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Hepatoprotective Efficacy of Residual Aqueous Fraction of *Bombax costatum* Pellegr. et Vuillet (Malvaceae) Stem Bark Against Carbon Tetrachloride Induced Acute Liver Toxicity in Mice

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Abstract

Introduction: Hepatic disease still remains a major public health challenge due to the liver's central role in metabolism and detoxification, which makes it highly susceptible to a variety of metabolic, circulatory, neoplastic, xenobiotic and microbial insults. The search for safe and efficacious hepatoprotective agents therefore remains a priority area of research.

Objectives: This study evaluated the hepatoprotective activity of residual aqueous fraction of *B. costatum* (RAF-BC) stem bark against carbon tetrachloride (CCl_4) induced acute liver injury in mice.

Methodology: Preliminary phytochemical screening and acute oral toxicity studies of the RAF-BC were conducted using standard procedures. Male Swiss albino mice were randomly assigned to six groups (n = 6). The normal control group received olive oil, while the toxic control group received CCl_4 (1 mL/kg, 1:1 in olive oil, intraperitoneally). The standard treatment group received silymarin (100 mg/kg, orally), whereas the test groups received the RAF-BC at doses of 125, 250, or 500 mg/kg orally once daily for seven consecutive days before CCl_4 administration. Twenty-four hours after CCl_4 intoxication, serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), and total bilirubin (TB) levels were determined. Histopathological examination of liver tissues was performed to assess hepatic structural changes.

Results: Phytochemicals present include tannins, alkaloids and saponins. Oral median lethal dose was estimated to be >5000 mg/kg. Pre-treatment of mice with the RAF-BC at 250 and 500 mg/kg significantly ($p < 0.05$) reduced CCl_4 mediated increase in ALT, AST, ALP and TB levels. Histopathological findings corroborated the biochemical results by demonstrating marked preservation of hepatic architecture and reduced hepatocellular necrosis in the treated groups compared with the toxic control.

Conclusion: The results suggest that RAF-BC stem bark possesses hepatoprotective activity against CCl_4 induced acute liver injury. These findings may be associated with the phytochemical constituents present in the fraction.

Keywords: *Bombax costatum*; Hepatoprotective; Carbon tetrachloride; Acute liver injury; Phytochemicals.

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Introduction

Liver is one of the major organs that maintain physiological processes of the body at equilibrium. It is an indispensable organ with capacity to perform many vital functions in the body (Okaiyeto et al., 2018). The liver also plays a central role in the metabolism of drugs, toxins and food substances which makes it at risk of damage from a wide variety of metabolic, toxic, microbial, chemotherapeutic, circulatory, and neoplastic insults (Crawford, 2004). Collectively, chronic liver diseases are extremely costly in terms of human suffering, management and premature loss of productivity (European Association for the Study of Liver Disease (EASL), 2017). The projected annual economic impact of non-alcoholic fatty liver disease burden alone has been estimated to be $103 billion

in the United States of America and €35 billion in the United Kingdom, Germany, France, and Italy combined (Younossi et al., 2016).

Carbon tetrachloride-induced hepatotoxicity is a well-established experimental model for evaluating potential hepatoprotective agents. Following hepatic metabolism, CCl_4 undergoes bioactivation mainly through cytochrome P450 enzymes, particularly CYP2E1, producing highly reactive trichloromethyl radicals and reactive oxygen species. These reactive intermediates initiate lipid peroxidation, oxidative stress, disruption of hepatocyte membranes, and eventual leakage of intracellular enzymes into the circulation (McCay et al., 1984). Medicinal plants have been used by both urban and rural dwellers, thereby constituting acceptable, accessible and affordable therapy (Jordan et al., 2010). The use of medicinal plants to treat various forms of liver diseases is becoming increasingly popular and has received wide acceptance (Oyagbemi & Odetola, 2010). *Bombax costatum* is a tree usually reaching a height of 5–15 m. It flowers in the dry season before the leaves appear. The bark of *Bombax costatum* is thick, gray brown and corky with sharp pointed spines on the stem and branches. The leaves are digitately compound, with 5–7 leaflets. It grows abundantly in the savanna zones of West Africa from Senegal to the Central African Republic (Dalziel, 1985). The English name for *Bombax costatum* is red-flowered silk cotton tree. The local names for *Bombax costatum* are *Kuriya* or *Gurjiya* in Hausa and *Joohi* in Fulfulde. The stem bark is used to treat fever, skin disease, pain, abscess, oedema, hernia, and epilepsy (Assogba et al., 2017). Crude methanol stem bark extract of *B. costatum* was previously reported to possess hepatoprotective activity against carbon tetrachloride (CCl_4) induced acute hepatotoxicity in rats (Mohammed et al., 2018), while ethyl acetate fraction of *B. costatum* stem bark was reported to be hepatoprotective against CCl_4 induced acute hepatotoxicity in mice (Mohammed et al., 2023). However, the biological activity of the residual aqueous fraction obtained after solvent partitioning of the methanol extract remains poorly characterized. Since aqueous fractions often contain polar bioactive constituents such as tannins, saponins, glycosides, and alkaloids, evaluation of this fraction may provide additional insight into the hepatoprotective potential of *B. costatum* and its traditionally reported medicinal applications.

