x 100 mL), ethyl acetate (7 x 100 mL) and n-butanol (2 x 100 mL), the resulting fractions were concentrated using rotary evaporator to obtain n-hexane, ethyl acetate and n-butanol fractions respectively. The dried fractions were stored at 4°C in a refrigerator for further analysis.

Hepatoprotective evaluation using Paracetamol-induced hepatotoxicity model

Thirty-five (35) Albino Wister rats with body weights ranging from 116.2g to 130.6g sourced from the Department of Pharmacology, Enugu State University of Science and Technology, Agbani, were used for the experiment. The rats were allowed to acclimatize in the experimental lab for 5 days, during which they were fed on a standard pellet diet and given water ad libitum. The food was withdrawn 12 hours before the time of the experiment; however, they were allowed free access to water. The animals were divided into seven (7) groups of five (5) animals each and subjected to an 8-day study. The rats received treatment for 7 days, then paracetamol (3g) was administered orally to induce hepatotoxicity on the ^{eighth} day, as presented in Table 1. Thereafter, blood samples were collected 24 hours after the administration of paracetamol through ocular puncture into sample bottles, allowed to coagulate, and centrifuged at 3000 rpm for 15 min to obtain a clear supernatant. The resulting supernatant was carefully decanted and placed on ice for the assay for liver marker enzymes such as Aspartate Aminotransferase (AST), Alanine Aminotransferase (ALT), Alkaline Phosphate (ALP), and serum Bilirubin. The liver enzymes were determined spectrophotometrically using Randox analytical kits according to the manufacturer's standard protocols.

Table 1: Drug administration for the paracetamol-induced model

Groups	Treatment received
A (negative control)	1ml/kg water for 7 days and paracetamol 3g/kg orally administered on the eighth day
B (induced and treated)	200mg/kg extract for 7 days and paracetamol 3g/kg orally administered on the eighth day
C (induced and treated)	400mg/kg extract for 7 days and paracetamol 3g/kg orally administered on the eighth day
D (induced and treated)	400mg/kg hexane fraction for 7 days, and paracetamol 3g/kg administered on the eighth day
E (induced and treated)	400mg/kg ethyl acetate fraction for 7 days, and paracetamol 3g/kg administered orally on the eighth day
F (induced and treated)	400mg/kg butanol for 7 days and paracetamol 3g/kg orally administered on the eighth day
G (positive control)	25mg/kg silymarin for 7 days and paracetamol 3g/kg orally administered on the eighth day

3. Statistical Analysis

All the experimental data are expressed as mean ± standard deviation. For data processing and analysis, SPSS version 21.0 was used and presented as mean ± standard deviation. The comparisons and significant differences between control and treated groups were determined using one-way ANOVA followed by a post hoc test. The results were considered significant at $p<0.05$.

4. Results

The results of hepatoprotective activity of *C. bauchiense* indicated a significant reduction in liver enzymes at a dose of 400mg/kg of the methanolic extract, which was more effective than the fractions and standard drug, as presented in Table 2 and fig. 1

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Table 2: Effect of extract and fractions on paracetamol-induced hepatotoxicity

Group/Treatment	ALT (U/L)	AST (U/L)	ALP (U/L)	BILI (g/dl)
A (1mL water + paracetamol 3g/kg)	41.25 ± 1.92^a	61.25 ± 2.38^a	89.25 ± 3.56^a	8.78 ± 0.3^b
B (200mg/kg ME + paracetamol 3g/kg)	25.75 ± 1.48^b	35.75 ± 1.48^b	43 ± 2.24^b	5.83 ± 0.33^b
C (400mg/kg ME + paracetamol 3g/kg)	21.75 ± 1.48^b	32 ± 1.22^b	46 ± 1.22^b	5.63 ± 0.15^b
D (400mg/kg HF + paracetamol 3g/kg)	40.52 ± 1.30^a	53.61 ± 4.03^a	86.07 ± 0.88^a	5.23± 0.56^b
E (400mg/kg EAF + paracetamol 3g/kg)	39.51 ± 1.19a	44.83 ± 3.31^b	85.33 ± 1.04^a	5.33 ± 0.55^b
F (400mg/kg BF + paracetamol 3g/kg)	42.61 ± 4.14^a	52.99 ± 3.70^a	85.74 ± 0.95^a	5.59 ± 0.06^b
G (100mg/kg SILYM + paracetamol 3g/kg)	23.25 ± 1.92^b	34.25 ± 1.48^b	45 ± 2.24^b	5.83 ± 0.29^b

Data expressed as (mean ± SD). Superscript a indicates no significant difference between treated and untreated groups, and superscript b indicates a significant difference between treated and untreated groups at P< 0.05. ALT = Alanine Aminotransferase; AST = Aspartate Aminotransferase; ALP = Alkaline phosphate; BILI = Bilirubin, ME = methanol extract, HF hexane fraction, EAF ethyl acetate fraction, BF = butanol fraction, SILYM silymarin.

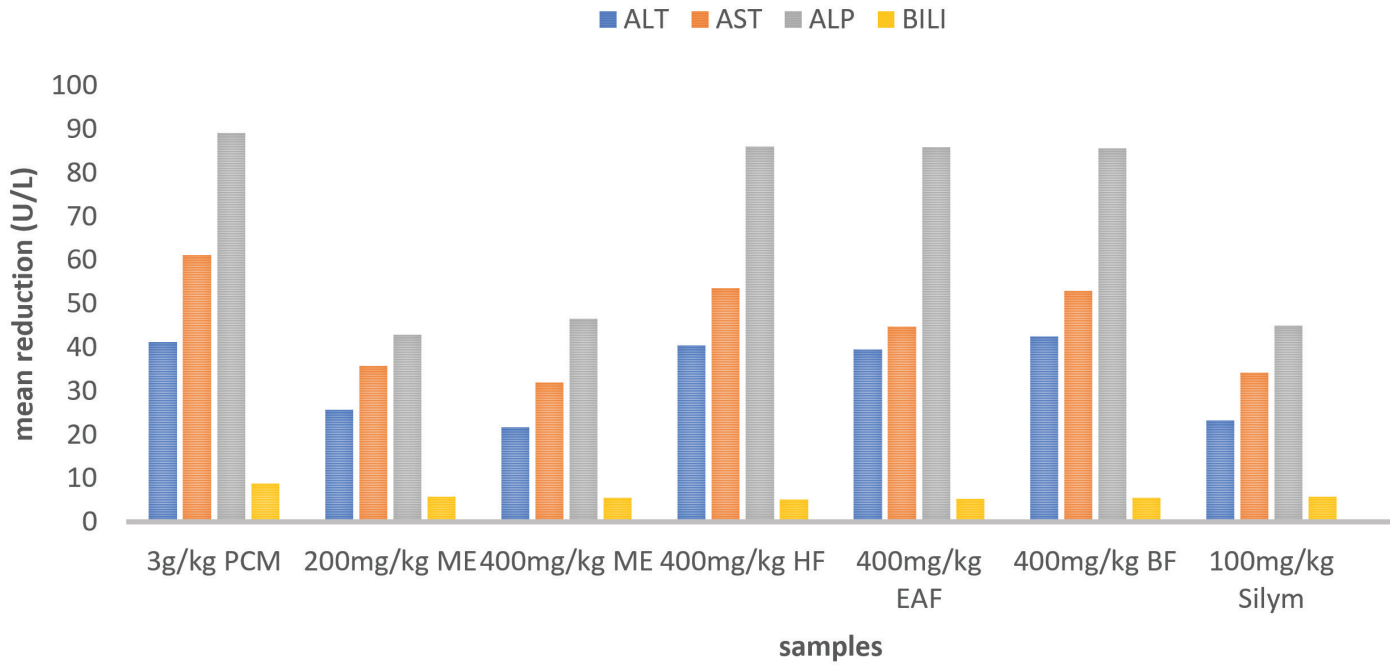

Figure 1: Effect of extract and fractions of C. bauchiense leaves on paracetamol-induced hepatotoxicity

5. Discussion

Paracetamol exerts its toxic effect through the release of toxic metabolite NAPQI, usually detoxified by conjugation with glutathione, but at high concentrations, glutathione may be insufficient to detoxify NAPQI, leading to liver damage. The manifestation of liver damage results in an increase in the serum transaminase levels as a marker of hepatotoxicity (15). In this study, *Combretum bauchiense* demonstrated potential for the attenuation of hepatotoxicity caused by paracetamol overdose, as presented in Table 2. The group administered with paracetamol showed elevated levels of serum transaminase, 41.25 ± 1.92 ALT, 61.25 ± 2.38 AST, 89.25 ± 3.56 ALP, and 8.78 ± 0.3 bilirubin, indicating liver damage. The elevated levels of ALT (41.25 ± 1.92) and AST (61.25 ± 2.38), the key indicators of cellular necrosis, are evidence of liver injury due to paracetamol intoxication as reported by …. Our results showed that there was no significant difference between the effect of the paracetamol-induced group and the group treated with the fractions. This suggests that none of the fractions possess hepatoprotective activity as presented in Figure 1. It is interesting to note that administration of 200 and 400mg/kg of

the methanol extract of *C. bauchiense* reduced the elevated level of liver enzyme in a dose-dependent manner better than the fractions. Our findings showed no significant difference between the standard drug silymarin 100mg/kg and the effects of methanolic extract at 200 and 400mg/kg at $P < 0.05$, whereas a significant difference was observed between hepatotoxic groups treated with methanolic extract see Fig. 1. The high level of bilirubin in the group treated with paracetamol is indicative of hepatotoxicity because of erythrocyte breakdown. The administration of the methanolic extract of *C. bauchiense* restored the bilirubin level to normal, suggesting hepatoprotection. Different fractions were evaluated to establish which of the fractions had the best hepatoprotective activity. All the fractions showed comparable reduction of the hepatic enzymes at 400mg/kg; however, the methanol extract at both 200 and 400mg/kg outperformed all individual fractions. The implication could be that the constituents of the extract have a synergetic effect, suggesting the use of the plant extract rather than fractions in the treatment of liver injury. The present study corroborates previous research on Combretum species that demonstrated significant hepatoprotection against liver damage (12)(9)(10). Our study showed that *C. bauchiense* contains putative agents capable of protecting and maintaining the functional integrity of damaged hepatic cells. Earlier phytochemical investigations revealed the presence of flavonoids and terpenoids, which are known for their hepatoprotective activity (16)(17). These bioactive constituents have been reported to alleviate liver injury by modulating key signaling pathways such as Nrf2, NF-κB, autophagy, free radical scavenging, and apoptosis (18). Our research confirms the hepatoprotective potential of *C. bauchiense* leaves against paracetamol-induced liver damage. Therefore, developing and standardizing the plant extract-based phytomedicine from the leaves of *C. bauchiense* could offer an alternative option to alleviate hepatotoxicity and oxidative stress.

