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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.)**

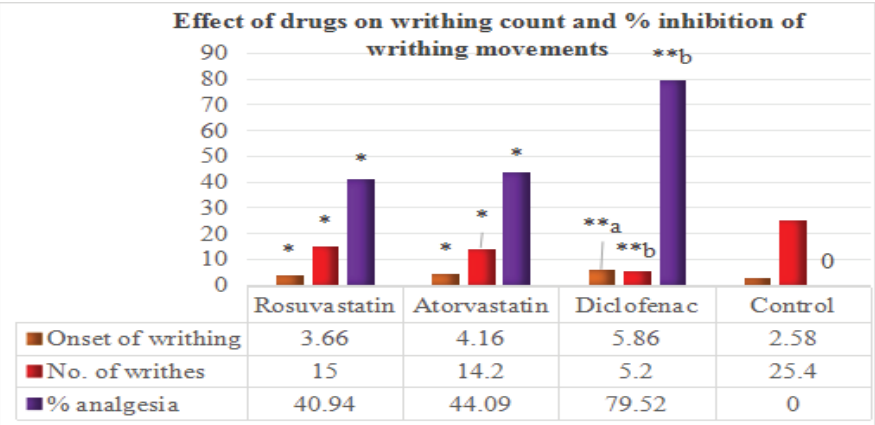
| Treatment groups | Mean reaction time (in secs.) ±SD | | | |
|---|---|---|---|---|
| | After 0 min | After 20 min | After 60 min | After 90 min |
| Rosuvastatin | 2.18 ± 0.18 | 3.13 ± 0.15* | 6.3 ± 0.43** | 8.61 ± 0.68** |
| Atorvastatin | 2.12 ± 0.16 | 3.16 ± 0.19* | 6.0 ± 0.33** | 8.23 ± 0.45** |
| 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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