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ANTI-HEPATOFIBROTIC EFFECT OF METHANOL STEM BARK EXTRACT OF *BOMBAX COSTATUM* AGAINST CCL_4 INDUCED LIVER FIBROSIS IN MICE

N. Mohammed[*a], B.N. Umar[b], A. Saleh[c], J.Yau [d] , R.M. Hamza[e], K.Usman [d], N. S. Ismail[f]

[a]Department of Clinical Pharmacology and Therapeutics, Ahmadu Bello University Zaria
[b]Department of Veterinary Microbiology, Ahmadu Bello University Zaria, Nigeria
[c]Department of Veterinary Pathology, Ahmadu Bello University Zaria, Nigeria
[d]Department of Pharmacology and Therapeutics, Ahmadu Bello University Zaria, Nigeria
[e]Department of Human Anatomy, Federal University Dutse, Nigeria
[f]Ahmadu Bello University Medical Centre, Ahmadu Bello University Zaria, Nigeria

Corresponding author: N. Mohammed, nuhumuhd8@gmail.com, 08069675540

ABSTRACT

Background: Liver fibrosis is a sequelae of wound healing from chronic liver injury. *Bombax costatum* (BC) stem bark was reported to possess hepatoprotective activity in rats.
Aim: This study was conducted to evaluate anti-hepatofibrotic effect of methanol stem bark extract of BC against CCl_4 induced hepatofibrosis in mice.
Methods: The mice were divided in to 7 groups (n=5). Normal control (Group I) mice were administered 0.4 ml/kg of olive oil twice weekly for 6 weeks while mice in the remaining groups (Group II-VII) received 0.4 ml/kg of Carbon tetrachloride (CCl_4) in olive oil (1:1, v/v) twice weekly for 6 weeks via intraperitoneal route. Mice in toxic control group (Group II) were euthanized 72 hours after the last dose of CCl_4 while mice in CCl_4 control group (Group III) were observed for another 2 weeks for spontaneous resolution of fibrosis. Mice in standard control group (Group IV) received silymarin (100 mg/kg) daily for 2 weeks. Mice in the treatment groups (Group V-VII) received methanol stem bark extract of BC once daily orally for two weeks at doses of 31.25, 62.5 and 125 mg/kg body weight respectively. Effect of treatment on tumour necrosis factor-α (TNFα), transforming growth factor-β_1 ($TGF\beta_1$), malondialdehyde (MDA), reduced glutathione (GSH), catalase levels and liver histopathology were evaluated.
Results: This study demonstrated that methanol stem bark extract of BC reversed CCl_4 induced hepatofibrosis as demonstrated by significant reduction ($p<0.001$) in $TGF\beta_1$, MDA, GSH and collagen deposition in liver when compared with CCl_4 control group.
Conclusion: The reversal of liver fibrosis by BC stem bark could be due of its antioxidant activity and its ability to down regulate the $TGF\beta_1$ signalling pathway of liver fibrosis.
Keywords: Antioxidant, *Bombax costatum*, Carbon tetrachloride, Fibrosis, Hepatoprotective

INTRODUCTION

The liver is an indispensable organ of the body that metabolise drugs, toxins and food substances. These vital functions of the liver make it susceptible to metabolic, infective, oxidative stress and circulatory insult [1]. Liver disease still remains a major public health problem. It accounts for 2 million deaths per year globally [2]. According to the European Association for Study of Liver Disease (EASL) [3], liver disease is extremely costly in terms of human suffering, management and premature loss of productivity. It is an important cause of morbidity and mortality globally and its pattern varies in different geographical locations [4].