Therefore, the present study was designed to investigate the acute toxicity profile, preliminary phytochemical constituents, and hepatoprotective activity of the residual aqueous fraction of *B. costatum* stem bark against CCl_4-induced acute liver injury in mice. The protective effects were assessed through biochemical markers of hepatic injury and histopathological evaluation of liver tissue.

Methods

Equipment and Chemicals

Silymarin (Micro Labs Limited, India), Biochemical kits (Randox, UK), Olive oil (Metaluni S.P.A., Italy), Carbon tetrachloride (BDH Ltd Poole, England), Ethyl acetate, Methanol, Hexane (Sigma Aldrich, USA), Haematoxylin and Eosin stain, Scopetek DCM500 Camera (Hangzhou S. Digital Technology Ltd. China), Leitz Microscope (Optical Institute, Germany), Spectrophotometer (Metsar Technologies Pvt. Ltd., Hyderabad).

Experimental Animals

Male Swiss Albino mice weighing between 25–30 g were purchased from the Animal House of the Department of Pharmacology and Therapeutics, Ahmadu Bello University Zaria. They were kept in clean metal cages in a well-ventilated room and allowed to acclimatize for 14 days. They were fed with standard commercial mice feed (Vital feed®, Zaria). The mice were allowed free access to feed and drinking water *ad libitum*. All experimental protocols were performed according to regulations governing the care and use of experimental animals as contained in "Principles of Laboratory Animal Care" published by the National Institute of Health (NIH Publication No. 85-23, revised, 1996) and were approved by the Ahmadu Bello University Committee for Animal Use and Care (Approval number: ABUCAUC/2020/50).

Preparation of Residual Aqueous Fraction from Stem Bark of *B. costatum*

The stem bark of the plant was air dried under shade, mechanically powdered using mortar and pestle, and stored in an air-tight container. Methanol extract of *B. costatum* stem bark was prepared by subjecting 11 kg of the powdered stem bark to maceration using 40 L of 70% v/v methanol for 72 hours. The mixture was intermittently stirred during the 72-hour period and then filtered using Whatman filter paper (No. 1). The

filtrate was concentrated to dryness over a water bath maintained at 50°C. The extract was kept in an air-tight container and then stored in a desiccator until required for further studies. To obtain the RAF-BC, 460 g of methanol stem bark extract of *B. costatum* was subjected to liquid–liquid partitioning to separate the extract into different fractions. For each session, about 100 g of methanol stem bark extract of *B. costatum* powder was dissolved in distilled water (500 mL) and exhaustively extracted by successive liquid/liquid partitioning with hexane (500 mL), chloroform (500 mL) and ethyl acetate (500 mL) using a separating funnel (1000 mL). For each solvent, the mixture was allowed to stand for 30 minutes in the separating funnel until a clear separation line appeared. The solvent fractions and the residual aqueous fraction were evaporated to dryness to obtain their corresponding fractions (Gandhi et al., 2003).

Acute Toxicity Study

Acute toxicity study of RAF-BC was carried out in mice according to Organisation for Economic Co-operation and Development (OECD) guideline 423 (Organisation for Economic Co-operation and Development, 2001). Before oral administration of a single dose of the fraction, the mice were weighed and deprived of food for 3 hours. Limit test at a dose of 5000 mg/kg was conducted using 3 mice. The first mouse was dosed at 5000 mg/kg and observed. The remaining 2 mice were also dosed at 5000 mg/kg. All the mice were observed for general behavioural changes, symptoms of toxicity and mortality after treatment for the first four hours, then over a period of 24 hours, and thereafter daily for 14 days. The body weights of the mice were also taken on day 7 and day 14 of the study.

