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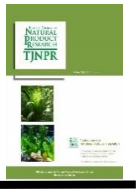

Bombax costatum Pellegr. and Vuillet Stem Bark Extract Prevents Paracetamol and Carbon Tetrachloride-Induced Liver Injury in Rats

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ABSTRACT

Bombax costatum is a commonly used medicinal plant in African traditional medicine in the treatment of liver disease. This study was aimed at investigating the hepatoprotective and antioxidant activities of methanol extract of *Bombax costatum* stem bark against paracetamol (PCM) and carbon tetrachloride (CCl_4) induced hepatotoxicity in Wistar rats. Phytochemical analysis and acute toxicity studies of the plant extract were performed. The hepatoprotective activity of the plant extract was evaluated *in vivo* using PCM (3g/kg orally on the 4th day of the study) and CCl_4 (1 mL/kg, 1:1 in olive oil, intraperitoneally on the 3rd and 4th day of the study) induced liver damage in rats while *in vitro* 1,1-diphenyl-2-picrylhydrazyl radical scavenging method was used for antioxidant study. Phytochemical screening of the extract showed the presence of tannins, sterols, alkaloids, saponins, cardiac glycosides, triterpenes and flavonoids. Oral median lethal dose of the extract in rats was estimated to be >5000 mg/kg. The extract (250 and 500 mg/kg) showed significant (p<0.05) reduction in alanine transaminase levels when compared with PCM toxic group. Significant (p<0.05) reduction in serum alkaline phosphatase, aspartate transaminase, alanine transaminase and total bilirubin were observed in the extract treated groups (250, 500 and 1000 mg/kg) when compared with CCl_4 toxic group. Histopathological findings showed that *B. costatum* extract at 250 and 500 mg/kg protected the liver tissues from damage induced by PCM and CCl_4. In conclusion, the findings of this study showed that the methanol stem bark extract of *Bombax costatum* possesses hepatoprotective and antioxidant properties.

Keywords: *Bombax costatum*, Hepatoprotective, Antioxidant, Paracetamol, Carbon tetrachloride.

Introduction

The liver is an important organ in the body involved in homeostasis, metabolism of drugs, toxins and food substances, and is one of the major organs prone to oxidative insults by the attack of reactive oxygen species.[1] Liver disease is a major cause of morbidity and mortality among human population and has a worldwide distribution.[2] The dominant primary diseases of the liver are viral hepatitis, alcoholic liver disease and hepatocellular carcinoma.[1] The conventional drugs used in the treatment of liver diseases are sometimes inadequate and disappointing due to serious adverse effects produced following their prolonged usage.[3,4] In this view, medicinal plants have gained wide patronage in the treatment of liver diseases and studies have geared towards investigating such plants as sources of hepatoprotective agents.

Medicinal plants have been widely used by urban and rural populations in treating various diseases, constituting an effective and less expensive therapy.[5] According to the World Health Organization, about 80% of the population in developing countries rely on traditional medicine for their healthcare needs.[6]

Studies have also shown that African indigenous herbal medicines are widely used throughout the African continent, despite an apparent lack of scientific evidence for their quality, safety and efficacy.[7] This can be explained due to the fact that traditional medicine is more affordable, accessible and culturally acceptable in many parts of the world.

Bombax costatum Pellegr. and Vuillet (family: *Bombacaceae*) is known as red-flowered silk cotton tree in English and kapokier in French. It is locally called *Kuryaa* or *Gurjiiyaa* and *Joohi* in "Hausa" and "Fulfulde" languages, respectively. Its distribution is restricted to the savanna zones of West Africa from Senegal to the Central African Republic.[8] Several parts of *Bombax costatum* are used in traditional medicine against variety of illnesses. The bark of both stem and roots are reported to have diuretic properties, and are used in the treatment of skin diseases, epilepsy, insanity, yellow fever and headache; and to promote lactation and wound healing.[8] Similarly, the leaves of the plant are used in the treatment of hemorrhage, convulsions, fever, diarrhea, jaundice and liver problems.[8] Previous scientific studies have reported that the fruits of *Bombax costatum* possess good *in vitro* antioxidant potentials.[9] Ogbobe *et al*.,[10] also reported the presence of caproic, caprylic, palmitic, stearic, oleic, linoleic, arachidic and lignoceric acids in *Bombax costatum* seeds. However, there is no scientific report validating the use of the stembark in treatment of liver diseases and in this regard, this study was aimed at investigating the hepatoprotective and antioxidant potentials of the methanol extract of *Bombax costatum* stem bark against paracetamol and carbon tetrachloride-induced liver injury in rats.

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Materials and Methods

Drugs and chemicals

1,1-diphenyl-2-picrylhydrazyl radical (DPPH) powder, paracetamol powder (Sigma Chemicals Co, USA), Carbon tetrachloride (BDH Ltd Poole, England), Silymarin (Micro Labs Limited, India), Olive oil (Metaluni S.P.A., Italy), Normal saline (0.9%$^{w}/_{v}$ NaCl Isotonic Solution).