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The high morbidity and mortality attributable to liver diseases in developing countries could be due to high cost of care, inadequate effective drugs, poor immunization coverage, lack of awareness about liver disease and late presentation to healthcare facilities. Chronic hepatitis is a type of liver disease that may arise from different aetiology and of varying intensity in which hepatic inflammation and necrosis persist for at least 6 months. Severe form of chronic hepatitis may be associated with excessive collagen deposition and architectural reorganization, which, when advanced, lead ultimately to cirrhosis [5]. It is worthy of note that the final outcome of progressive liver injury, independent of the aetiological agent, is liver fibrosis and this process may progress and ultimately lead to development of hepatocellular carcinoma (HCC) [6]. Chronic infections with hepatitis viruses (HBV and HCV) are major risk factors for HCC development [7]. Other risk factors include chronic alcohol abuse, toxicants, biliary disease, metabolic disorders, drugs, and genetic conditions [8]. Chronic inflammation of the liver is characterized by the continued expression of cytokines and recruitment of immune cells to the liver. Activated inflammatory cells release reactive oxygen species (ROS), nitric oxide (NO) reactive species and cytokines such as tumour necrosis factor alpha (TNF-α) and transforming growth factor beta-1 (TGFβ1). Transforming growth factor (TGF)- β1 is the major fibrogenic cytokine in the liver [9] and it has been clearly demonstrated to play a crucial role in the process of HSC activation to myofibroblast [10]. ROS formation is also a key driver of hepatic inflammation and fibrosis. Under normal physiological conditions, oxygen-containing reactive molecules control key physiological activities in the body such as cell growth, proliferation, migration, differentiation, and apoptosis [11]. However, elevated intracellular ROS concentrations induce damage to cell structures. Free radicals can initiate the oxidation of biomolecules, such as protein, lipid, amino acids and nucleic acid thereby leading to cell injury. In the liver, ROS can induce apoptosis and necrosis of hepatocytes. Apoptosis or necrosis of hepatocytes will further stimulate the production of profibrogenic mediators (by Kupffer cells) and recruitment of circulating inflammatory cells leading to direct activation of HSC [12]. The role of oxidative stress and inflammation in the pathogenesis of liver diseases has been well established and accordingly, blocking or retarding the chain reactions of oxidation and inflammatory processes could be a promising therapeutic strategy for prevention and treatment of liver injury [13].

Despite the remarkable advancement in the understanding of cellular and molecular mechanisms of hepatic fibrosis, [14], effective anti-hepatofibrotic agents have not been developed yet [15], hence the search for new effective drug is still ongoing. Eliminating the cause(s) of liver injury and their mediators, reversing hepatic inflammation, inhibiting HSC activation, inhibiting the fibrogenic activities of HSC, and stimulating matrix degradation have become an attractive target for anti-hepatofibrotic therapy [16]. Plants serve as a source to discovery of new drug for the treatment of a variety of diseases that affect human health. *Bombax costatum* is a traditionally used medicinal plant for the treatment of liver diseases [17]. It is often found on rocky hills or lateritic crusts. Its distribution is restricted to the savanna zones of West Africa from Senegal to the Central African Republic

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[17]. Methanol stem bark extract of *Bombax costatum* was found to possess hepatoprotective properties against Paracetamol induced liver damage as well as Carbon tetrachloride (CCl_4) induced liver damage in rats [18], but its anti-hepatofibrotic effect have not been scientifically evaluated. In this regard, this study was aimed at investigating the anti-hepatofibrotic effect of *Bombax costatum* stem bark against CCl_4 induced hepatofibrosis in mice.

MATERIALS AND METHODS

Equipment and Chemicals

Carbon tetrachloride (BDH Ltd Poole, England), Silymarin (Micro Labs Limited, India), Olive oil (Metaluni S.P.A., Italy), Biochemical kits (Randox, UK), ELISA kits (Wuhan Fine Biological Technology Co., Ltd. China), Masson trichrome stain, Haematoxyline and Eosin stain, ScopetekDCM500 Camera (Hangzhou S. Digital Technology Ltd. China), Leitz Microscope (Optical Institute, Germany), Spectrophotometer (Metsar Technologies Pvt. Ltd., Hyderabad), Water Bath (Meditech, Chennai India)

