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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.

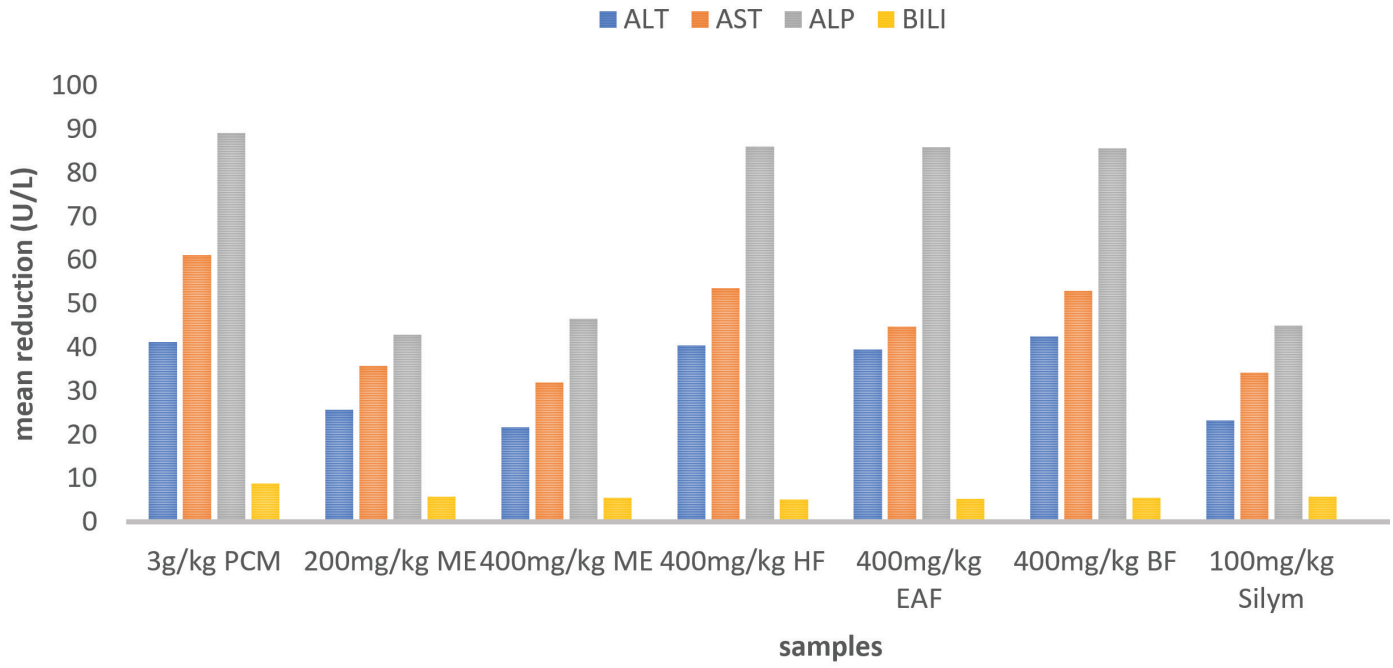

Figure 1: Effect of extract and fractions of C. bauchiense leaves on paracetamol-induced hepatotoxicity

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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