Experimental animals

Wistar rats of both sexes (180-220 g) obtained from Animal Facility of Department of Pharmacology and Therapeutics, Bayero University, Kano, were used for the study. The animals were kept in clean cages with metal coverlids and bedded with soft wood shavings, which were replaced every three days. They were maintained in a well-ventilated room and fed on standard commercial rat feed (Vital feed®, Kano) with free access to drinking water *ad libitum*. All experiments performed on the laboratory animals were approved by the University Animal Ethics Committee and the animals were handled in accordance with 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).

Plant material and preparation of extract

The plant was collected from Hanwa area of Sabon Gari local Government, Kaduna State in July, 2015. It was authenticated by Musa Muhammad, a taxonomist at the Herbarium section of the Department of Botany, Ahmadu Bello University, Zaria and assigned a voucher number (No. 1211). A voucher specimen of the plant was deposited in the Herbarium for future reference. 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 *Bombax costatum* stem bark was prepared by subjecting 1.29 kg of the powdered stem bark to maceration using 10 L of 70% methanol (in 30% water) for 72 hours. The mixture was intermittently stirred during the 72 hours period and then filtered using Whatman filter paper No. 1. The filtrate was concentrated to dryness over a water bath maintained at 50ºC. The extract obtained was weighed, kept in an air tight container and then stored in a desiccator until required for further studies.

Phytochemical screening

B. costatum stem bark extract was subjected to standard phytochemical screening[11] to test for the presence or absence of phytochemicals including tannins, alkaloids, flavonoids, glycosides, anthraquinones, sterols and saponins.

Acute toxicity study

Oral acute toxicity study in rats was carried out according to the method described by Lorke.[12] The median lethal dose (LD_{50}) evaluation was carried out in two stages and the animals were fasted overnight prior to administration of the extract. In the first stage, three groups of three rats each were treated orally with the stem bark extract at doses of 10, 100 and 1000 mg/kg body weight respectively. The animals were then observed for signs of toxicity including death for 24 hours. In the second phase, three rats were used, and each rat was treated with the extract at doses of 1600, 2900 and 5000 mg/kg respectively. The rats were also observed for 24 hours for signs of toxicity and mortality. The LD_{50} value was then calculated as the geometric mean of the highest non-lethal dose (with no death) and the lowest lethal dose (where death occurred).

Paracetamol-induced hepatotoxicity

Paracetamol-induced hepatotoxicity was carried out according to the method described by Ajith et al.,[13] with some modifications which were validated by pilot survey. In this study, the rats were divided into 6 groups with 5 rats in each. Group I served as normal control and received Normal saline, 1 mL/kg, *p.o.* for 5 days. Group II (Toxic control) animals received paracetamol (3 g/kg, *p.o.*) on the 4th day. Group III (Standard) was treated with standard drug silymarin (100 mg/kg *p.o.*) for 5 days and on the 4th day, paracetamol (3 g/kg, *p.o.*) was given 1 hour after the treatment with the drug. Groups IV, V and VI were treated with the extracts at doses of 250, 500 and 1000 mg/kg body weight *p.o.* per day respectively for 5 days and on the 4th day paracetamol (3 g/kg, *p.o.*) was given 1 hour after treatment with the extracts. The animals in all the groups were sacrificed 48 hours after the dose of paracetamol under mild chloroform anaesthesia. The blood was collected in a plain sample bottle and then centrifuged at a speed of 4000 revolution per minute to separate the serum. The serum samples were stored at −80°C. Biochemical parameters such as aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), as well as serum determination of total bilirubin (TB) and conjugated bilirubin (CB) were then analysed using spectrophotometer.

Carbon tetrachloride (CCl_4)-induced hepatotoxicity

CCl_4-induced hepatotoxicity was carried according to the method described by Jain et al.,[14] with some modifications which were validated using pilot survey. The dose of CCl_4 used for this study was 1 mL/kg. The rats were divided into six groups with each group containing 5 rats. Group I (Normal control) received Normal saline, 1 mL/kg, *p.o.* for 5 days. Group II (Toxic group) animals received CCl_4 (1mL/kg, 1:1 in olive oil, *i.p.*), on the 3rd and 4th days. Group III (Standard) was treated with standard drug silymarin (100 mg/kg *p.o.*) for 5 days and on the 3rd and 4th day CCl_4 (1 mL/kg, 1:1 in olive oil, *i.p.*) was given 1 hour after the treatment with the drug. Group IV, V and VI animals received 250, 500 and 1000 mg/kg of the extract *p.o.* per day, respectively for 5 days and on the 3rd and 4th days CCl_4 (1 mL/kg, 1:1 in olive oil, *i.p.*) was given 1 hour after the treatment with extract. The animals in all the groups were sacrificed 48 hours after the last injection of CCl_4 under mild chloroform anaesthesia. The blood was collected in a plain sample bottle and then centrifuged at a speed of 4000 revolutions per minute to separate the serum. The serum samples were stored at −80°C. Biochemical parameters such as AST, ALT, ALP, as well as serum determination of TB and CB were then analysed using a spectrophotometer.