Experimental Animals

Wistar albino male mice (20-30 g) obtained from Animal Facility of Department of Pharmacology and Therapeutics, Ahmadu Bello University Zaria, were used for the study. The mice were kept in clean cages with metal coverlids and bedded with soft wood shavings, which was replaced every three days. They were maintained in a well-ventilated room and fed on standard commercial rat feed (Vital feed®, Zaria) with free access to drinking water *ad libitum*. All experiments performed on mice were approved by the Ahmadu Bello University Committee for Animal Use and Care (Approval number: ABUCAUC/2020 /50).

Plant material and preparation of extract

The plant was collected from Hanwa of Sabon Gari local government area of Kaduna State in July, 2018. It was authenticated by Mallam Namadi (taxonomist) of the Herbarium section of the Department of Botany, Ahmadu Bello University, Zaria. It was compared with a voucher specimen deposited in the Herbarium and a voucher number (No. 1211) was assigned to it 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 5.5 kg of the powdered stem bark to maceration using 20 L of 70% v/v 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.

Anti-hepatofibrotic Studies

The anti-fibrotic study was conducted as previously described [19] but with some modifications. The mice were divided in to 7 groups of five mice each and Sylimarin was used as standard treatment. Mice in group I received 0.4ml /kg of olive oil via intraperitoneal (*i.p*) injection twice weekly (Normal control). Mice in group II received 0.4 ml/kg of CCl_4 in olive oil (1:1, v/v) by *i.p.* injection twice weekly for six weeks and were euthanised 72 hours after the last dose of CCl_4. Mice in group

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III received 0.4 ml/kg of CCl_4 in olive oil (1:1, v/v) by *i.p.* injection twice weekly for six weeks and were observed for spontaneous resolution of fibrosis for the another two weeks. Mice in group IV received 0.4 ml/kg of CCl_4 in olive oil (1:1, v/v) by *i.p.* injection twice weekly for six weeks and also received oral silymarin (100 mg/kg) daily for two weeks. Mice in group V-VII received 0.4 ml/kg of CCl_4 in olive oil (1:1, v/v) by *i.p* injection twice weekly for six weeks and then received methanol stem bark of BC per oral (*p.o.*) at 31.25, 62.5 and 125 mg/kg respectively daily for two weeks. At the end of the 8 weeks period, the remaining mice were sacrificed 24 hours after the last treatment. The liver and blood samples were collected for analysis. The blood samples were collected and allowed to clot at room temperature for 2 hours and centrifuged at 1000xg for 20 minutes to obtain serum for determination of TNF-α, hepatic malondialdehyde (MDA) levels (as free radicals increased in liver disease), hepatic reduced glutathione (GSH) and catalase (CAT). The liver tissues were rapidly dissected and the left lobe was fixed in a buffer solution of 10% formalin for histological analysis with Masson trichrome stain. For each liver, about 0.7g of the liver tissue was homogenized in a PBS (0.01 M, pH 7.4) with a glass homogenizer. The homogenate was centrifuged at 5000 x g for 5 minutes. The supernatant was stored at -20◦C for determination of liver TGFβ1 content using ELISA kit according to manufacturer's instruction.

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 Masson's trichrome stain for examination. The stained tissues were observed through a Leitz microscope at x250 magnification and photographed with Scope tek DCM500 camera.

Determination of TGFβ1

TGFβ1 level determination was conducted using ELISA kit according to manufacturer's instruction.

Determination of TNFα level

TNFα level determination was conducted using ELISA kit according to manufacturer's protocol.