6. Conclusion

The present study demonstrated that the leaves of *Combretum bauchinese* exhibit significant hepatoprotective properties. The methanol extract showed greater effectiveness in restoring liver function enzyme levels compared to fractions, suggesting a possible synergetic effect of the bioactive constituents present in the extract. This has offered scientific validation for the traditional belief in the efficacy of *C. bauchinese* in treating various ailments. We recommend further research to establish mechanisms of hepatoprotection and standardization of the extract for possible development of phytomedicine.

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Conflict of interest

The authors declared no conflict of interest.

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Recent Advanced Technologies for Ocular Drug Delivery: The Transformative Impact of Nanotechnology on Treating Eye Disorders

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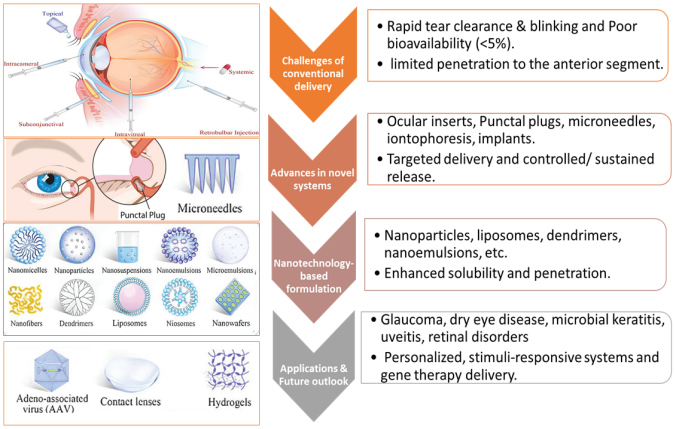
ABSTRACT

Ocular drug delivery is profoundly challenging due to natural barriers like the corneal epithelium and blood-ocular barriers, which restrict drug penetration, resulting in low bioavailability (<5%) and frequent dosing required by conventional eye drops, thus hindering therapeutic efficacy. To overcome these limitations, innovative delivery platforms, most notably nanotechnology-based systems (including nanoparticles, liposomes, and cubosomes) and advancements like microneedles and sustained-release implants, are being developed to ensure longer residence duration, tailored drug release, and improved penetration for diseases spanning the anterior and posterior segments. While these nanocarriers have demonstrated clinical potential and are already licensed for use, significant obstacles related to long-term safety, cost-effectiveness, and large-scale manufacturing must be standardized. Ultimately, the future integration of smart stimuli-responsive systems, gene therapy, and personalized platforms promises to transform ophthalmic care by delivering safer, more effective, and sustained patient-specific treatments.

KEYWORDS

Ocular, Nanotechnology, Nanoparticles, Glaucoma, Retinopathy.

Graphical Abstract



1. Introduction

The challenge of delivering drugs to the eye

1.1. The Eye's Fortifications

The eye is comprised of three layers: connective, vascular, and neural tissues. The connective tissue consists of the transparent cornea, which is connected to the white sclera through the limbus. The vascular tissue is composed of the choroid, as well as two ciliary bodies in the middle, connected at the front by the iris. The retina constitutes the neural tissue, which functions to transmit electrical impulses to the brain through the optic nerve (1).

The lens is another key transparent structure inside the eye. It is located beneath the iris and is suspended between the ciliary bodies by two ligaments known as the zonule of Zinn (2). The ocular globe is divided into two segments: an anterior segment (filled with aqueous humour)

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and a posterior segment (filled with vitreous humour). The anterior portion of the eye is composed of the cornea, conjunctiva, iris–ciliary body (ICB), lens, and aqueous humour. The posterior segment, on the other hand, is the primary ocular structure, consisting of the sclera, choroid, and retina, which surround the vitreous cavity filled with vitreous humour (3,4).

The eye has various distinct anatomical and physiological barriers that dramatically reduce the bioavailability of medicines, particularly those applied topically. The cornea is one of the principal barriers, as it is part of the static anatomical barriers and is made up of tightly packed epithelial cells and stromal tissue that prevent medicines from entering the anterior chamber of the eye. Furthermore, tear film dynamics, such as tear turnover, nasolacrimal drainage, and blinking, serve as crucial physiological barriers that rapidly wash away delivered medicines, minimizing their contact duration with ocular surfaces (5). Another key barrier is the blood-ocular barrier (BOB), which consists of both the blood-aqueous barrier (BAB) and the blood-retinal barrier (BRB). These barriers consist of tight junctions between retinal capillary endothelial cells and the retinal pigment epithelium (RPE), thereby preventing systemically or topically administered medications from entering the retina and vitreous compartments (6,7,8). The BRB, in particular, is essential for maintaining retinal homeostasis and preventing harmful substances from reaching sensitive neural tissues.

Furthermore, the mucin layer on the corneal and conjunctival surfaces functions as an additional permeability barrier, especially for large molecules, although the exact impact on topical medication bioavailability is unknown (9). Efflux transporters such as P-glycoprotein (P-gp), multidrug resistance protein (MRP), and breast cancer resistance protein (BCRP) actively expel medicines from intraocular tissues, creating another layer of defense that prevents efficient medication absorption (10). These complex and interrelated barriers underscore the inherent difficulties in attaining therapeutic medication concentrations in ocular tissues.

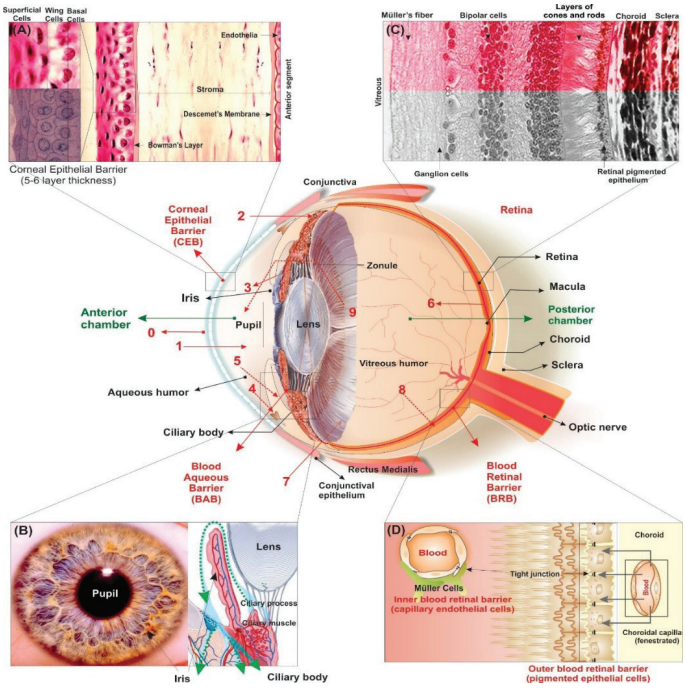

Figure 1: Overview of the anatomical structure of the eye, highlighting physiological barriers that hinder drug delivery (11).

1.2. *The downfall of Droplets*

Conventional methods of ocular drug delivery systems, such as eye drops and ointments, make up about 70% of the ophthalmic medications available in the pharmaceutical market. Among these, eye drops represent nearly 95% of marketed ocular products and remain the most commonly used method. However, despite their widespread use, eye drops are associated with various limitations (12, 13).

Eye drops are favored because they are noninvasive, practical, and safe. However, they suffer from a pulsatile release pattern, where the drug concentration spikes immediately after instillation and then rapidly declines due to physiological clearance, preventing sustained therapeutic levels (14, 15, 16).

Besides the liquid eye drops, there are other forms, such as suspension and emulsion. Ocular suspensions depend on the dispersion of the hydrophobic drug in aqueous solvent; therefore, particle size will be crucial for the physicochemical properties of the formulation. Generally, it's preferred to maintain a particle size <10 µm due to greater solubility, enhanced dissolution rates, but still exhibiting poor retention on the ocular surface. On the other hand, ocular emulsions are a solubilized biphasic system by the presence of surfactants; they provide delivery of hydrophobic drugs as oil-in-water emulsions (O/W), which

exhibit enhanced contact time, bioavailability, and less irritation if compared to water-in-oil emulsions (W/O). In ocular drug delivery, anionic surfactants are generally preferred. Cationic surfactants interact strongly with the negatively charged ocular tissues. This interaction can disrupt cell membranes and cause irritation and toxicity. In contrast, anionic surfactants are much safer and better tolerated.

Another form of eye medication is ointments, which are made with semisolid hydrocarbons that melt at body temperature, to make them more comfortable and less irritating for the patient. Once applied, the ointment melts into small droplets that collect in the cul-de-sac, creating a reservoir for the medication and allowing sustained release. While ointments offer some advantages, they also have some drawbacks. Common problems include blurred vision and discomfort. An alternative to ointments is eye gels, which are also a semisolid dosage form with added polymers to enhance the viscosity and increase bioavailability. They still cause mild and temporary blurred vision, but less prominently than ointments (13). A more comparative overview of conventional ocular drug delivery systems, highlighting their respective advantages, limitations, and future prospects, is illustrated in **Table 1.**

Table 1: Comparative overview of different conventional ocular drug delivery systems.