Histopathological analysis

Sections of tissue from liver 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, embedded in paraffin and routinely processed for histological analysis. Sections of 5 µm thickness were cut and stained with haematoxylin-eosin for examination. The stained tissues were observed through a Leitz microscope at ×250 magnification and photographed by a Scope tek DCM500 camera.

DPPH radical scavenging assay

The DPPH radical scavenging activity of the methanol extract of *B. costatum* stem bark was determined by the DPPH radical scavenging method.[15] DPPH solution was prepared by dissolving 6 mg of DPPH in 100 mL of methanol. To 1 ml of various concentrations of the methanol extract of the stem bark (20, 40, 60, 80, 100 µg/mL), 2 ml of DPPH solution (0.1 mM) was added. An equal amount of methanol and DPPH served as control. The mixture was shaken vigorously after being kept for 30 minutes. The absorbance of the resulting solution was taken using a UV spectrophotometer at 520 nm. The experiments were performed in triplicate and the percentage scavenging activity of the extract on DPPH radical was calculated using the following formula:

Radical scavenging activity (%) = $[AB - AE/AB] \times 100$

Where AB = absorbance of control, AE = absorbance of the extract.
DPPH radical scavenging activity of the extract was expressed in terms of IC_{50} (concentration of the extract required to inhibit DPPH radical formation by 50%). This was calculated after plotting the graph of % inhibition against sample concentration using Microsoft excel software.

Statistical analysis

Data were presented using tables, charts and plates where applicable. Serum biochemistry data were presented as Mean ± Standard Error of the Mean (S.E.M.). Analysis of data was done using minitab version 17. Significant differences in biochemical parameters of each extract treated group were compared with that of toxic group using one-way ANOVA followed by Dunnett's post-hoc test. The results were considered significant at p-values of ≤ 0.05.

Results and Discussion

The present study was designed to establish a scientific justification for the ethnomedical use of *B. costatum* stem bark in treatment of liver diseases. The hepatoprotective effect was studied in PCM and CCl_4-induced liver injury. The preliminary phytochemical analysis of *B. costatum* stem bark extract revealed the presence of alkaloids, flavonoids, tannins, carbohydrates, cardiac glycosides, triterpenes and saponins (Table 1). Some of these constituents are active secondary metabolites shown to be responsible for some important pharmacological activities of medicinal plants.[16,17] The phytochemical constituents present may also be responsible for the observed hepatoprotective and antioxidant activities of *B. costatum* extract in this study.

The oral LD_{50} of the methanol extract of *B. costatum* stem bark in rat was found to be greater than 5000 mg/kg body weight, and no sign or symptoms of toxicity were observed. This implies that the extract is practically non-toxic[18] in rats when administered orally as no death was recorded in both phases of the study.

In the paracetamol-induced hepatotoxicity studies, administration of PCM caused significant ($p< 0.05$) increase in ALT, AST and ALP levels when compared to control rats treated with normal saline. Pre-treatment of the rats with sylimarin (100 mg/kg) caused significant ($p< 0.05$) decrease in ALT levels (24.8±0.8) as compared with PCM toxic group (38.0±2.5). Similarly, pre-treatment of rats with 250 and 500 mg/kg of *B. costatum* extract also caused significant ($p< 0.05$) reduction in serum ALT levels (25.6±1.3 and 30.2±0.49 respectively) when compared with PCM toxic group (38.0±2.5) (Table 2). However, pretreatment with *B. costatum* extract (250, 500 and 1000 mg/kg) did not cause significant ($p> 0.05$) decrease in serum AST and ALP levels when compared to the PCM toxic group (Table 2). Liver section from saline control group showed normal lobular architecture and normal hepatic cells (Plate Ia). Administration of PCM at a dose of 3 g/kg caused moderate hepatocellular necrosis with kupffer cell hyperplasia (Plate Ib). Liver section from Silymarin treated group showed mild hepatocellular necrosis (Plate Ic) while the group treated with *B. costatum* at a dose of 250 mg/kg showed slight increase in pyknotic nuclei (Plate Id). Liver section of rats treated with 500 mg/kg of *B. costatum* showed slight hepatocellular necrosis (Plate Ie) while liver section of rats treated with 1000 mg/kg of *B. costatum* showed intense hepatocellular necrosis with kupffer cell hyperplasia (Plate If).