In Vivo Anti-Oxidant Studies:

Determination of reduced glutathione (GSH)

Determination of reduced glutathione content (GSH) in serum was done according to the colorimetric method of Ellman as previously described [20]. One hundred and fifty microlitres (150µl) of serum from each mouse was added to 1.5ml of 10% trichloroacetic acid and centrifuged at 1500g for 5minutes. One millilitre (1ml) of the supernatant was treated with 0.5ml of Ellman's reagent [Ellman's reagent is 5,50-dithiobis-2-nitrobenzoic acid (0.1 mM) prepared in 0.3 M phosphate buffer with 1% of sodium citrate solution] and 3ml of phosphate buffer (0.2M, pH 8.0). Absorbance of solutions was measured at 412 nm against blank. Absorbance values were compared with a standard curve generated from known GSH.

Determination of catalase

This was measured using Abebi's method [21]. Ten microliters (10µl) of serum was

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added to a test tube containing 2.8ml of 50mM potassium phosphate buffer (pH 7.0). The reaction was initiated by adding 0.1ml of freshly prepared 30Mm hydrogen peroxide (H_20_2) and the decomposition rate of H_20_2 was measured at 240nm for 5minutes on a spectrophotometer. A molar extinction coefficient € of $0.041nM^{-1}$ was used to calculate the catalase activity.

Determination of hepatic malondialdehyde (MDA)

The lipid peroxides content in the serum were determined by monitoring the thiobarbituric acid (TBA) reactive substance formation as described by Okhawa [22] with slight modification by Atawodi *et al*., [23]. Two millilitres (2ml) of 15% trichloroacetic acid was measured in to a test tube, 2ml of TBA was added and 100µl of serum was added. The mixture was incubated at $80°C$ for 30 minutes in a water bath and allowed to cool, followed by centrifugation at 3000rpm for 10 minutes. After centrifugation at 3000 rpm for 10 min, the upper organic layer was taken and its absorbance was taken at 535 nm against an appropriate blank without the sample. The levels of lipid peroxides was expressed as n moles of thiobarbituric acid reactive substances (TBARS)/mg protein using an extinction coefficient of 1.56×10^5 ML cm^{-1}.

Statistical Analysis of Data

Data were presented using tables and plates where applicable. Data were presented as Mean ± Standard Error of the Mean (S.E.M.). Analysis of data were done using minitab (version 17), Microsoft excel (version 2010) and SPSS (version 20). Significant differences were compared using one way ANOVA followed by Tukey's post hoc test (where significant difference exist). The results were considered significant at *p*-values ≤ 0.05.

RESULTS

Percentage yield of MSBE of BC

The % yield of MSBE of BC was found to be 5.10%.

Effect of MSBE of BC on Fibrogenic Cytokine in CCl_4 induced liver

Intraperitoneal administration of CCl_4 at a dose of 0.4ml/kg twice weekly for 6 weeks caused significant ($p \leq 0.001$) increase in level of TGF-β1 compared to normal control group that received olive oil. Administration of MSBE of BC at dose of 125 mg/kg for 2 weeks after induction of liver fibrosis caused significant ($p \leq 0.001$) reduction in the level of TGF-β1 compared to CCl_4 toxic group. No significant reduction in level of TGF-β1 was observed after 2 weeks treatment with silymarin at 100 mg/kg compared with CCl_4 toxic group. There was also no significant decrease in level of TGF-β1in CCl_4 control group when compared with CCl_4 toxic group (Figure 1).

Effect of MSBE of BC on Inflammatory Cytokine in CCl_4 induced liver fibrosis

Intraperitoneal administration of CCl_4 at a dose of 0.4ml/kg twice weekly for 6 weeks caused significant ($p \leq 0.05$) increase in level of TNFα compared to normal control group that received olive oil. Administration of silymarin at dose of 100 mg/kg for 2 weeks after induction of liver fibrosis caused significant ($p \leq 0.05$) reduction in the level of TNFα compared to CCl_4 toxic group. There was also significant ($p \leq 0.05$) decrease in level of TNFα in CCl_4 control group compared

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with CCl_4 toxic group. No significant reduction in level of TNFα was observed after 2 weeks treatment with MSBE of BC at 31.25 mg/kg, 62.5 mg/kg and 125 mg/kg compared with CCl_4 toxic group (Figure 2).