Preliminary Phytochemical Screening

The presence of tannins, alkaloids, flavonoids, glycosides (reducing sugars), anthraquinones, sterols and saponins were tested by simple qualitative methods (Trease & Evans, 1996; Wahab et al., 2010).

In Vivo Hepatoprotective Study of Residual Aqueous Fraction of *B. costatum* Against CCl_4 Induced Acute Hepatotoxicity in Mice

CCl_4 induced acute liver injury was carried out according to a method previously described (Arshad et al., 2024), with some modifications. The mice were divided into 6 groups with each group containing 6 mice. Group I (Normal control) received olive oil, 1 mL/kg, *i.p.*, on the 8th day of the study. Mice in group II (Toxic group) received CCl_4 (1 mL/kg, 1:1 in olive oil, *i.p.*) on the 8th day of the study. Group III (Standard group) mice were treated with silymarin (100 mg/kg *p.o.*) daily for 7 days followed by CCl_4 (1 mL/kg, 1:1 in olive oil, *i.p.*) on the 8th day. Mice in groups IV, V and VI received 125, 250 and 500 mg/kg of RAF-BC *p.o.* per day respectively for 7 days followed by CCl_4 (1 mL/kg, 1:1 in olive oil, *i.p.*) on the 8th day of the study. The mice in all the groups were euthanized 24 hours after injection of CCl_4. Liver tissues were harvested and the left lobes were fixed in a buffer solution of 10% formalin for histological analysis while blood samples were collected in plain sample bottles and then centrifuged at a speed of 4000 revolutions per minute to obtain serum. The sera were used for determination of AST, ALT, ALP and TB using biochemical kit (Randox reagent UK) and a spectrophotometer for reading the absorbance.

Histopathological Study

Sections of liver tissue were used for histopathological examination after dissecting the animals. Tissues were fixed in 10% buffered formalin (pH 7.2) and dehydrated through a series of ethanol solutions (70%, 90%, 96% and 100%). The tissues were cleared in xylene and embedded in paraffin. Sections of 5 μm thickness were cut using a rotary microtome and stained with haematoxylin and eosin for examination. The stained tissues were observed through a Leitz microscope at $\times$250 magnification and photographed with a Scopetek DCM500 camera.

Statistical Analysis of Data

Data were presented using tables and plates where applicable. Analyses of data were done using SPSS (version 26). Biochemical data were presented as Mean $\pm$ Standard Error of Mean (S.E.M.). Differences between means were analyzed using one-way ANOVA followed by Tukey's or Games-Howell's post hoc test. Games-Howell's post hoc test was used to analyse results of one-way ANOVA when the variances were not equal (Levene's test $p < 0.05$). The results were considered significant at p-values ≤ 0.05.

Results

Acute Toxicity Study

The oral LD_{50} for residual aqueous fraction of *B. costatum* stem bark in mice was estimated to be >5000 mg/kg body weight. No significant changes in body weight (Table 1) or other signs and symptoms of toxicity were observed for up to 2 weeks.

Table 1: Effect of Oral Administration of RAF-BC on Body Weight of Mice During Acute Toxicity Study

S/N	Day 0 (g)	Day 7 (g)	Day 14 (g)
1	26	28	30
2	28	29	31
3	25	27	29

RAF-BC = Residual aqueous fraction of *Bombax costatum* stem bark.

Phytochemical Constituents of RAF-BC

Preliminary phytochemical screening of residual aqueous fraction of *B. costatum* stem bark showed the presence of tannins, saponins, glycosides (reducing sugars), and alkaloids. Flavonoids, anthraquinones, and triterpenes were absent (Table 2).

Table 2: Phytoconstituents of Residual Aqueous Fraction of *Bombax costatum* Stem Bark

Phytoconstituent	Test	Aqueous fraction of BC
Flavonoids	Shinoda	−
Saponins	Frothing	+
Tannins	Ferric chloride	+
Anthraquinones	Borntrager's	−
Glycosides (reducing sugars)	Molisch	+
Triterpenes	Liebermann-Burchard	−
Alkaloid	Dragendorff	+

+ = Present; − = Absent

Effect of RAF-BC on ALT, AST, ALP and TB

Single intraperitoneal administration of CCl_4 to the mice in the CCl_4 toxic group on the 8th day of the study caused a statistically significant ($p < 0.01$) increase in serum levels of ALT, AST and ALP (121.67 $\pm$ 6.08, 102.33 $\pm$ 2.64 and 108.79 $\pm$ 5.62, respectively) when compared with the normal control group (38.5 $\pm$ 2.80, 66.00 $\pm$ 2.79 and 62.05 $\pm$ 2.19, respectively). A statistically significant ($p < 0.05$) increase in TB (1.33 $\pm$ 0.07) was also observed when compared with that of the normal control group mice (0.68 $\pm$ 0.02).