Types	Brief description	Bioavailability	Advantages	Limitations	Applications	Future Prospects
Eye Drops [17,18]	Clear, sterile aqueous solutions in which the drug is completely dissolved. The most widely used ocular dosage forms are due to simplicity and ease of use. Simplicity.	Very low, around 1–5%, due to rapid precorneal elimination by tear drainage and blinking.	Rapid onset, good tolerability, and excellent patient compliance; ideal for acute treatment of anterior eye conditions.	Necessitates frequent dosing, ineffective for poorly water-soluble drugs.	- Glaucoma - Ocular Infections such as bacterial and viral conjunctivitis or keratitis. - Ocular inflammation due to surgery, trauma, or uveitis. - DED (Dry Eye Disease) to improve lubrication.	- Personalized eye drops based on diagnostic AI tools. - Eye drop delivery of gene-editing technology, such as CRISPR (Clustered regularly interspaced short palindromic repeats), to treat genetic ocular disorders - The delivery of biologics such as Anti-VEGF (anti-vascular endothelial growth factor) is now being explored for delivery through eye drops.
Eye Suspension [19]	A dispersed system containing micronized solid particles intended for drugs with limited water solubility.	Slightly improved over solutions due to slower dissolution and longer retention, especially when particle size is optimized.	Formulations of lipophilic agents can remain longer on the ocular surface due to slower dissolution.	Coarser particles may trigger a foreign-body sensation or mild irritation.	Common in the treatment of ocular inflammation, such as *Pred Forte®* (Prednisolone Acetate Ophthalmic Suspension 1%).	The use of Nanosuspensions containing 100% pure drug in the nano range, by reducing the particle size, increases the surface area and concentration of the drug in the infected area.
Eye Emulsion [20, 21, 22]	Biphasic systems, typically (O/W), are used to solubilize lipophilic drugs for ocular delivery.	Enhanced bioavailability due to better corneal penetration, prolonged contact time, and interaction with the tear film lipid layer.	Ideal for hydrophobic drugs and chronic inflammatory conditions, with low irritation potential.	Require emulsifying agents for stability; may cause mild blurring post-application.	Treatment of dry eye syndrome with an anionic lipid emulsion containing cyclosporine A 0.05% *Restasis™* was approved for clinical use by the FDA (Food and Drug Administration) in December 2002. Also, a non-medicated anionic emulsion formulation, *Refresh Endura®*, for moderate to severe dry-eye syndrome.	- A new generation of artificial tears based on emulsions supplements the tears with lipids acting as a lubricant and, more importantly, as a barrier against evaporation and a tear film stabilizer. - Microemulsions and nanoemulsions, which enhance ocular penetration.

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Eye Gels [19,23,24]	Semisolid formulations with added polymers (e.g., polyacrylic acid, acrylic acids) to enhance the viscosity.	Gels offer higher bioavailability than drops or suspensions.	Gels reduce the frequency of administration, increase patient compliance, and can be tailored for controlled release.	May cause temporary blurring or irritation; formulation challenges include ensuring optimum gelling and clarity.	- Topical anesthesia for surgery or foreign body removal (e.g., *Akten®* FDA-approved ophthalmic gel). - Postoperative inflammation (e.g., *LOTEMAX®* loteprednol etabonate).	- The use of hydrogel-based drug carriers for the delivery of biologic agents in the eye. - The use of in situ-forming gel as a vehicle for loading nano and micro particles to treat ocular diseases.
Eye Ointments [25]	Semisolid formulations using petrolatum or lanolin as a base are ideal for lipophilic drugs and long-term ocular residence.	High bioavailability due to extended retention on the ocular surface and protective barrier effect.	Good choice for lipophilic and moisture-sensitive drugs. Provides prolonged release, enhances drug absorption, and protects the eye post-surgery or during sleep.	Their greasy nature causes vision blurring, limiting daytime use and reducing patient compliance.	- Herpetic keratitis treatment via Avaclyr®, an ocular ointment containing the antiviral acyclovir that was approved in 2019. - Prophylaxis of ophthalmia neonatorum in newborns via Erythromycin 0.5% ophthalmic ointment.	Ointments are currently said to follow a patient-centric approach, with the advancements in nanotechnology and bioengineering.

1.3. The Dawn of a New Era

These previously discussed challenges have created a demand for more advanced, targeted, and sustained release drug delivery technologies. Recent advancements, particularly in nanotechnology, have marked the dawn of a new era in ocular therapeutics. Nanotechnology-based systems, which use nanoscale carriers to increase drug solubility, stability, and targeted administration while minimizing systemic side effects, are among the most promising advances (19, 21, 26). These techniques have shown tremendous promise in both preclinical and clinical contexts, with certain nanocarriers currently approved for use in ophthalmology. The purpose of this review article is to investigate the most recent technological advances in ocular drug delivery, with a special emphasis on nanotechnology, and to assess their therapeutic influence on the treatment of various eye diseases, paving the way for more successful and patient-specific therapies.

2. Advances in Ocular Drug Delivery: Beyond the Nanoscale

For decades, the treatment of ocular disorders has heavily relied on conventional ocular drug delivery systems. As previously discussed, these systems present multiple limitations, including poor drug bioavailability, rapid tear clearance, and issues with patient compliance. Consequently, there has been a pressing need for more advanced ocular drug delivery platforms that offer prolonged residence time, targeted delivery, and enhanced patient adherence. This shift marks the beginning of a new era in ophthalmic care.

2.1. Ocular inserts

Ocular inserts are sterile, thin, multilayered devices with either solid or semisolid consistency. They are designed for placement in the conjunctival cul-de-sac, with careful consideration of Size and shape to ensure suitability for ophthalmic use. These inserts are generally composed of polymers, which may or may not be drug-loaded.

Ocular inserts aim to overcome several disadvantages associated with conventional delivery systems, most notably the “pulse entry” drug release profile. In contrast, they provide controlled, sustained, and continuous drug delivery. A significant advantage of ocular inserts is the reduction in dosing frequency, which contributes to improved patient compliance. However, one of the main limitations of ocular inserts is their solid nature. Patients may perceive them as a foreign body, which can be a barrier to both physical comfort and psychological acceptance. Ocular inserts can be broadly classified based on their solubility into three types: insoluble, soluble, and bioerodible, as shown in **Figure 2.**

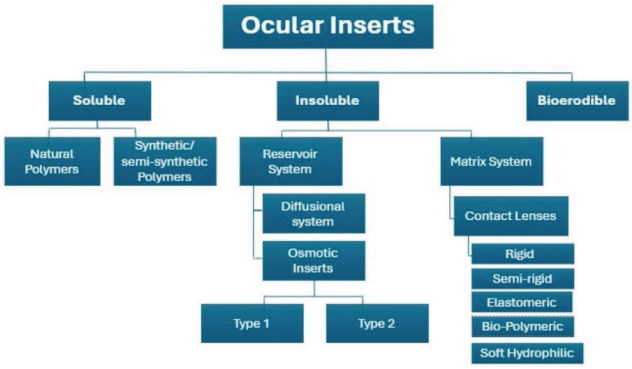

Figure 2: Classification of medicated ocular inserts.

Insoluble inserts are made up of insoluble polymers and may be classified as a reservoir or matrix system, where each system follows a different releasing pattern. Reservoir systems release drugs through either diffusion or osmosis. A prominent example is the *Ocusert®* system, which is a novel ocular delivery platform featuring a porous membrane that regulates the release of a drug reservoir through diffusion at a constant rate, as illustrated in **Figure 3.** The *Ocusert®* pilocarpine system is designed to deliver time-independent drug concentration to ocular tissues. This controlled delivery minimizes side effects such as miosis and myopia, while maintaining effective intraocular pressure (IOP) reduction in glaucoma patients (27).

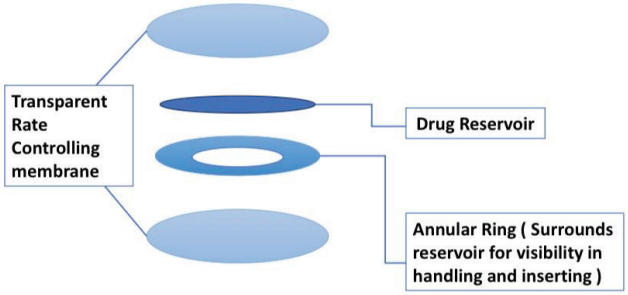

Figure 3: Structure of a reservoir-type ocular insert system, specifically the Ocusert® system.

On the other hand, the osmotic inserts include a central core surrounded by a peripheral layer and exist in two types, as shown **in Figure 4.**

Type 1: Contains a single compartment in which the drug is dispersed throughout a polymer matrix. As osmotic pressure builds, small ruptures form in the semipermeable membrane, allowing the drug to be released near the surface.

Type 2: Consists of two compartments, one for the drug and another for the osmotic solute. Tear fluid enters the solute chamber, generating pressure that stretches an elastic membrane and compresses the drug chamber, facilitating drug release (27).

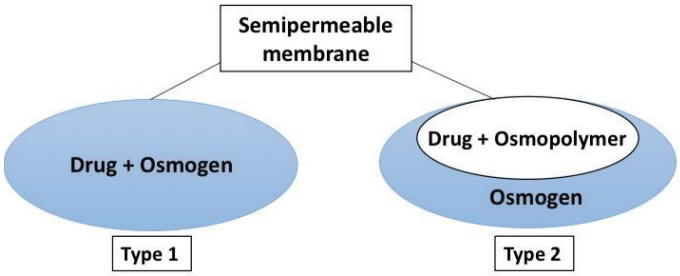

Figure 4: Comparative representation of the two fundamental osmotic insert designs.