Effect of MSBE of BC on MDA, CAT and GSH in CCl_4 induced liver fibrosis

Intraperitoneal administration of CCl_4 at a dose of 0.4ml/kg twice weekly for 6 weeks caused significant ($p \leq 0.001$) increase in level of MDA compared to normal control group that received olive oil. Administration of silymarin at dose of 100 mg/kg for 2 weeks after induction of liver fibrosis caused significant ($p \leq 0.001$) reduction in the level of MDA compared to CCl_4 toxic group. There was also significant ($p \leq 0.001$) reduction in level of MDA after 2 weeks treatment with MSBE of BC at 125 mg/kg.

Significant ($p \leq 0.001$) decrease in CAT and GSH were observed after intraperitoneal administration of CCl_4 at a dose of 0.4ml/kg twice weekly for 6 weeks (5.49±1.15 and 9.55±0.45 respectively) compared to normal control group (14.00±0.65 and 19.99±0.54 respectively). Administration of silymarin at dose of 100 mg/kg for 2 weeks after induction of liver fibrosis caused significant ($p \leq 0.001$) increase in the level of GSH compared to CCl_4 toxic group. There was also significant ($p \leq 0.001$) increase in level of GSH after 2 weeks treatment with MSBE of BC at 62.5 and 125 mg/kg when compared with CCl_4 toxic (Table 1).

Effect of MSBE of BC on histophathological changes in CCl_4 induced liver fibrosis

Administration of 0.4 ml of CCl_4 twice weekly for six weeks to the mice in toxic control group caused moderate deposition of collagen at the portal triad extending into the liver parenchyma (Plate Ib). Subsequent observation for 2 weeks period after induction of fibrosis for spontaneous resolution of fibrosis and administration of silymarin at 100 mg/kg for 2 weeks showed persistence of moderate porto hepatic fibrosis (Plate Ib and Ic respectively). Administration of MSBE of BC at 31.25 mg/kg for 2 weeks after induction of fibrosis caused moderate portal fibrosis with slight hepatic fibrosis (Plate Id) while administration of MSBE of BC at 62.5 mg/kg and 125 mg/kg caused slight portal fibrosis (Plate Ie and Plate If respectively).

DISCUSSIONS

Cold maceration of dried powdered material of the stem bark of *B. costatum* yielded an extract weighing 560.9g (5.10%) at the end of the extraction.

The oral LD_{50} for MSBE of *Bombax costatum* in mice was reported to be >5000 mg/kg body weight per oral and no sign and symptom of toxicity were observed for up to 2 weeks [18].

Administration of CCl_4 to mice can cause bioactivation of cytochrome P450 2E1, resulting in the formation of trichloromethyl free radicals and reactive oxygen species (ROS), which initiate lipid peroxidation, protein oxidation and damage to hepatocellular membranes [24]. This is followed by the release of inflammatory mediators from activated hepatic macrophages that potentiate the CCl_4-induced hepatic injury [25, 26]. Kupffer cells can release pro-inflammatory mediators either in response to necrosis or in direct response to liver toxicants thereby aggravating CCl_4-induced hepatic injury [27].

CCl_4 is a fast acting toxicant and can cause morphological changes in the liver to start appearing at 15 minutes after administration. The effects of CCl_4 on