Pre-treatment of the mice with silymarin (100 mg/kg) caused a significant ($p < 0.01$) reduction in serum levels of ALT, AST and ALP when compared with that of the CCl_4 toxic group. Additionally, pre-treatment of the mice with 500 mg/kg of RAF-BC stem bark caused a significant ($p < 0.01$) reduction in serum levels of ALT, AST and ALP when compared with that of the CCl_4 toxic group. However, no significant ($p > 0.05$) reduction in serum levels of ALT (100.50 $\pm$ 5.02) and ALP (96.37 $\pm$ 3.38) were observed after pre-treatment with 125 mg/kg of RAF-BC when compared with the CCl_4 toxic group (121.67 $\pm$ 6.08 and 108.79 $\pm$ 5.62, respectively). Pre-treatment with RAF-BC at all tested doses also caused a significant ($p < 0.001$) reduction in the level of AST in a dose-dependent manner when compared with the CCl_4 toxic group. Statistically significant ($p < 0.05$) reductions in the levels of TB were also noted following treatment of the mice with silymarin and RAF-BC at all tested doses compared with the CCl_4 toxic group (Table 3).

Table 3: Effect of Intraperitoneal Administration of Carbon Tetrachloride on Liver Enzymes and Total Bilirubin in Mice

Treatment	**ALT (U/L)**	**AST (U/L)**	**ALP (U/L)**	**TB (mg/dL)**
OO	38.5±2.80	66.00±2.79	62.05±2.19	0.68±0.02
CCl_4	121.67±6.08$^{\#\#}$	102.33±2.64$^{\#\#}$	108.79±5.62$^{\#\#}$	1.33±0.07$^{\#}$
SLY	58.17±5.64**	64.90±1.69**	80.47±3.03**	0.79±0.04*
RAF-BC125	100.50±5.02^{ns}	78.10±3.51**	96.37±3.38^{ns}	0.68±0.02*
RAF-BC250	82.83±3.47**	73.97±3.61**	84.33±3.60*	0.64±0.01*
RAF-BC500	66.33±2.72**	63.58±3.46**	65.38±4.07**	0.61±0.01*

Data are presented as Mean ± S.E.M. (n = 6). One-way ANOVA followed by Tukey's and Games-Howell's post hoc tests for liver enzymes and total bilirubin, respectively. $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ compared to normal control group. *p <0.05, **p <0.01 compared to CCl_4 toxic group. ns = not significant compared to CCl_4 toxic group. RAF-BC = Residual aqueous fraction of *Bombax costatum* stem bark; OO = Olive oil; SLY = Silymarin; CCl_4 = Carbon tetrachloride.

Effect of RAF-BC on Histopathological Changes in Liver

Liver histopathological sections from mice of the CCl_4 toxic group showed intense hepatocellular necrosis (Plate Ib), whereas pre-treatment of the mice with silymarin prior to CCl_4 intoxication showed vascular congestion of the liver (Plate Ic). Pre-treatment of the mice with RAF-BC at doses of 125 mg/kg and 250 mg/kg prior to CCl_4 intoxication resulted in moderate hepatocellular necrosis (Plates Id and Ie), while the histopathological slide of the liver tissue of mice pre-treated with 500 mg/kg of RAF-BC before CCl_4 intoxication showed slight hepatocellular necrosis (Plate If). Normal liver architecture was observed in the normal control group (Plate Ia).