Liver cell damage can lead to loss of functional integrity of parenchymal cells and leakage of liver enzymes resulting in their elevation in the serum.[19] In this study, a single toxic dose of paracetamol (3 g/kg) was used to induce liver damage in the rats of toxic, silymarin and extracts treated groups which resulted in serum elevation of biochemical markers of liver damage (ALT, AST, ALP, conjugated and total bilirubin) as well as hepatocellular necrosis. Paracetamol is a well-known antipyretic and analgesic agent which is harmless at therapeutic doses but can produce fatal hepatic necrosis in experimental animals as well as humans. It is employed as a well-established experimental hepatotoxic agent for pre-clinical research.[20,21] Conjugation with glucuronic acid and sulfuric acid constitute the major metabolic pathways of paracetamol. At therapeutic doses, a small proportion of paracetamol is metabolized by cytochrome P450 to form the highly reactive species, *N*-acetyl-*p*-benzoquinone imine (NAPQI), which under normal conditions is readily detoxified by conjugation with glutathione (GSH) to form harmless water soluble mercapturic acid, which undergoes renal excretion.[20] Paracetamol administration in high dose can cause necrosis of the centrilobular hepatocytes which is characterized by nuclear pyknosis and eosinophilic cytoplasm followed by large excessive hepatic lesion.[22] The mechanism by which over dosage with paracetamol leads to hepatocellular injury and death involves its conversion to the toxic NAPQI metabolite. This toxic metabolite accumulates as a result of saturation of the glucuronide and sulfate conjugation pathways. In the setting of paracetamol overdose, hepatocellular levels of GSH become depleted. The highly reactive NAPQI metabolite binds covalently to cell macromolecules, leading to dysfunction of enzymatic systems and structural and metabolic disarray. Furthermore, depletion of intracellular GSH renders the hepatocytes highly susceptible to oxidative stress and apoptosis.[20] Pre-treatment of the rats with silymarin (100 mg/kg) and *B. costatum* extract (250 and 500 mg/kg) protected the rats liver from oxidative stress and apoptosis caused by toxic dose of paracetamol. The methanol extracts also protected the liver against hepatocellular necrosis, pyknotic nuclei, vacoulation and kupffer cell hyperplasia caused by PCM in dose-dependent manner. However, 1000 mg/kg of *B. costatum* tend to cause hepatic damage as evidenced by elevated serum hepatic enzymes and moderate hepatocellular necrosis of the liver.

The recovery from paracetamol induced hepatocellular necrosis observed from histopathological results has supported the biochemical analysis findings which further confirmed the hepatoprotective activity of methanol stem bark extract of *B. costatum* against PCM-induced hepatic damage. The hepatoprotective activities of the methanol extract of *B. costatum* against PCM-induced liver damage correlated well with the research findings of similar study[23] where *Moringa oleifera* hydroethanolic extracts were found to effectively alleviate PCM-induced hepatotoxicity in experimental rats through their antioxidant nature. The hepatoprotective mechanism of action of methanol stem bark extract of *B. costatum* could be as a result of the antioxidant properties of its phytochemical constituents.

In the CCl_4-induced hepatotoxicity studies, administration of CCl_4 caused significant ($p< 0.05$) increase in ALT, AST, ALP, CB and TB levels when compared to control rats treated with normal saline. Treatment with sylimarin (100 mg/kg) caused significant ($p< 0.05$) reduction in serum ALT, AST, ALP and TB when compared with CCl_4 toxic group (Table 3). Similarly, the extract (250, 500 and 1000 mg/kg) caused significant ($p< 0.05$) decrease in serum ALT (24.0±3.3, 31.4±1.5 and 32.8±5.8), serum AST (74.0±8.0, 66.2±1.8 and 76.8±3.5) and serum ALP (353.6±17.0, 329.6±20.0 and 385.6±7.6 respectively) and serum TB levels (11.2±3.2, 4.8±3.2 and 9.6±3.0) respectively when compared with CCl_4 toxic group. However, no significant ($p> 0.05$) reduction in serum conjugated bilirubin was observed when compared with CCl_4 toxic group (Table 3). Liver sections from saline control group showed normal lobular architecture and normal hepatic cells (Plate IIa). Section of the liver tissue from CCl_4 toxic group showed moderate hepatocellular necrosis (Plate IIb). Liver section from Sylimarin treated group showed focal necrosis with vacoulation and intense pyknotic nuclei (Plate IIc). Histopathological study of liver tissue from *B. costatum* treated group at a dose of 250 mg/kg showed slight hepatocellular necrosis (Plate IId). Liver section from group treated with 500 mg/kg of *B. costatum* showed focal necrosis with intense kupffer cell hyperplasia (Plate IIe). Treatment with 1000 mg/kg of *B. costatum* caused vacoulation in the liver with moderate hepatocellular necrosis and kupffer cell hyperplasia (Plate IIf).