Matrix systems are primarily represented by contact lenses. Although traditionally used for vision correction, contact lenses have been repurposed for drug delivery by presoaking them in medicated solutions. They are categorized into five types as mentioned in **Figure 2.**

Rigid lenses, typically made from polymers such as polymethyl methacrylate (PMMA), have poor moisture and oxygen permeability, often causing discomfort. Gas-permeable alternatives, such as cellulose acetate butyrate, offer improved breathability but remain unsuitable for sustained drug delivery. To address this, soft hydrophilic contact lenses have been developed. These offer greater comfort and prolonged drug release for agents including pilocarpine, chloramphenicol, tetracycline, and prednisolone sodium phosphate. Common materials include hydroxyethyl methacrylate, often copolymerized with polyvinylpyrrolidone to increase water content, or ethylene glycol dimethacrylate to reduce it. Drug loading depends on the lens's hydrophilicity, soaking time, drug concentration, and water content.

Now, for the soluble inserts, they dissolve completely in the ocular environment, eliminating the need for removal. These inserts are classified based on the type of polymer used. Type 1 is the Natural polymers (e.g., collagen), while type 2 uses Semi-synthetic polymers (e.g., cellulose derivatives) or Synthetic polymers (e.g., polyvinyl alcohol). Their complete solubility makes them convenient and well-tolerated by patients. However, they may offer lower drug loading capacity and mechanical strength compared to insoluble systems (28).

Bioerodible inserts are formed from polymers that undergo hydrolysis and dissolve over time. A major advantage of these systems is that their erosion rate can be modulated through structural modifications during synthesis and by the addition of anionic or cationic surfactants. However, erosion rates can vary significantly

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based on individual patient physiology. Several commercial and experimental systems include:

SODI (Soluble Ophthalmic Drug Insert): A small, oval, bioerodible insert made from a specially engineered ABE copolymer. This type of copolymer contains Acrylmide derivatives for hydrophilicity and softness, Butyl for controlled erosion, and Ethyl-based monomer for mechanical strength. It softens quickly upon insertion and dissolves within an hour, releasing the drug in a controlled manner.

Collagen Shields: Made from purified animal collagen, these inserts resemble contact lenses and slowly dissolve on the ocular surface. Providing high drug levels in ocular tissues comparable to subconjunctival injections. However, they can cause discomfort, affect vision, and are unsuitable for damaged corneas.

Ocufit: A rod-shaped, insoluble silicone-based insert designed to fit in the conjunctival fornix. It offers extended retention (up to two weeks) and sustained drug release. The cylindrical design improves comfort and reduces the risk of expulsion (28).

Table 2: Comparison between soluble inserts and bioerodible inserts.

Feature	Soluble Inserts	Bioerodible Inserts
Drug-Release Duration	Short-term (minutes to a few hours). For rapid, bulk drug release.	Long-term (hours to days). For sustained, controlled drug release.
Primary Use Case	Acute conditions: postoperative care, infections, immediate relief.	Chronic conditions: glaucoma, dry eye, long-term inflammation.
Patient Tolerance	Generally high. Due to rapid dissolution, minimizing foreign body sensation is important.	Variable; may cause initial discomfort.
Mechanical Strength	Lower. Softer, more fragile polymer structure.	Higher. More durable and robust.
Drug Loading Capacity	Lower. Limited by the small, fast-dissolving matrix.	Higher. Larger and durable matrix supports higher dose.
Example Systems	Basic polymer inserts (e.g., polyvinyl alcohol).	SODI, collagen shields.

2.2. Punctal plugs and Intraocular injections

Another contributor to the advancement in ocular drug delivery is Punctal Plugs, initially developed for the treatment of DED. Punctal plugs are miniature medical implants placed at the punctal opening with an umbrella-like design, with a head, narrow neck, and conical base, which facilitates retention and removal, as seen **in Figure 5.** They are manufactured from materials such as collagen, silicone, hydrogel, polydioxanone, and acrylic. Their primary function is to occlude the lacrimal drainage system, thereby enhancing tear retention and improving the efficacy of artificial lubricants and medications. Punctal plugs have recently gained attention as potential drug delivery systems, especially for conditions like glaucoma. By improving drug retention on the ocular surface, they offer a promising route for sustained therapy. However, they are relatively contraindicated in patients with ocular inflammation, as blocked tear drainage can lead to the accumulation of inflammatory cytokines, worsening symptoms.

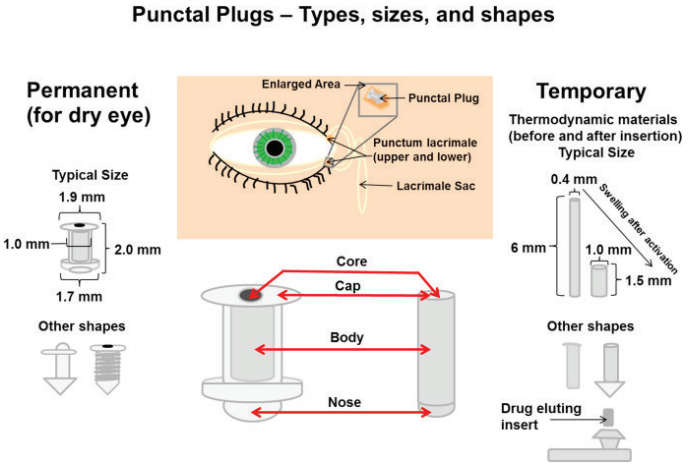

Figure 5: Types, sizes, and structural components of punctal plugs (30).

Among the various novel drug delivery systems, injectable formulations known as intraocular injections have the most impactful application as they can deliver the right amount of drug in the desired area of the eye. Considering this, some of the disadvantages associated with intraocular injections are their invasive nature, frequent application of injections leads to non-compliance, and also less bioavailability, which is where iontophoresis and micro needles came in display (31).

2.3. Iontophoresis and Microneedles

Iontophoresis is a noninvasive drug delivery technique that employs a low-intensity electric current to enhance the penetration of the drug through physiological barriers. By following the basic electrochemical principle that like charges repel and opposite charges attract, iontophoresis facilitates the targeted migration of charged

molecules through biological membranes, including skin, mucosa, joints, nails, and ocular tissues. Compared to conventional topical administration, this approach can achieve drug delivery rates 10–2000 times greater, with dosing directly proportional to the applied current, duration of application, and surface area in contact with the drug reservoir (32).

Two principal electrical modalities are utilized: direct current (DC), which remains the most widely applied in clinical and experimental contexts, and alternating current (AC). The drug transport process is governed by three complementary and synergistic mechanisms. The direct-field effect (Nernst–Planck effect) refers to the electrophoretic movement of charged molecules in response to the applied potential gradient, with ionized substances migrating toward the oppositely charged electrode. This mechanism is particularly significant for small ions. Electro-osmosis involves bulk solvent flow induced by a potential difference across a charged membrane, promoting the movement of both ionic and neutral drugs, and is especially relevant for the delivery of large monovalent ions. Electro-permeabilization describes the transient alteration of membrane porosity and transport pathway characteristics under an electric field, thereby increasing permeability to both charged and neutral molecules during and after current application.

In ophthalmology, iontophoresis has been extensively investigated as an alternative to invasive intravitreal injection for both anterior and posterior segment drug delivery. Transcorneal iontophoresis targets the anterior segment, enabling delivery of antibiotics such as gentamicin, tobramycin, ciprofloxacin, and vancomycin across the cornea despite the formidable barrier posed by its stratified squamous epithelium and tight junctions, as shown in **Figure 6**, but still due to the lens barrier, drugs administered transcorneally rarely achieve therapeutically relevant concentrations in the posterior segment.

To address this limitation, transscleral iontophoresis has been developed, capitalizing on the sclera's higher hydration, lower cellular density, and larger surface area (~17 cm^2 vs. ~1.3 cm^2 for the cornea) to facilitate diffusion of both small and high molecular weight compounds. This route enables drug passage to the posterior segment via the choroid, bypassing the lens and iris diaphragm, as shown in **Figure 6**. Low current transscleral iontophoresis using hydrogel probes has been shown to achieve high intravitreal and retinal drug concentrations in short treatment times, offering a promising therapeutic alternative for posterior uveitis, scleritis, and endophthalmitis conditions traditionally managed by invasive intravitreal injection with associated pain and risk of complications (33).

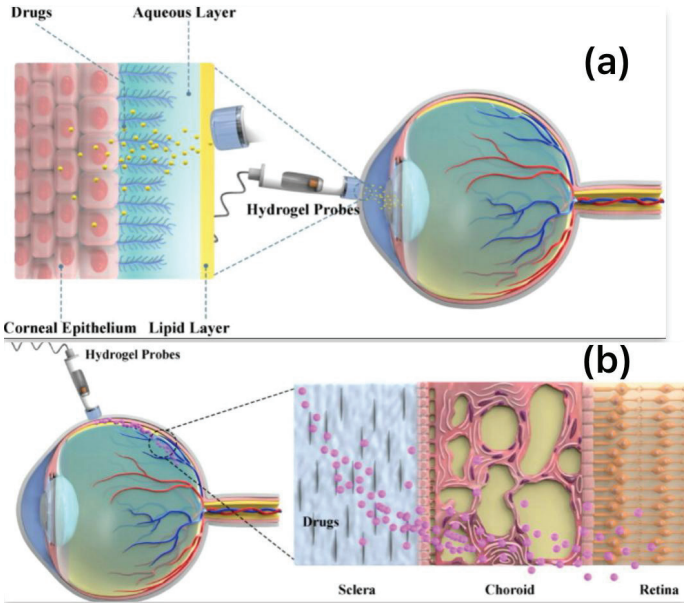

Figure 6: Drug release and penetration by: (a) Transcorneal Iontophoresis (b) Transscleral Iontophoresis (33).