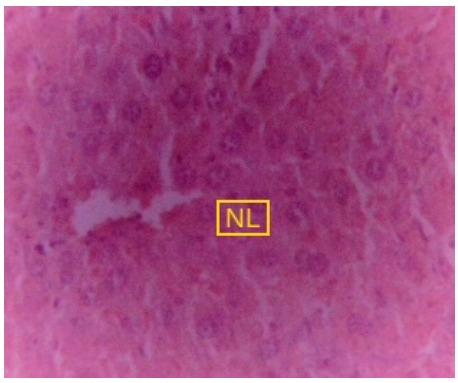

Plate Ia: Normal control (NL = Normal liver)

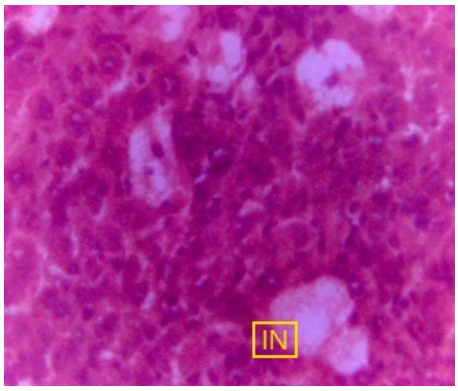

Plate Ib: CCl_4 toxic group (IN = Intense necrosis)

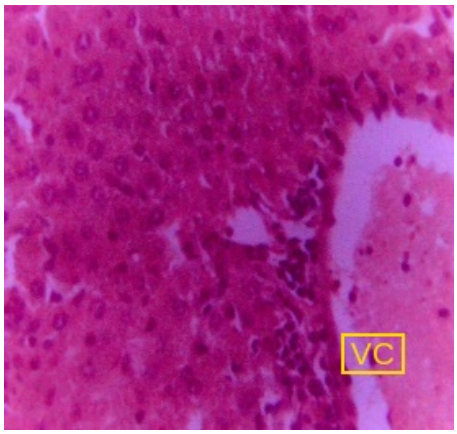

Plate Ic: Silymarin group (VC = Vascular congestion)

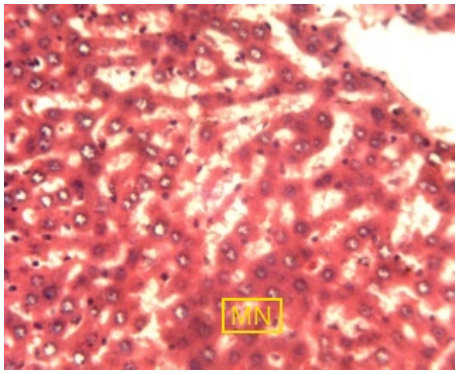

Plate Id: RAF-BC 125 mg/kg (MN = Moderate necrosis)

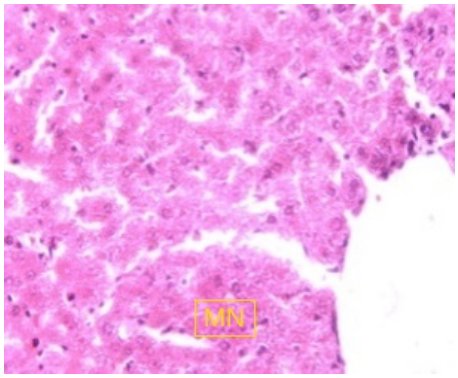

Plate Ie: RAF-BC 250 mg/kg (MN = Moderate necrosis)

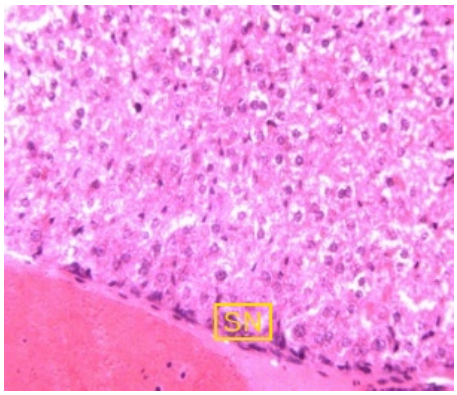

Plate If: RAF-BC 500 mg/kg (SN = Slight necrosis)

Figure 1: Liver histopathological slides (H and E ×250) showing the effect of residual aqueous fraction of *Bombax costatum* stem bark (RAF-BC) on CCl_4-induced acute liver injury in mice. (Ia) Normal control showing normal liver architecture. (Ib) CCl_4 toxic group showing intense hepatocellular necrosis. (Ic) Silymarin standard group showing vascular congestion. (Id, Ie) RAF-BC at 125 and 250 mg/kg showing moderate hepatocellular necrosis. (If) RAF-BC at 500 mg/kg showing only slight hepatocellular necrosis, indicating dose-dependent hepatic architectural preservation.