CCl_4 induces hepatotoxicity through bioactivation of cytochrome P450 2E1 enzymes (CYP2E1). This leads to the formation of trichloromethyl free radicals and reactive oxygen species (ROS), which initiate lipid peroxidation, protein oxidation and damage the hepatocellular membranes.[24] This process is followed by the release of inflammatory mediators from activated hepatic macrophages, which are believed to potentiate the CCl_4-induced hepatic injury.[25,26] The effects of CCl_4 on hepatocytes, depending on dose and exposure time, can manifest histologically as hepatic steatosis, centrilobular necrosis, and ultimately, cirrhosis. The damage to the liver is associated with leakage of hepatic enzymes into serum resulting in elevation in their serum concentration. The dose of CCl_4 that induces hepatotoxicity ranges from 0.1 to 3 mL/kg when administered intraperitoneally.[22]

Table 1: Phytochemical constituents of methanol stem bark extract of *Bombax costatum.*

Phytochemicals	Inference
Alkaloids	Present
Anthraquinones	Absent
Flavonoids	Present
Glycosides	Present
Saponins	Present
Triterpenes	Present
Tannins	Present
Carbohydrates	Present

Table 2: Effect of pretreatment with methanol extract of *Bombax costatum* stem bark against paracetamol-induced hepatotoxicity on enzyme and non-enzyme markers of liver damage in rats.

Treatment (mg/kg)	ALT (U/L)	AST (U/L)	ALP (U/L)	CB (µmol/L)	TB (µmol/L)
NS	17.6 ± 1.9	40.4 ± 6.5	184.8 ± 21.0	1.6 ± 0.9	4.8 ± 3.2
PCM	38.0 ± 2.5**	90.8 ± 1.9**	344.8 ± 41.0**	5.6 ± 1.6	4.6 ± 5.5
SLY + PCM	24.8 ± 0.8*	71.8 ± 1.8	331.0 ± 12.0	1.6 ± 1.6	4.8 ± 3.2
BC (250) + PCM	25.6 ± 1.3*	74.8 ± 1.5	372.2 ± 20.0	2.4 ± 1.6	6.4 ± 3.0
BC (500) + PCM	30.2 ± 0.5*	93.2 ± 1.0	344.2 ± 39.0	2.8 ± 1.7	9.8 ± 6.6
BC (1000) + PCM	37.2 ± 1.1	104.8 ± 1.4	306.4 ± 14.0	3.2 ± 2.0	6.4 ± 3.9

Data were presented as Mean ± SEM. (n = 5),*$p < 0.05$ when compared with PCM toxic group, **$p < 0.05$ when compared with normal control group (One way ANOVA followed by Dunnett's post-hoc test), ALT = Alanine transaminase, ALP = Alkaline phosphatase, AST = Aspartate transaminase, BC = *Bombax costatum*, CB = Conjugated bilirubin, TB=Total bilirubin, PCM = Paracetamol, NS= Normal saline, SLY = Silymarin.

Table 3: Effect of methanol extract of *Bombax costatum* stem bark on CCl_4-induced hepatotoxicity on enzyme and non-enzyme markers of liver damage in rats.

Treatment (mg/kg)	ALT (U/L)	AST (U/L)	ALP (U/L)	CB (µmol/L)	TB (µmol/L)
NS	13.2 ± 1.4	69.0 ± 4.0	153.4 ± 18.0	1.6 ± 1.6	4.8 ± 3.2
CCL_4	96.2 ± 9.4**	126.4 ± 17.0**	464.4 ± 20.0**	8.0 ± 2.5**	21.2 ± 5.0**
SLY + CCl_4	18.6 ± 0.8*	72.0 ± 4.2*	249.0 ± 15.0*	3.2 ± 2.0	6.4 ± 3.9*
BC (250) + CCl_4	24.0 ± 3.3*	74.0 ± 8.0*	353.6 ± 17.0*	4.8 ± 2.0	11.2 ± 3.2*
BC (500) + CCl_4	31.4 ± 1.5*	66.2 ± 1.8*	329.6 ± 20.0*	3.2 ± 2.0	4.8 ± 3.2*
BC (1000) + CCl_4	32.8 ± 5.8*	76.8 ± 3.5*	385.6 ± 7.6*	4.0 ± 1.8	9.6 ± 3.0*

Data are presented as Mean ± S.E.M., *$p < 0.05$ when compared with CCl_4 toxic group, **$p < 0.05$ when compared with normal control group (One way ANOVA followed by Dunnett's post-hoc test), n = 5, ALT = Alanine transaminase, ALP = Alkaline phosphatase, AST = Aspartate transaminase, CB = Conjugated bilirubin, TB=Total bilirubin, BC = *Bombax costatum*, NS= Normal saline, CCl_4 = Carbon tetrachloride, SLY = Silymarin.

Table 4: Quantitative DPPH assay of the stem bark extract of *Bombax costatum*.