Microneedles are devices made up of polymer or metal having dimensions in the range of a few micrometres to 200 μm, offering a minimally invasive strategy for enhancing ocular drug penetration by creating micro-scale channels in the cornea or sclera, thereby improving tissue permeability and targeted delivery. These microneedles are able not only to overcome the disadvantages associated with conventional delivery systems but also to cross the ocular barriers to specifically target the drugs at the needed site of action. There are three main microneedle types that play a substantial role in drug delivery to ocular tissues. These types include solid coated, hollow, and microneedles of dissolving polymers, as illustrated in **Figure 7**.

Solid coated microneedles are fabricated from non-biodegradable materials such as stainless steel or silicon, and function by piercing ocular tissue, after which the surface coating rapidly dissolves to release the drug. While manufacturing complexity limits their use, they have demonstrated efficacy in enhancing the absorption of agents such as pilocarpine for glaucoma and bevacizumab for corneal neovascularization.

Hollow microneedles, typically made from borosilicate or stainless steel, encapsulate the drug formulation within their lumen.

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Upon insertion, the drug is delivered directly into ocular tissues. These devices can be loaded with nanoparticles, liposomes, emulsions, or microparticles to enhance therapeutic activity. For example, hollow microneedle delivery of triamcinolone acetonide (TA) into the suprachoroidal space effectively managed posterior uveitis for up to three days without elevating intraocular pressure or damaging retinal structures.

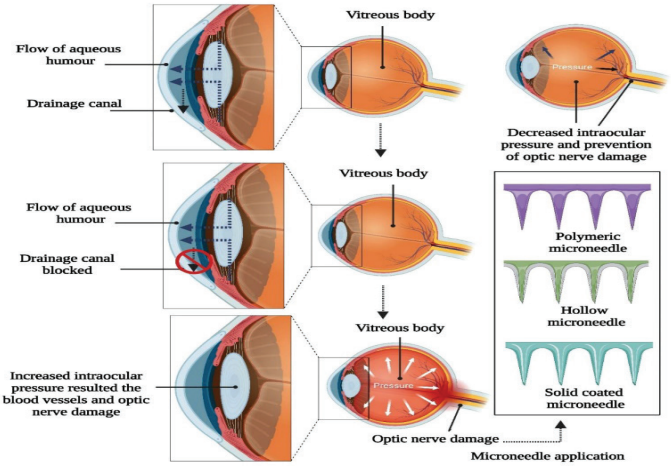

Figure 7: Main types of microneedles and their application in glaucoma treatment (34).

2.4. Ocular Implants

Ocular implants are solid drug delivery devices designed to provide controlled, sustained release of therapeutic agents from either biodegradable or non-biodegradable polymeric matrix over extended periods ranging from several months to years. Implants can be positioned at multiple ocular sites, and they possess the advantage of bypassing the BOB, delivering precise drug doses directly to the target tissue for prolonged durations. Intravitreally placed implants, in particular, can localize therapy to the vitreous with minimal systemic exposure, potentially reducing risks such as infection or retinal detachment.

Biodegradable implants, fabricated from polymers such as polycaprolactone, polyglycolic acid, polylactic acid, polylactic-co-glycolic acid (PLGA), and polyanhydrides, gradually degrade in situ, eliminating the need for surgical removal, a requirement for non-biodegradable counterparts. However, biodegradable systems may exhibit variable drug release kinetics.

By providing continuous medication delivery, ocular implants reduce the frequency of interventions and are therefore well-suited for managing chronic ophthalmic conditions. One notable platform, Durasert™, utilizes a solid polymer matrix capable of releasing small molecule drugs for up to three years. This technology underpins three FDA-approved products, Iluvien®, Retisert®, and Vitrasert®, which have demonstrated clinical utility in long-term treatment of ocular diseases (35).

3. Nanotechnology in Ocular Drug Delivery

In recent decades, the field of ophthalmology has witnessed a transformative shift towards nanotechnology-based formulations for drug delivery to both the anterior and posterior segments of the eye. This innovative approach leverages the unique properties of nanomaterials to overcome the inherent challenges associated with conventional ocular drug administration, offering enhanced therapeutic outcomes and improved patient compliance.

3.1. Why go Nano?

The small Size of nanoparticles, typically ranging from 10 nm to 1000 nm, allows for improved drug penetration into the deeper layers of the ocular structure, including the aqueous humor. This enhanced penetration is crucial for treating conditions affecting the posterior segment of the eye, which are often difficult to reach with conventional formulations (36, 37). Furthermore, nanoparticles can be designed to facilitate enhanced cellular uptake, allowing for more efficient delivery of therapeutic agents into target cells. This ability to overcome ocular barriers and increase drug penetration is a significant advantage over traditional eye drops, which often suffer from limited drug absorption.

(37). Also, one of the critical challenges in ocular drug delivery is the rapid clearance of formulations from the eye due to tear fluid turnover and blinking. Nanoparticles have various types, as seen in **Figure 8**, and some types showcase mucoadhesive properties.

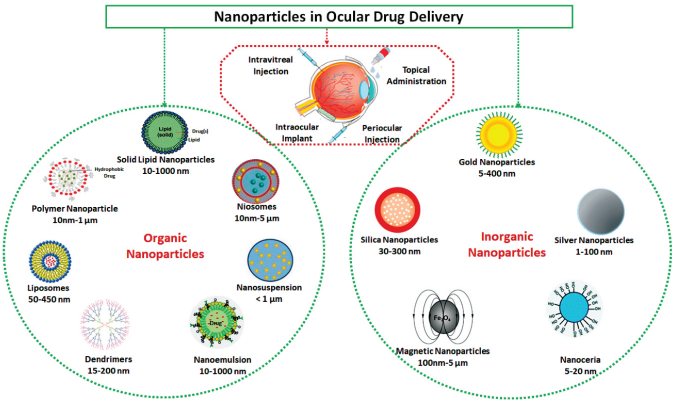

Figure 8: Overview and classification of nanoparticles in ocular drug delivery (38).

3.2. A Menagerie of Nanocarriers

3.2.1. Liposomes

Liposomes are spherical vesicles composed of one or more lipid bilayers, which encapsulate an aqueous core. They vary in Size, ranging from 10 nm to over 1 µm. This unique structure allows them to carry both hydrophilic drugs within their aqueous core and hydrophobic or amphiphilic drugs embedded within their lipid bilayers. They are highly versatile, biocompatible, biodegradable, and generally non-toxic, making them attractive candidates for drug delivery (39).

Structurally, they are classified as unilamellar vesicles (ULVs), possessing a single lipid bilayer, or multilamellar vesicles (MLVs), which consist of multiple concentric lipid bilayers separated by aqueous compartments. ULVs are further categorized by Size into small unilamellar vesicles (SUVs), large unilamellar vesicles (LUVs), and giant unilamellar vesicles (GUVs). Liposomes with multiple compartments (MLVs) generally have a greater capacity for entrapping hydrophilic drugs due to their larger aqueous volume (39).

The primary building blocks of liposomes are phospholipids, which can be naturally occurring (e.g., egg phosphatidylcholine, brain and synthetic phosphatidylserine, sphingomyelin, ovolecithin) or synthetic (e.g., synthetic dipalmitoyl-dl-α-phosphatidylcholine). To introduce surface charge, other lipids are often incorporated: stearylamine for positive charge, and diacetylphosphate, phosphatidyl glycerol, or phosphatidylserine for negative charge. One of the major limitations is their stability. Liposomes may become chemically unstable due to hydrolysis or oxidation of their constituent unsaturated lipids. They may also become physically unstable due to the leakage of the entrapped drug. Therefore, Incorporation of Cholesterol is frequently added to enhance stability, improve fluidity, and reduce drug leakage (39).

Liposomes still may aggregate to form larger particles that interfere with ocular absorption and also make them susceptible to phagocytosis by phagocytic cells. In general, charged liposomes resist aggregation and fusion better compared to uncharged liposomes, and positively charged liposomes provide greater duration of action and higher drug delivery compared to negatively charged liposomes. This is because positively charged liposomes intimately interact with the negatively charged cornea, leading to prolonged residence time. It has also been suggested that a cationic vehicle slows down the drug drainage with lacrimal fluid by increasing the viscosity and interaction with negative charges of the mucus. The effect of the surface charge of liposomes on ocular irritation has also been evaluated. Positively charged liposomes significantly increase the rabbit eye blinking rate compared to neutral liposomes; however, the mean total score on the Draize test remains below "practically non-irritating level," and no corneal histological changes appeared. Cationic liposomes can also serve to deliver genetic material; they consist of positively charged lipids that interact with and neutralize the negatively charged deoxyribonucleic acid (DNA) and hence, condense the DNA into a more compact structure. Such lipid complexes provide protection to entrapped genetic material and enhance its intracellular delivery. They are of sufficient flexibility to allow synthesis in various sizes and can be formulated as eye drops, gels, and ointments for topical delivery.

3.2.2. Nanoparticles

Polymeric nanoparticles are solid colloidal particles ranging from 10 to 1000 nm, formed from natural or synthetic polymers. They are widely explored for drug delivery due to their versatility, stability, and ability to provide controlled release of encapsulated therapeutics. These nanoparticles are typically composed of biodegradable or non-biodegradable polymers. Common examples include poly (lactic-co-glycolic acid) (PLGA), a synthetic biodegradable polymer widely used due to its biocompatibility and tunable degradation rates, and chitosan, a natural biodegradable polymer derived from crustacean exoskeletons and fungal cell walls. Other polymers like polycaprolactone (PCL) are also utilized (40).

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Drugs can be loaded into polymeric nanoparticles either by encapsulation within the polymer matrix during their formation or by adsorption onto the nanoparticle surface. The method depends on the drug's properties and the desired release profile. For instance, hydrophobic drugs are often encapsulated within the polymer core, while hydrophilic drugs might be loaded onto the surface or within a hydrophilic matrix.