Discussion

The present study investigated the hepatoprotective potential of the residual aqueous fraction of *Bombax costatum* stem bark against carbon tetrachloride (CCl_4)-induced acute liver injury in mice. The findings demonstrated that pretreatment with the residual aqueous fraction significantly attenuated CCl_4-induced alterations in serum biochemical markers and preserved hepatic architecture, suggesting a protective effect against chemically induced hepatocellular injury. Furthermore, the fraction exhibited a favourable acute safety profile, with an estimated oral median lethal dose greater than 5000 mg/kg.

The absence of mortality and observable signs of toxicity following oral administration of the residual aqueous fraction supports its relative safety at the tested dose. This finding is consistent with the traditional use of *B. costatum* in various communities for the management of different ailments. The wide safety margin observed in the present study provides preliminary evidence supporting further pharmacological investigations of the plant. However, additional subacute and chronic toxicity studies are required to establish its long-term safety profile.

Preliminary phytochemical screening of the residual aqueous fraction revealed the presence of tannins, saponins, alkaloids, and carbohydrates. These secondary metabolites have previously been associated with several biological activities, including antioxidant and anti-inflammatory effects. Tannins possess free radical scavenging properties and may contribute

to cellular protection by reducing oxidative damage, while saponins and alkaloids have been reported to exhibit antioxidant and membrane-stabilizing activities (Panche et al., 2016). The hepatoprotective activity observed in this study may therefore be partly associated with the presence of these phytoconstituents. Nevertheless, further phytochemical characterization using quantitative methods such as total phenolic content determination, chromatographic profiling, and isolation of bioactive compounds is necessary to identify the constituents responsible for the observed activity.

The significant elevation of serum ALT, AST, ALP, and total bilirubin observed in the CCl_4-treated group in the present study confirms successful induction of acute hepatic injury.

Alanine aminotransferase and aspartate aminotransferase are important biochemical indicators of hepatocellular damage. Their increased activity in serum reflects disruption of hepatocyte membrane integrity and leakage of intracellular enzymes into the bloodstream. In this study, pretreatment with the residual aqueous fraction significantly reduced CCl_4-induced elevation of ALT and AST, particularly at doses of 250 and 500 mg/kg. This suggests that the fraction may protect hepatocytes against membrane damage and reduce the extent of cellular leakage caused by CCl_4 toxicity. Similar hepatoprotective effects have been reported previously with other medicinal plants possessing antioxidant constituents (Sharida et al., 2012).

Alkaline phosphatase is an important marker associated with hepatobiliary and cholestatic injury. The significant increase in serum ALP following CCl_4 administration was significantly reduced by pretreatment with RAF-BC, particularly at 500 mg/kg. Similarly, RAF-BC administration reduced total bilirubin levels.

The biochemical findings were further supported by histopathological observations. Liver sections from CCl_4-intoxicated mice showed marked hepatocellular necrosis and distortion of normal hepatic architecture, confirming the hepatotoxic effect of CCl_4. In contrast, animals pretreated with RAF-BC showed progressive improvement in hepatic morphology, with the highest dose (500 mg/kg) producing substantial preservation of liver architecture and only slight hepatocellular necrosis. The consistency between biochemical parameters and histological findings strengthens the evidence supporting the hepatoprotective activity of the residual aqueous fraction.

The hepatoprotective effect observed in the present study is consistent with previous reports from our research group demonstrating protective effects of crude methanol extract and ethyl acetate fraction of *B. costatum* stem bark against experimentally induced liver injury (Mohammed et al., 2018; Mohammed et al., 2023). However, the present study provides additional information regarding the biological activity of the residual aqueous fraction, which represents the more polar component remaining after solvent partitioning. The activity observed suggests that water-soluble constituents of *B. costatum* may contribute significantly to its hepatoprotective properties.

The observed hepatoprotective activity of the residual aqueous fraction of *B. costatum* stem bark could possibly be related to its natural antioxidant properties.

Conclusions

The results suggest that RAF-BC possesses hepatoprotective activity against CCl_4 induced acute liver toxicity in mice. Further studies are needed to elucidate the underlying mechanism of the hepatoprotective effect of RAF-BC.

What Is Known About the Topic

- Carbon tetrachloride is a potent hepatotoxicant used for induction of hepatotoxicity in animal models.
- Silymarin is a standard drug used as a hepatoprotective agent.
- Loss of hepatocellular membrane integrity is accompanied by a rise in serum levels of liver enzymes and bilirubin.