Concentration (µg/mL)	AB	AE	% Inhibition
20	0.469	0.284	39.45
40	0.809	0.320	60.44
60	0.827	0.127	84.64
80	0.914	0.225	75.38
100	0.796	0.288	63.82

Where AB = absorbance of control. AE = absorbance of the Extract. Wavelength = 520 nm

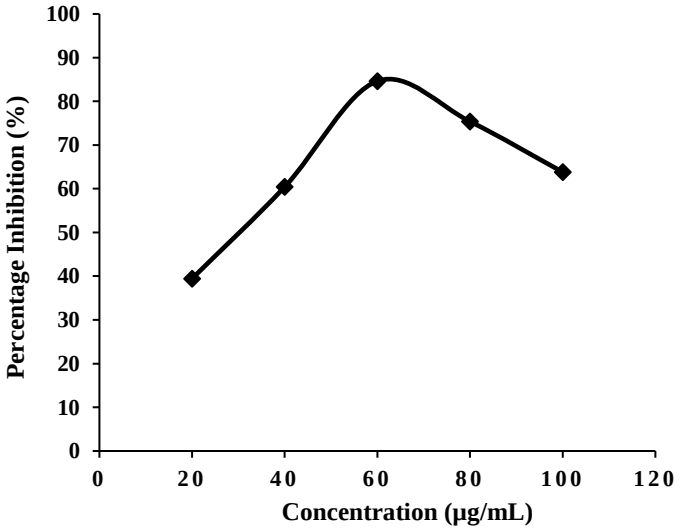

Figure 1: DPPH radical scavenging activity of methanol extract of *B. costatum* stem bark. Results represents mean of triplicates of different concentrations analysed. Wavelength = 520nm.

In this study, 1 ml/kg of CCl_4-induced liver damage in the experimental rats. In agreement with results obtained in similar investigations,[27,28] treatment with CCl_4 in the present study elicited increase in the levels of serum ALT and AST but pre-treatment of the rats with silymarin and methanol extract of *B. costatum* protected the liver against CCl_4-induced hepatic damage as evidenced by significant reduction in serum ALT, AST, ALP and total bilirubin levels. The methanol stem bark extracts of *B. costatum* also protected the liver against hepatocellular necrosis caused by CCl_4 which further confirmed its hepatoprotective activity against CCl_4 induced hepatic damage. Hepatoprotective activities of the methanol stem bark extracts of *B. costatum* against CCl_4 induced liver damage correlated well with the research findings of similar study.[27] The possible hepatoprotective mechanism of action of the *B. costatum* extracts may be associated with scavenging of free radicals that are responsible for CCl_4 toxicity.

The DPPH scavenging assay showed IC_{50} value for *B. costatum* to be 53.15µg/mL (Table 4; Fig. 1). Antioxidants appear to act against disease processes by increasing the levels of endogenous antioxidant enzymes and decreasing toxic products such as lipid peroxidation by products. Free radicals are easily formed when a covalent molecular bond is broken and one electron remains with each newly formed atom. DPPH is a stable free radical which produces deep purple colour in methanol. The principle of this assay is based on the reduction of purple coloured methanolic DPPH solution in the presence of hydrogen donating

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antioxidants by the formation of yellow coloured diphenyl-picryl hydrazine. When DPPH accepts an electron donated by an antioxidant compound, the DPPH is decolorized, which can be quantitatively measured from the changes in absorbance.[29] The IC_{50} of the methanol stem bark extract of *B. costatum* was found to be 53.15 µg/mL and this further validate previous report that *B. costatum* possess good *in vitro* antioxidant potential.[9] Antioxidants in food appear to play an essential role in the prevention of oxidative stress-related diseases and in the reduction of total mortality.[30] Typical phenolics that possess antioxidant activity are mainly phenolic acids and flavanoids. Flavonoids commonly accumulate in the epidermal cells of plant organs such as flowers, leaves, stems, roots, seeds and fruits. In this study, the plant extract was found to possess antioxidant and hepatoprotective activities against PCM and CCl_4 -induced liver damage and this could be due to presence of phenolic compounds such as flavonoids. Previous studies have shown that hepatoprotective effects are associated with phytocompounds rich in natural antioxidants.[23]

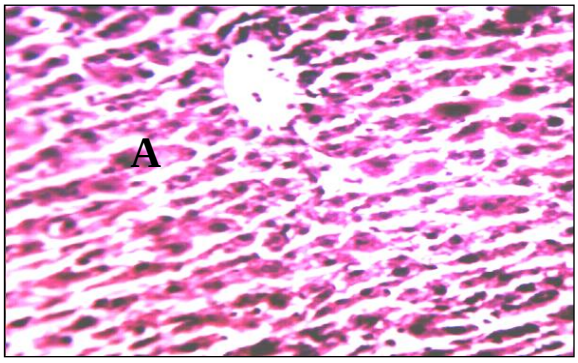

Plate Ia: Rat liver administered normal saline showing normal hepatocyte (A).