Surface functionalization is a key strategy to enhance the targeting, stability, and drug delivery efficiency of polymeric nanoparticles. This involves modifying the nanoparticle surface with specific ligands, polymers, or other molecules. For example, chitosan's positively charged nature allows for strong mucoadhesive interactions with the negatively charged ocular mucosa, enhancing drug retention and permeability by transiently relaxing tight junctions between cells. The molecular weight and deacetylation degree of chitosan can influence its mucoadhesion to ocular tissues (40). Surface functionalization can also help in evading the body's immune response, improving cellular uptake, or enabling specific targeting to diseased cells or tissues.

Polymeric nanoparticles offer a wide range of advantages, including biodegradability and biocompatibility, especially those made from PLGA and chitosan, leading to minimal toxicity, Controlled and Sustained Release. Therefore, these systems help reduce dosing frequency and improve patient compliance. Polymers like chitosan enhance drug permeability by modulating tight junctions, leading to improved penetration. Chitosan-based nanoparticles also exhibit strong mucoadhesive properties, which increase bioavailability. However, despite these advantages, several limitations exist. The synthesis and functionalization of polymeric nanoparticles can be complex, requiring precise control over formulation parameters. Ensuring consistent particle size, drug loading, and release profiles is often challenging, leading to batch-to-batch variability. Moreover, while generally biocompatible, some synthetic polymers or their degradation products might pose toxicity concerns at high concentrations or over long periods.

Solid Lipid Nanoparticles (SLNs) and Nanostructured Lipid Carriers (NLCs) represent advanced lipid-based colloidal drug delivery systems. SLNs are typically spherical nanoparticles with a solid lipid core matrix at both body and room temperatures. This solid core can effectively solubilize lipophilic drug molecules. The lipid component can be a triglyceride, diglyceride, monoglyceride, fatty acid, steroid, or wax. The solid lipid core is stabilized by surfactants, which prevent aggregation and maintain particle size. SLNs are prepared from physiological lipids, contributing to their low bio-toxicity (41). NLCs are a second generation of lipid nanoparticles, designed to overcome some limitations of SLNs. Unlike SLNs, NLCs are composed of a mixture of solid and liquid lipids in their core. This blend creates an imperfect crystal structure within the lipid matrix, which provides more space for drug loading and reduces the risk of drug expulsion during storage. Similar to SLNs, NLCs are stabilized by surfactants and water (42).

As shown in **Table 2**, both SLNs and NLCs primarily load lipophilic drugs by dissolving them within their lipid core during the formulation process. For hydrophilic drugs, strategies like surface adsorption or creating a hydrophilic shell around the lipid core can be employed. The unique structure of NLCs, with their disordered lipid matrix, allows for higher drug loading capacity and prevents drug leakage more effectively than SLNs.

NLCs offer several advantages over SLNs, making them a preferred choice in many applications. The blend of solid and liquid lipids in NLCs creates an amorphous or imperfect crystal structure, leading to more void spaces within the lipid matrix. This allows for a higher incorporation of drug molecules compared to the highly ordered crystalline structure of SLNs. The disordered matrix of NLCs minimizes drug expulsion during storage, a common issue with SLNs, where drugs can crystallize out of the solid lipid matrix over time, and the disordered matrix can also lead to a more controlled and sustained release of the encapsulated drug, as the drug molecules have to diffuse through a more complex and less uniform matrix. This leads to better long-term stability of the encapsulated drug (42).

3.2.3. Nanoemulsions and Nanosuspensions

Nanoemulsions are submicron emulsions of oil and water stabilised using surfactants. They are especially useful in ocular drug administration because they increase the solubility of poorly water-soluble medicines, improve corneal penetration, and prolong precorneal residency (43, 44). Their small droplet size improves the bioavailability and stability of labile compounds. However, they require rather high concentrations of surfactants, which may cause irritation, and their long-term stability might be influenced by environmental conditions (45).

Nanosuspensions are dispersions of pure

medication nanoparticles stabilised with appropriate agents, making them suited for pharmaceuticals with low water solubility. Compared to conventional formulations, they allow for larger drug loading, faster dissolution, and better absorption (46, 47). Furthermore, they are rather simple to prepare. Nonetheless, the drawbacks of nanosuspensions include particle agglomeration, burst release, and inadequate control over sustained drug administration (48).

3.2.4. Dendrimers

Dendrimers are three-dimensional, highly branched macromolecules with a well-defined architecture and surface groups that can be modified. Their distinct structure allows for significant drug-loading capacity via both encapsulation and surface conjugation, making them appealing carriers for eye therapy (49, 50). They can stay on the ocular surface for longer periods of time, increase corneal permeability, and be functionalised with ligands to deliver drugs to specific tissues (51). Several studies have shown that dendrimers can help manage glaucoma, ocular inflammation, and retinal disorders by increasing therapeutic efficacy and decreasing dose frequency (52, 53). However, their manufacturing is complicated and expensive, and higher-generation dendrimers with positively charged surfaces may cause cytotoxicity or ocular discomfort. Surface changes like PEGylation or acetylation can mitigate these negative effects, increasing their clinical applicability (54).

3.2.5. Niosomes and Cubosomes

Niosomes are vesicular systems composed of non-ionic surfactants and cholesterol, similar in structure to liposomes but more stable and cost-effective. They can encapsulate both hydrophilic and lipophilic medicines, increasing bioavailability and providing prolonged release, thus lowering the need for frequent ocular dosage. Their advantages include high biocompatibility and biodegradability; nevertheless, they sometimes have poorer drug entrapment efficiency than liposomes and may aggregate during storage (52).

Cubosomes, on the other hand, are nanoparticles with a cubic liquid crystalline structure that provide a large surface area, strong bioadhesion, and diversity in drug encapsulation. They show considerable potential in ocular administration because of their capacity to sustain release and increase corneal penetration (53). Despite these advantages, difficult production procedures and stability issues limit cubosomes' clinical application (54, 55).

Table 3: Comparison of different nanoparticle-based drug delivery systems.

Types	Composition	Size and Surface Properties	Drug Loading and Release	Biocompatibility and toxicity	Targeting ability	Applications
Liposome nanoparticles [39]	Spherical vesicles composed of one or more lipid bilayers encapsulating an aqueous core. With other lipids like stearylamine (positive charge), phosphatidyl glycerol, or phosphatidyl serine (negative charge) for surface modification. Cholesterol is often added for stability.	- Size: from 10 nm to over 1 µm. - Surface Properties: Can be cationic or anionic depending on the incorporated lipids. Cationic liposomes show strong interaction with the negatively charged cornea.	It can encapsulate both hydrophilic drugs and hydrophobic/amphiphilic drugs. Stability issues due to hydrolysis/oxidation of unsaturated lipids and drug leakage can occur, but cholesterol addition improves stability and reduces leakage.	Generally biocompatible, biodegradable, and non-toxic. Positively charged liposomes can increase ocular irritation, but often remain below the 'practically non-irritating level'.	Cationic liposomes show enhanced interaction with the negatively charged cornea, leading to prolonged residence and potentially better targeting to the ocular surface.	- Investigated for cancer chemotherapy, it can be formulated as eye drops, gels, and ointments for topical delivery. - Cationic liposomes can deliver genetic material by condensing negatively charged DNA.
Polymeric nanoparticles [40]	Solid colloidal particles formed from natural or synthetic polymers. Common examples include PLGA and chitosan.	- Size: 10 to 1000 nm. - Surface Properties: Surface functionalization is key to enhancing targeting, stability, and drug delivery. Chitosan's positive charge allows for strong mucoadhesive interactions with the negatively charged ocular mucosa.	Drugs can be encapsulated within the polymer matrix or adsorbed onto the surface. They provide controlled and sustained release.	Many are biodegradable and biocompatible, leading to minimal toxicity. Some synthetic polymers or their degradation products might pose toxicity concerns.	Surface functionalization can enhance targeting. Chitosan can improve permeability by relaxing tight junctions.	Widely explored for drug delivery due to its versatility and stability. Chitosan-based nanomedicines are explored for ocular applications in glaucoma.