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hepatocytes, depending on dose and exposure time, can manifest histologically as hepatic steatosis, necrosis, fibrosis or ultimately cirrhosis [28]. The trichloromethyl radical formed during the metabolism of CCl_4 is capable of binding to lipids, and this binding can initiate lipid peroxidation that leads to liver damage [29]. Liver fibrosis develops progressively during repetitive administration of CCl_4 [30,31]. Fibrosis appears initially in pericentral areas, and can progress to bridging fibrosis, cirrhosis, and eventually hepatocellular carcinoma [6]. The dose of CCl_4 that induces hepatotoxicity ranges from 0.1 to 3 ml/kg when administered intraperitoneally [32]. In this study, intraperitoneal administration of CCl_4 at 0.4ml/kg twice weekly for 6 weeks resulted in significant increase in levels of TNF-α ($p \leq 0.05$) and TGF-β1 ($p \leq 0.001$) 72 hours after withdrawal of CCl_4 when compared with normal control group. This is in agreement with findings of previous studies [33,34,35] where TNF-α and TGF-β1 were also observed to be significantly elevated following repeated intraperitoneal administration of CCl_4. TNF-α level in CCl_4 control group was observed to spontaneously normalize although the progression of liver fibrosis was observed on histopathological examination.

The significant ($p \leq 0.05$) reduction in TNF-α level observed in the CCl_4 control group when compared with CCl_4 toxic group has demonstrated that cessation of further liver damage by toxicants could lead to some resolution of inflammation in the liver but not spontaneous resolution of advanced fibrosis. Once advanced fibrosis is established, collagen deposition will progress to the formation of hepatic nodules and decreased blood supply to the liver [10,36].

The significant ($p \leq 0.001$) reduction in TGF-β1 level with corresponding resolution of established fibrosis observed in BC treated groups could be due to down regulation of the TGFβ1 signalling pathway.

Hepatic MDA is a marker of lipid peroxidation. The level of hepatic MDA was significantly ($p \leq 0.05$) reduced in sylimarin and BC (125 mg/kg) treated groups when compared with CCl_4 toxic groups. This could be due to presence of flavonoid in them. MSBE of BC was reported to possess bioactive constituents such as flavonoids and tannins [18]. Flavonoids have antioxidant properties due to their free radical scavenging effect [37]. Hepatic MDA levels are reduced by agents having antioxidant properties as seen in results of previous studies [33,38].

Findings from the liver sections on histopathological analysis revealed that chronic administration of CCl_4 caused marked increase in collagen deposition, as evidenced by the blue colouration around the portal triad after Masson's trichrome staining. This finding is consistent with the observed significant ($p \leq 0.001$) elevation in TGF-β1 levels. However, treatment with BC markedly ameliorated the severity of liver fibrosis and restored the architecture of the liver in dose dependent manner.

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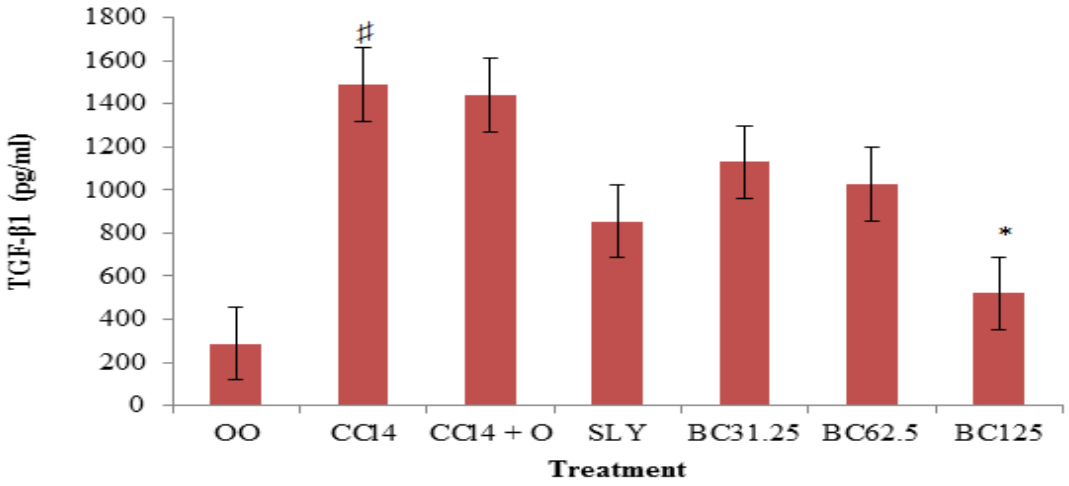