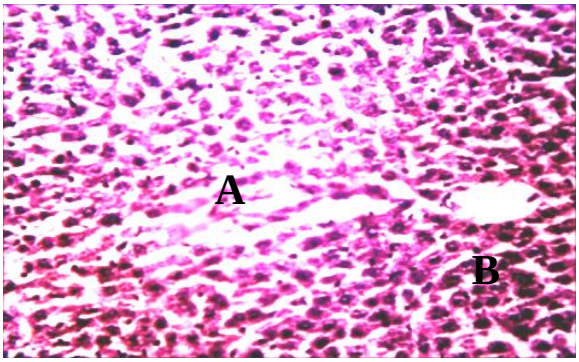

Plate Ib: Rat liver administered PCM showing moderate hepatocellular necrosis (A) with kupffer cell hyperplasia (B).

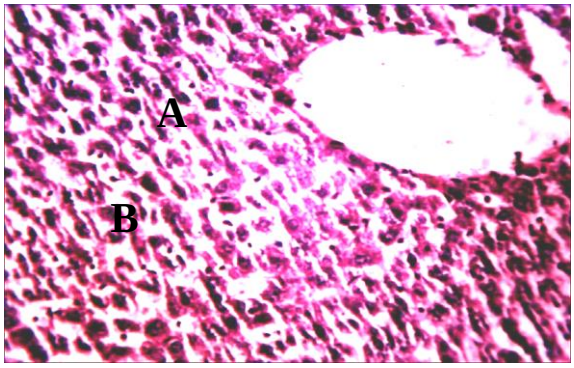

Plate Ic: Rat liver administered Silymarin and PCM showing intense pyknosis of nucleus (B) and slight necrosis of hepatocytes (A).

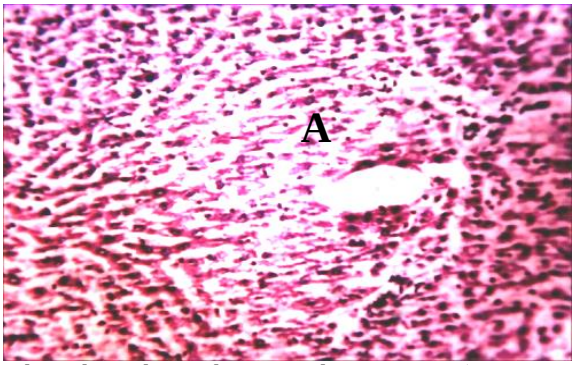

Plate Id: Rat liver administered *B. costatum* (250 mg/kg) showing slight pyknosis of nucleus (A).

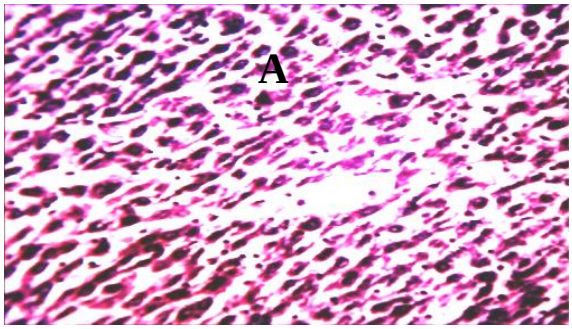

Plate Ie: Rat liver administered *B. costatum* (500 mg/kg) showing slight hepatocellular necrosis (A).

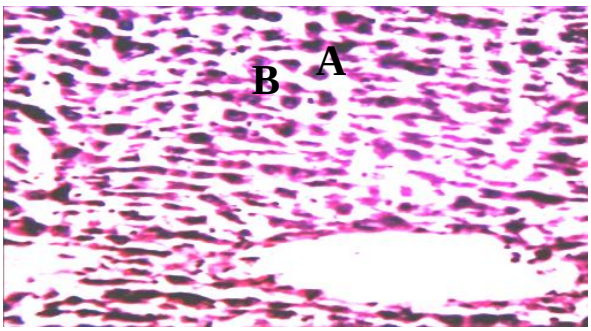

Plate If: Rat liver administered *B. costatum* (1000 mg/kg) showing intense hepatocellular necrosis (A), kupffer cell hyperplasia (B).

Plate I: Effects of methanol extract of *Bombax costatum* stem bark on histopathological changes in liver of rats in paracetamol-induced hepatotoxicity. Magnification (H and E ×250)

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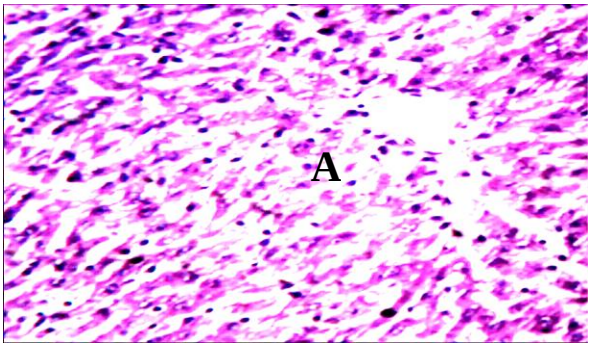

Plate IIa: Rat liver administered normal saline showing normal hepatocyte (A).