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SLNs & NLCs [41, 42]	- SLNs: Spherical nanoparticles with a solid lipid core matrix stabilized by surfactants. - NLCs: Composed of a mixture of solid and liquid lipids in their core, creating an imperfect crystal structure, also stabilized by surfactants and water.	- Size: both generally range in Size from 50 to 1000 nm. - Surface Properties: Both are stabilized by surfactants. NLCs show good interaction with corneal mucosa due to biocompatibility and mucoadhesive properties.	Both primarily load lipophilic drugs by dissolving them in the lipid core. Hydrophilic drugs can be loaded via surface adsorption or a hydrophilic shell. NLCs offer higher drug loading capacity and reduced drug expulsion compared to SLNs due to their disordered lipid matrix. Both provide prolonged drug release.	Both are prepared from physiological lipids, making them highly biocompatible and non-toxic.	Both achieve targeting through passive EPR-based accumulation, active ligand-mediated uptake, and stimulus-triggered release. NLCs generally have an edge in loading efficiency and flexibility for functionalization, enhancing their targeting abilities compared to SLNs.	- SLNs: anti-glaucoma drugs (e.g., Methazolamide) and anti-inflammatory drugs (e.g., Cyclosporine A). - NLCs: ocular delivery of poorly water-soluble drugs (e.g., hydrocortisone, estradiol, pilocarpine, propranolol hydrochloride).
Nanoemulsions & Nanosuspensions [43, 44, 46, 47]	- Nanoemulsions are composed of oil and water stabilized by surfactants. - Nanosuspensions are pure drug nanoparticles dispersed with stabilizers.	- Size: for nanoemulsions 20-200nm, nanosuspensions <1000 nm. - Surface Properties: both have High surface area; stability depends on surfactants.	Nanoemulsions and nanosuspensions are both suitable for lipophilic drug loading.	Both are generally biocompatible, but in nanoemulsions, surfactants may cause irritation, and in nanosuspensions, there's a risk of aggregation.	Nanosuspensions achieve targeting mainly through surface modification, charge control, and Size for passive or active delivery, whereas nanoemulsions rely on droplet composition, surface ligands, and Size to direct lipophilic drugs to specific tissues or enhance lymphatic transport.	- Nanoemulsions enhance solubility and corneal penetration, and sustained ocular delivery. - Nanosuspensions improve dissolution and absorption; simple prep for hydrophobic drugs.
Dendrimers [49, 50]	Highly branched synthetic macromolecules (e.g., Polyamidoamine (PAMAM)).	Size: 1-10 m (depends on generation) Surface Properties: Functional surface groups modifiable with ligands.	High encapsulation & conjugation.	Biocompatible if surface-modified; risk of cytotoxicity at higher generations.	High targeting abilities via surface ligand conjugation.	For treatment of glaucoma, uveitis, and retinal diseases, sustained release is required.
Niosomes & Cubosomes [51, 52, 57, 58]	Niosomes are made of non-ionic surfactants + cholesterol vesicles, while Cubosomes are Cubic liquid crystalline lipid nanoparticles.	- Size: Niosomes range from 100-1000 nm and Cubosomes from 100-300nm. - Surface Properties: Niosomes are Similar to liposomes, stable and flexible. Cubosomes have a high surface area and strong bioadhesion.	Niosomes' drug loading is moderate for hydrophilic and lipophilic drugs. On the other hand, Cubosomes' drug loading is high and diverse.	Both are Biocompatible, but niosomes suffer from lower entrapment efficiency than liposomes, and cubosomes may face some stability issues.	Generally limited for both unless modified.	- Niosomes can be used for Glaucoma management, sustained-release eye drops - Cubosomes offer sustained ocular delivery and improved corneal penetration.

4. Clinical Impact of Nanotechnology in Ocular Diseases

The unique properties of nanoparticles offer a promising solution by enhancing drug penetration into the anterior segment of the eye and enabling targeted delivery, thereby improving therapeutic outcomes for chronic eye conditions.

Glaucoma is a known cause of irreversible blindness; the main goal in managing glaucoma is to decrease IOP. However, the efficacy of standard treatments, which are mainly through topical hypotensive agents, is fundamentally limited by previously discussed barriers. Surgical interventions, on the other hand, are effective but compromised by the body's natural fibrotic response (56).

Current research leverages more advanced, biocompatible materials such as biodegradable polymers and specially functionalized nanoparticles. Among the most promising are polymer-based carriers and lipid nanoparticles (LNPs), which have demonstrated excellent biocompatibility for ocular use. Polymeric nanoparticles, especially those made from PLGA, have shown success in preclinical models for the sustained release of IOP-lowering agents like brimonidine.

LNPs are found to be beneficial for glaucoma treatment. Their lipid structure enhances penetration, shields drugs from degradation, and allows for controlled and sustained release. This reduces the need for frequent applications, a major benefit for patient adherence. Furthermore, LNPs can be modified with a polyethylene glycol (PEG) coating to improve their bioavailability and targeting ability. These carriers are now being used to deliver a wide range of small-molecule drugs like prostaglandin analogs to advanced nucleic acid-based therapies, including DNA, small-interfering RNA (siRNA), and messenger RNA (mRNA). For example, siRNA delivered via LNPs has been shown to silence genes responsible for fibrosis, a common complication of glaucoma surgery, leading to better surgical outcomes. Despite this progress, challenges remain in ensuring the long-term stability of lipid formulations, as degradation could compromise drug effectiveness. Additionally, formulations must be carefully designed to avoid triggering an immune response and to optimize drug release for sustained effect (57).

DED is a growing health issue characterized by a loss of homeostasis in the tear film, leading to discomfort and visual problems. Several topical treatments are commonly used to treat DED; however, poor bioavailability is achieved by the majority of eye drops in the market. In this context, there's an indication for enhancing the drug's ability to overcome ocular barriers. Several nanotechnology-based products for DED have already received FDA approval and are available to patients (60).

Restasis®, the first FDA-approved nanoemulsion for DED, delivers cyclosporine (CsA) in an (O/W) formula. Other products like Cationorm® and Ikervis® use the Novasorb technology, which employs electrostatic attraction to prolong the drug's residence time on the negatively charged ocular surface.

Cequa®, a nanomicellar formulation of CsA, was developed to improve drug solubility and bioavailability, demonstrating a higher concentration of CsA in ocular tissues compared to earlier nanoemulsions.

Liposomal sprays such as Tears Again® (marketed as Optrex ActiMist™ in the UK) are applied to the closed eyelids, allowing phospholipids to migrate to the tear film and enhance its stability. Other liposomal products deliver vitamins A, E, and B12 to address deficiencies associated with DED.

Hydrogel formulations like Vidisc® and GelTears® are commercially available and valued for their biocompatibility and ability to provide sustained drug release. Eysuvis®, another innovation, uses mucus-penetrating nanoparticles to deliver loteprednol etabonate for the short-term treatment of DED (58).

Researchers are also exploring novel nanocarriers like niosomes, which are cost-effective and can entrap both water-soluble and fat-soluble drugs, and cubosomes, which offer a large surface area for drug delivery (59).

Microbial keratitis (MK) is a severe infection of the cornea that is caused by a range of microorganisms, such as bacteria, viruses, fungi, and protozoa. It can lead to blindness if not treated promptly and effectively. The rise of antimicrobial resistance has made conventional treatments less reliable, creating an urgent need for new therapeutic strategies. Nanotechnology offers powerful tools to manage MK by improving drug delivery and introducing novel treatment modalities (60).

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Beyond simply acting as delivery vehicles, some nanoparticles have intrinsic therapeutic properties. Innovations in nanomedicine have led to the development of several advanced treatments, including Photothermal Therapy (PTT) and Photodynamic Therapy (PDT). These therapies use nanoparticles that, when activated by light, either generate heat in the case of PTT or produce reactive oxygen species (ROS) in the case of PDT to destroy pathogens. Gold nanoparticles, for example, can convert light energy into heat to kill bacteria and fungi. PDT, which uses a light-activatable dye, has shown promise as an alternative to traditional antibiotics.

Another promising therapy is the use of nanozymes, which are nanomaterials with enzyme-like properties that can combat infection by reducing oxidative stress and promoting tissue repair. Treatments based on multienzyme-like nanozymes are being explored to provide combined antibacterial and anti-inflammatory effects.

Moreover, there are other distinct treatments that differ based on the anatomical target. For instance, in treating anterior segment diseases like infectious keratitis, metal ion therapy has emerged as a powerful antimicrobial strategy. Nanoparticles composed of metals such as silver, copper, or zinc exhibit potent, broad-spectrum activity by generating reactive oxygen species (ROS) and disrupting microbial cell membranes, making them effective against a wide range of pathogens (61).

In contrast, for diseases affecting the posterior segment, nanotechnology focuses on overcoming drug delivery challenges. Anti-VEGF nanocarriers, for example, are designed to manage retinal conditions that currently require frequent intravitreal injections. By encapsulating anti-VEGF compounds in platforms like PLGA microspheres or liposomes, these nanocarriers provide sustained drug release over an extended period. This approach reduces the treatment burden associated with injections every 4–8 weeks, marking a significant improvement in patient care for chronic retinal diseases (62). However, they also share common hurdles, as critical challenges related to toxicity, biocompatibility, and regulatory approval must be addressed to ensure their safe and effective clinical translation. For example, PLGA nanospheres and microspheres can inhibit VEGF for a long time following intravitreal injection (63), whereas pegylated liposome–protamine–hyaluronic acid nanocarriers loaded with siRNA against VEGFR1 have considerably reduced choroidal neovascularisation in animal models (64). Dendrimer-based carriers also have shown long-term suppression of CNV after intravitreal administration (64). Such technologies have the potential to increase treatment intervals and improve patient adherence while remaining effective.

Sustained corticosteroid delivery is essential for treating posterior uveitis because it reduces inflammation while avoiding repeated injections and systemic complications. Nanoparticles and implanted devices have been studied to administer medications such as TA and dexamethasone directly to the vitreous. Ozurdex® (dexamethasone) and Retisert® (fluocinolone acetonide) are FDA-approved implants that offer long-term medication release (months to years) and have been used successfully to treat uveitis (64, 66).

Emerging nanocarrier technologies, such as thermo-responsive hydrogels loaded with PLGA microspheres, can encapsulate drugs like ranibizumab, aflibercept, or corticosteroids and release them for up to 200 days (65). Such platforms reduce the risk of ocular hypertension and cataract advancement caused by repeated corticosteroid bolus injections, providing a safer and more long-lasting treatment option.

5. From Bench to Bedside: Challenges in Clinical Translation

5.1. The Regulatory Hurdle

The regulatory approval process for ocular nanomedicines is particularly complex, owing to the unique barriers of the eye, as well as the inherent novelty of nanoscale drug delivery systems. Nanomedicines have very different pharmacokinetics (PK) and pharmacodynamics (PD) than standard drug molecules. This is due to their complex nature, which varies significantly in structure, shape, Size, surface properties, and other physicochemical characteristics. This inherent complexity makes it difficult for regulatory agencies, such as the United States FDA and the European Medicines Agency (EMA), to define, classify, and establish standardized PK and PD profiles across the wide range of nanomedicine types.