Figure 1: Effect of MSBE of BC on TGF-β1 in CCl_4 Induced Hepatofibrosis in mice

Data were presented as Mean ± SEM. (n = 5), One way ANOVA followed by Tukey's post hoc test, $^{\sharp}$= $p \leq 0.001$ compared to normal control group, * = $p \leq 0.001$ compared to CCl_4 toxic group. TGF-β1: Transforming growth factor β1, OO: Olive oil, SLY: Silymarin, BC: Methanol stem bark extract of *Bombax costatum*, O: Observation, CCl_4: Carbon tetrachloride

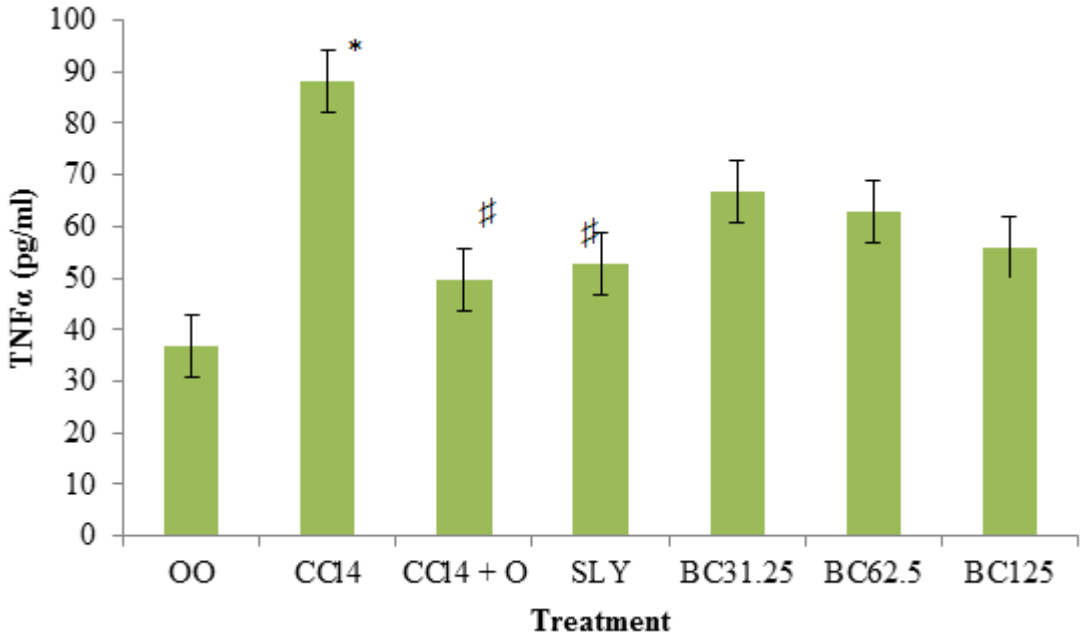

Figure 2: Effect of MSBE of BC on TNFα in CCl_4 Induced Hepatofibrosis in Mice

Data were presented as Mean ± SEM. (n = 5), One way ANOVA followed by Tukey's post hoc test, *=. $p \leq 0.05$ compared to normal control group, $^{\sharp}$ = $p \leq 0.05$ compared to CCl_4 toxic group. TNFα: Tumour necrosis factor alpha, OO: Olive oil, SLY: Silymarin, BC: Methanol stem bark extract of *Bombax costatum*, O: Observation, CCl_4: Carbon tetrachloride