What This Study Adds

The findings of this study demonstrate that the residual aqueous fraction of *Bombax costatum* stem bark possesses significant protective activity against CCl_4-induced acute liver injury in mice. The combination of biochemical improvement, histological preservation, and favourable acute toxicity profile supports further investigation of this fraction as a potential source of hepatoprotective agents.

Declarations

Author Contributions

- Nuhu Mohammed – Conceptualization and original draft preparation
- Umar Abdullahi – Visualization
- Ahmadu Saleh – Reviewing
- Hamza Rabiu Muhammad – Data analysis
- Umar Bala Ningi – Editing

Funding Disclosure

All authors declare that they have no financial relationship with any organization that might have an interest in the submitted work.

Conflicts of Interest

All authors declare no conflict of interest.

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References

Arshad, M., Kousar, S., Din, A., Afzaal, M., Faisal, M. N., Sharif, M. K., Rasheed, H., Saeed, F., Akram, N., Ahmed, F., & Khan, M. R. (2024). Hepatoprotective efficacy of quinoa seed extract against CCl4-induced acute liver toxicity in rat model. *Food Science & Nutrition*, *12*, 5007–5018. https://doi.org/10.1002/fsn3.4149

Assogba, G. A., Fandohan, A. B., Salako, V. K., & Assogbadjo, A. E. (2017). Uses of *Bombax costatum* (malvaceae) in the surrounding areas of the pendjari biosphere reserve, republic of benin. *Bois et Forêts des Tropiques*, *333*(3), 17–33. https://doi.org/10.19182/BFT2017.333.A31465

Chen, Y.-J., Chen, S.-C., Chen, C.-S., Kuo, C.-P., Wu, K.-C., Chen, C.-J., Tsai, J.-C., & Peng, W.-H. (2018). Hepatoprotective effect of the ethanol extract of *Polygonum orientale* on carbon tetrachloride-induced acute liver injury in mice. *Journal of Food and Drug Analysis*, *26*, 369–379. https://doi.org/10.1016/j.jfda.2017.04.007

Crawford, J. M. (2004). Liver and biliary tract. In V. Kumar, A. Abbas, & N. Fausto (Eds.), *Robbins and cotran's pathologic basis of disease* (7th, pp. 877–937). Saunders.

Dalziel, J. M. (1985). *The useful plants of west tropical africa* (Vol. 1). Royal Botanic Gardens.

Daniel, S. P. (2015). Evaluation of liver function. In A. S. Fauci, E. Braunwald, D. L. Kasper, S. L. Hauser, D. L. Longo, J. L. Jameson, & J. Loscalzo (Eds.), *Harrison's principles of internal medicine* (17th, pp. 1995–1999). The McGraw-Hill Companies.

European Association for the Study of Liver Disease (EASL). (2017). HEPAMAP. a roadmap for hepatology research in europe: An overview for policy makers.

Gandhi, A. P., Joshi, K. C., Jha, K., Parihar, V. S., Srivastav, D. C., Raghunadh, P., & Tripathi, R. N. (2003). Studies on alternative solvents for the extraction of oil-I soybean. *International Journal of Food Science and Technology*, *38*(3), 369–375. https://doi.org/10.1046/j.1365-2621.2003.00683.x

Jordan, S. A., Cunningham, D. G., & Marles, R. J. (2010). Assessment of herbal medicinal products: Challenges, and opportunities to increase the knowledge base for safety assessment. *Toxicology and Applied Pharmacology*, *243*, 198–216. https://doi.org/10.1016/j.taap.2009.12.005

Kim, S. H., Cheon, H. J., Yun, N., Oh, S. T., Shin, E., Shim, K. S., & Lee, S. M. (2009). Protective effect of a mixture of *Aloe vera* and *Silybum marianum* against carbon tetrachloride–induced acute hepatotoxicity and liver fibrosis. *Journal of Pharmacological Sciences*, *109*, 119–127. https://doi.org/10.1254/jphs.08189fp

Laurence, L. B., Keith, L. P., Donald, K. B., & Lain, L. O. B. (2008). *Goodman and gilman's manual of pharmacology and therapeutics*. McGraw-Hill Companies.

McCay, P. B., Lai, E. K., Poyer, J. L., DuBose, C. M., & Janzen, E. G. (1984). Oxygen and carbon-centered free radical formation during carbon

tetrachloride metabolism. observation of lipid radicals *In Vivo* and *In Vitro*. *Journal of Biological Chemistry*, *259*(4), 2135–2143.