A significant challenge is the lack of definitive and standardized protocols for assessing nanotoxicity across various ocular layers. Given the human eye's delicate and intricate structure, developing robust in vitro and in vivo protocols is critical for ensuring accurate

and comprehensive safety assessments of nanomedicines. The risk of retinal accumulation, which could cause toxicity in various retinal layers, as well as systemic accumulation that could impair normal ocular functions, highlights the importance of rigorous toxicity testing. Many nanomedicine formulations, particularly those that combine a drug and a delivery device (for example, a sustained-release eye implant), are classified as combination products. This classification introduces new regulatory criteria and frequently necessitates a collaborative review by multiple centers within regulatory bodies. For example, in the United States, the FDA's Center for Drug Evaluation and Research (CDER) and the Center for Devices and Radiological Health (CDRH) work together to determine the primary mode of action and the appropriate regulatory pathway. Similarly, in Europe, the EMA's Committee for Medicinal Products for Human Use (CHMP) assesses the proportion of drug and device components in such combined products. Ensuring manufacturing consistency is especially important for nanomedicines (67).

The physicochemical characteristics of nanoparticles can be dramatically changed by slight changes in the production process. To ensure batch-to-batch consistency, regulatory bodies require that manufacturing processes be thoroughly designed and verified. This includes thorough stability testing, guaranteeing sterility for ocular formulations, and adhering to stringent Good Manufacturing Practice (GMP) guidelines for scaling up nanoparticle production. Verifying the final product's quality frequently calls for specialized analytical methods, which further complicates the manufacturing and quality control procedures. As of right now, there isn't a single, internationally consistent regulatory framework for the clinical use of nanomedicines.

This lack of consistency can present additional challenges for developers seeking global market access, as they must navigate varying requirements and guidelines across jurisdictions. Both the FDA and the EMA have strict requirements for demonstrating the safety, efficacy, and manufacturing consistency of ocular nanomedicines. Extensive preclinical studies on safety are expected, including acute and chronic toxicity assessments, detailed ocular histopathology, and PK data that show drug distribution and elimination profiles within the eye. Efficacy demonstration necessitates strong clinical data demonstrating therapeutic benefit, with clinical endpoints relevant to patients. Manufacturing consistency necessitates validated processes that ensure batch-to-batch reproducibility, adhere to GMP guidelines, and employ specialized analytical techniques.

Developers must address these challenges proactively by generating robust safety and efficacy data, ensuring reproducible manufacturing processes, and collaborating early with regulatory bodies to clarify requirements and streamline the approval pathway for advanced therapies (67).

5.2. Safety and Biocompatibility

Clinical trials of nanomedicine formulations for ocular diseases provide important information about their safety and biocompatibility. Phase I/II trials focus on safety and biocompatibility, measuring visual comfort, vital signs, visual acuity, intraocular pressure, and the frequency of adverse events. Safety data for intravitreal injections focuses on inflammation, increased intraocular pressure, and retinal detachment, as well as monitoring for systemic effects. While nanoparticle formulation can prolong drug release and reduce injection frequency, it is critical to monitor for inflammation and immune responses. Dexamethasone intravitreal implants, for example, show sustained release and a consistent safety profile, but common side effects include increased intraocular pressure and cataract formation. Patient feedback is critical for improving delivery methods and formulations, informing the regulatory approval process, and refining therapeutic strategies.

Due to the delicate tissues of the eye, biocompatibility is an important consideration for ocular nanomaterials. This includes assessing nanoparticles' interactions with ocular structures such as the cornea, conjunctiva, vitreous humor, and retina. In vitro and in vivo models are used to assess safety by measuring oxidative stress, cellular viability, tissue integrity, and the absence of inflammatory responses. Nanoparticle toxicity is linked to their biophysical characteristics. Size, for example, influences nanoparticles' entry, cellular uptake, and overall toxicity. Research indicates a direct relationship between nanoparticle size and distribution, as well as the generation of ROS in organs such as the kidneys. Smaller nanoparticles frequently exhibit greater tissue distribution and more severe toxic effects. Beyond Size, nanoparticle shape influences distribution, deposition, and clearance; long, fibrous particles, such as single-walled nanotubes, are particularly difficult for the body to clear, resulting in significant organ deposition. Surface chemistry has a significant impact on pharmacokinetics, as charged nanoparticles accumulate more in target organs than uncharged counterparts. The dissolution of

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nanoparticles, particularly inorganic ones, can also influence acute toxicity, with the release of free ions contributing to the toxic effects (68).

Biodegradable biocompatible polymers are frequently chosen for ocular applications due to their documented safety and non-toxic byproducts. Surface modification, such as PEGylation or anti-inflammatory coatings, is used to reduce toxicity and inflammation. Another strategy is to adjust the surface charge, as highly positively charged particles cause more irritation.

Different types of nanoparticles, such as liposomes, polymeric, and metallic, can elicit a variety of immune responses. Nanoparticles can disrupt the eye's immune privilege, resulting in conditions such as uveitis or increased intraocular pressure. Metallic nanoparticles may cause greater oxidative stress and inflammation than biodegradable polymeric carriers. Understanding these interactions is critical for developing nanoparticles with minimal immunogenicity while maintaining therapeutic efficacy (67).

5.3. Scalability and Cost

Significant scalability and cost issues further complicate the development and commercialization of nanomedicines. Because nanomedicine products are inherently complex, they require careful engineering and design, rigorous physicochemical property characterization, and the development of repeatable scale-up and manufacturing procedures. These steps are critical for achieving a consistent product with stable physicochemical properties, biological behaviors, and pharmacological profiles.

Scaling nanoparticle production from laboratory research to commercial manufacturing presents a number of challenges. Stability and reproducibility are critical, as maintaining consistent nanoparticle physicochemical properties and drug encapsulation efficiency can be difficult during large-scale production. Small variations in process parameters, such as temperature and mixing speed, can have a significant impact on nanoparticle properties, affecting their safety and efficacy. Quality control is also critical, necessitating powerful analytical methods to monitor key characteristics and ensure batch-to-batch consistency. Furthermore, high production costs due to specialized equipment, raw materials, and quality control processes create economic challenges. Addressing these issues through process optimization and advanced manufacturing techniques is critical to the clinical success of nanoparticle-based therapies (67).

6. Future Perspectives in Ocular Drug Delivery

As eye disorders become more common and complex, the future of ocular therapeutics lies in the integration of modern biomaterials, molecular methods, and patient-specific tactics to create safer, more effective, and more convenient treatments. This section emphasises three promising directions: smart response systems, gene therapy delivery platforms, and personalized ocular drug delivery.

6.1. smart systems

Smart ocular drug delivery systems are designed to respond to local physiological cues (pH, temperature, enzymes, and light) or external stimuli, allowing regulated, on-demand drug release. Stimuli-responsive hydrogels can undergo sol-gel transitions or changes in mesh size in response to pH or temperature changes, allowing for reduced dosing frequency and enhanced patient adherence (69, 70). Contact lenses with drug reservoirs or integrated biosensors are another transformative approach: they can continuously monitor tear biomarkers and release therapeutic agents in a feedback-controlled manner, enabling both prophylactic and reactive treatment strategies for chronic ocular conditions (71). These platforms aim to improve local bioavailability while reducing systemic exposure and adverse effects.

6.2. Gene therapy delivery

Gene therapy has previously demonstrated therapeutic promise for hereditary retinal diseases, and optimizing delivery vehicles remains critical to building on these results. Viral vectors, notably adeno-associated viruses (AAVs), have been shown to produce effective transduction and long-term expression in retinal cells in landmark clinical studies (72). However, viral delivery can be hampered by cargo size, immunogenicity, and manufacturing complexity, prompting the development of non-viral nanocarriers. Lipid nanoparticles, polymeric nanoparticles, and dendrimer-based systems provide scalable, customizable, and potentially safer methods of delivering DNA, mRNA, or gene-editing components to retinal tissues (73, 74) Advances in ligand targeting, surface modification, and particle design are enhancing penetration into retinal layers and improving

cellular selectivity, which will be crucial for treating a wider range of inherited and acquired retinal diseases.

6.3. Personalized medicine

In ocular treatments, personalized medicine entails adapting both the drug and the delivery system to each patient's genetics, disease subtype, ocular surface features, and lifestyle. Integration of pharmacogenomic data with tear/blood biomarkers can inform drug selection and dosing, while modular delivery technologies (e.g., adjustable sustained-release implants, sensor-guided contact lenses) allow for therapeutic modifications over time (75). Long-acting ocular implants, for example, may help patients with rapid drug clearance or poor adherence, whereas biosensor-responsive devices may be better suited to individuals with changing disease activity. Personalized approaches have the potential to increase efficacy, reduce adverse effects, and optimize resource utilization in clinical practice; however, widespread implementation will require comprehensive biomarker validation, cost-effectiveness studies, and regulatory frameworks (76).

7. Conclusion

This review highlights the substantial progress made in developing ocular medication delivery systems, particularly those based on nanotechnology, which have shown significant potential to overcome the limitations of conventional eye treatments by enhancing drug penetration, residence time, and targeted delivery. Despite these promising developments, several critical gaps are preventing their widespread clinical adoption.

A primary concern is the lack of long-term safety data, especially regarding the potential for nanoparticle accumulation and chronic inflammation in sensitive ocular tissues. The majority of research remains in the preclinical stage, highlighting a pressing need for well-designed, large-scale human trials to validate both efficacy and safety. Furthermore, significant manufacturing challenges, including high costs, difficulty in scaling up production, and batch-to-batch variability, persist. Delivering drugs to the posterior segment of the eye non-invasively also remains a major, unsolved obstacle.

To move forward, future research must prioritize comprehensive long-term toxicity studies and the development of standardized, GMP-compliant manufacturing processes. Expanding clinical trials is essential to confirm therapeutic outcomes in humans. Looking ahead, the development of "smart" stimuli-responsive systems and the integration of gene therapies could offer even greater precision.

In summary, while nanotechnology is set to transform ophthalmic therapy, its successful clinical translation hinges on overcoming these key scientific, manufacturing, and regulatory hurdles. Continued collaboration between researchers, clinicians, and regulatory bodies is crucial to ensure these advanced treatments become safe, effective, and accessible for patients.

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