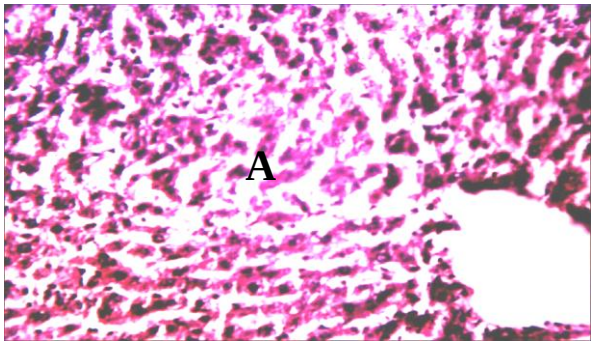

Plate IIb: Rat liver administered CCl_4 showing moderate hepatocellular necrosis (A).

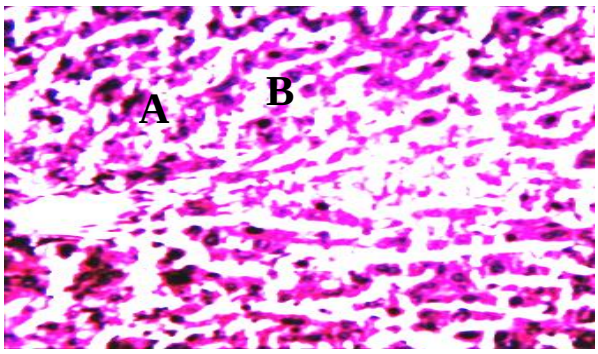

Plate IIc: Rat liver administered Silymarin showing vacoulation (A) with focal necrosis (B).

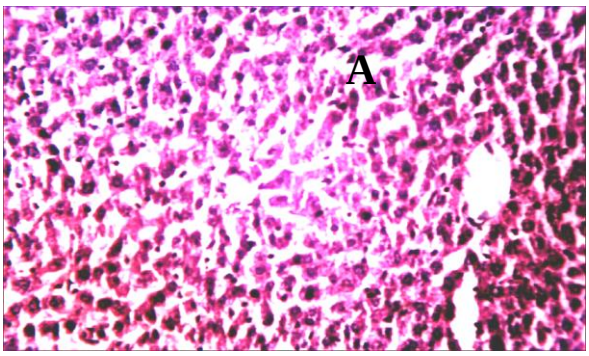

Plate IId: Rat liver administered *B. costatum* (250 mg/kg) showing slight hepatocellular necrosis (A).

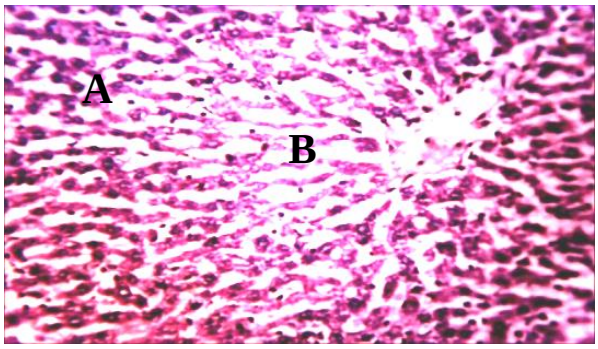

Plate IIe: Rat liver administered *B. costatum* (500 mg/kg) showing focal necrosis (A) with kupffer cell hyperplasia (B).

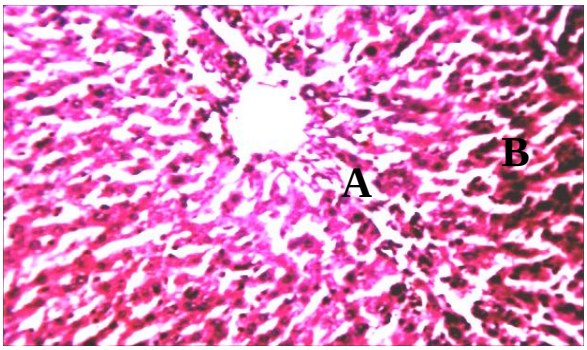

Plate IIf: Rat liver administered *B. costatum* (1000 mg/kg) showing moderate hepatocellular necrosis (A) with kupffer cell hyperplasia (B).

Plate II: Effect of *B. costatum* on histopathological changes in liver of rats in CCl_4-induced hepatotoxicity. Magnification (H and E ×250).

Conclusion

The results of this study showed that methanol extract of *Bombax costatum* stem bark possesses antioxidant and hepatoprotective activities against PCM and CCl_4-induced liver toxicity in rats. Thus, justify the use of the plant in herbal medicine for treatment of liver diseases.

Conflict of interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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