Mohammed, N., Ahmadu, S., Yau, J., Bala, N. U., Rabiu, M. H., Najib, S. I., Khadija, U., & Aliyu, M. M. (2023). Hepatoprotective activity of ethyl acetate fraction of *Bombax costatum* (pellegr. et vuillet) stem bark against carbon tetrachloride induced acute liver injury in mice. *Archives of Pharmaceutical Sciences and Biotechnology Journal*, *3*(2), 117–127.

Mohammed, N., Yaro, A. H., & Nazifi, A. B. (2018). *Bombax costatum* pellegr. and vuillet stem bark extract prevents paracetamol and carbon tetrachloride-induced liver injury in rats. *Tropical Journal of Natural Product Research*, *2*(5), 220–226. https://doi.org/10.26538/tjnpr/v2i5.4

Okaiyeto, K., Nwodo, U. U., Mabinya, L. V., & Okoh, A. I. (2018). A review on some medicinal plants with hepatoprotective effects. *Pharmacognosy Reviews*, *12*, 186–199.

Organisation for Economic Co-operation and Development. (2001). OECD guidelines for testing of chemicals, test no. 423, acute toxic class method.

Ouassou, H., Bouhrim, M., Daoudi, N. E., Mekhfi, H., Ziyyat, A., Legssyer, A., Aziz, M., & Bnouham, M. (2021). Evaluation of hepatoprotective activity of *Caralluma europaea* stem extract against CCl4-induced hepatic damage in wistar rats. *Advances in Pharmacological and Pharmaceutical Sciences*, *2021*. https://doi.org/10.1155/2021/8883040

Oyagbemi, A. A., & Odetola, A. A. (2010). Hepatoprotective effects of ethanolic extract of *Cnidoscolus aconitifolius* on paracetamol-induced hepatic damage in rats. *Pakistan Journal of Biological Sciences*, *13*(4), 164–169.

Panche, A. N., Diwan, A. D., & Chandra, S. R. (2016). Flavonoids: An overview. *Journal of Nutritional Science*, *5*(47), 1–15. https://doi.org/10.1017/jns.2016.41

Shahzad, A., Faisal, M. N., Hussain, G., Naqvi, S. A. R., & Anwar, H. (2021). Therapeutic potential of quinoa seed extract as regenerative and hepatoprotective agent in induced liver injury wistar rat model. *Pakistan Journal of Pharmaceutical Sciences*, *34*, 2309–2315.

Sharida, F., Syazana, A. S., & Palanisamy, A. (2012). *Moringa oleifera* hydroethanolic extracts effectively alleviate acetaminophen-induced hepatotoxicity in experimental rats through their antioxidant nature. *Molecules*, *17*, 8334–8350. https://doi.org/10.3390/molecules17078334

Trease, G. E., & Evans, W. C. (1996). *A text book on pharmacognosy* (12th). Bailliere-Tindall.

Wahab, O. M., Ayodele, A. E., & Moody, J. O. (2010). Thin layer chromatography phytochemical screening in some nigerian loranthaceae. *Journal of Pharmacognosy and Phytotherapy*, *2*(5), 64–70.

Weber, L. W., Boll, M., & Stampfl, A. (2003). Hepatotoxicity and mechanism of action of haloalkanes: Carbon tetrachloride as a toxicological model. *Critical Reviews in Toxicology*, *33*, 105–136.

Widodo, H., Sismindari, S., Asmara, W., & Rohman, A. (2019). Antioxidant activity, total phenolic and flavonoid contents of selected medicinal plants used for liver diseases and its classification with chemometrics. *Journal of Applied Pharmaceutical Science*, *9*(6), 99–105. https://doi.org/10.7324/JAPS.2019.90614

Xu, Y., Guo, W., Zhang, C., Chen, F., Tan, H. Y., Li, S., Wang, N., & Feng, Y. (2020). Herbal medicine in the treatment of non-alcoholic fatty liver diseases—efficacy, action mechanism, and clinical application. *Frontiers in Pharmacology*, *11*, 601. https://doi.org/10.3389/fphar.2020.00601

Younossi, Z. M., Blissett, D., Blissett, R., Henry, L., Stepanova, M., Younossi, Y., & Beckerman, R. (2016). The economic and clinical burden of nonalcoholic fatty liver disease in the united states and europe. *Hepatology*, *64*, 1577–1586. https://doi.org/10.1002/hep.28785