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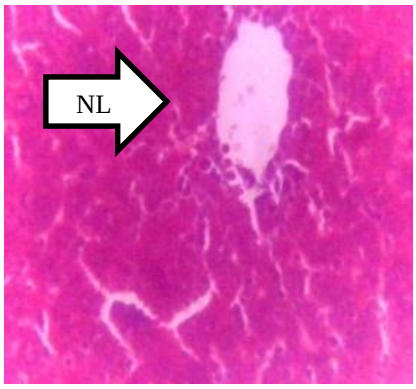
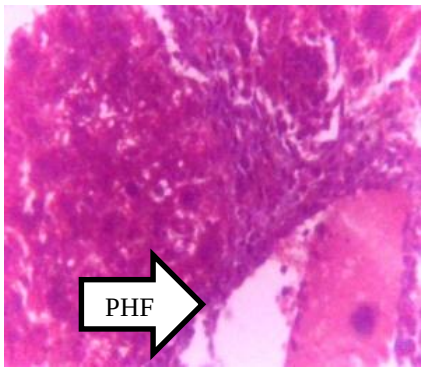
Table 1: Effect of MSBE of BC on MDA, CAT and GSH in CCl_4 Induced Hepatofibrosis in Mice

S/N	Treatment	MDA(nMol/ml)	CAT(U/ml)	GSH(U/ml)
1	OO	235.5±20.9	14.00±0.65	19.99±0.54
2	CCl_4	1153.0±320.0*	5.49±1.15*	9.55±0.45*
3	CCl_4 + O	776.0± 102.0	9.20±1.58	12.44±1.61
4	SLY	271.1±26.6#	13.05±1.09	18.79±0.29#
5	BC31.25mg/kg	661.9±34.5	8.30±1.38	15.03±2.12
6	BC62.5mg/kg	659.0±216.0	10.00±1.38	17.47±1.55#
7	BC125mg/kg	366.8±17.3#	10.59±2.59	17.84±0.94#

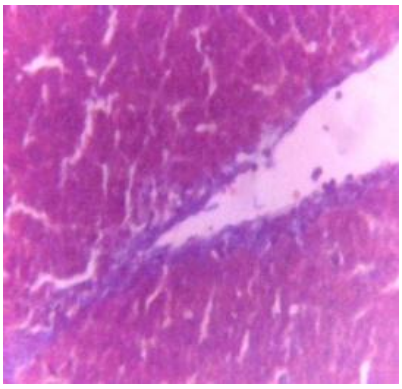
Data were presented as Mean ± SEM. (n = 5), One way ANOVA followed by Tukey's post hoc test.*= $p \leq 0.05$ when compared with normal control group, # = $p \leq 0.05$ compared to CCl_4 toxic group, MDA: Malondialdehyde, CAT: Catalase, GSH: Reduced glutathione, OO: Olive oil, SLY: Silymarin, BC: Methanol stem bark extract of *Bombax costatum*, O: Observation, CCl_4 : Carbon tetrachloride



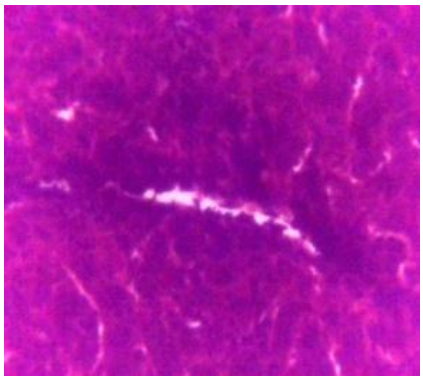
1a = OO: Normal Liver (NL)



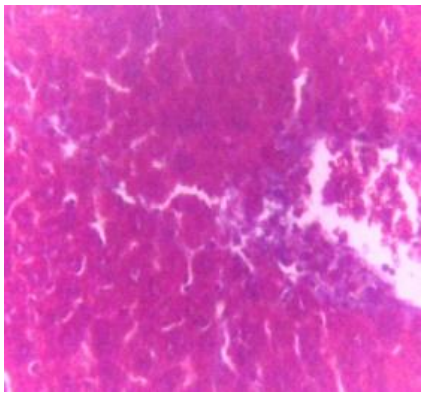
1b = CCl_4: Moderate porto-hepatic fibrosis (PHF)



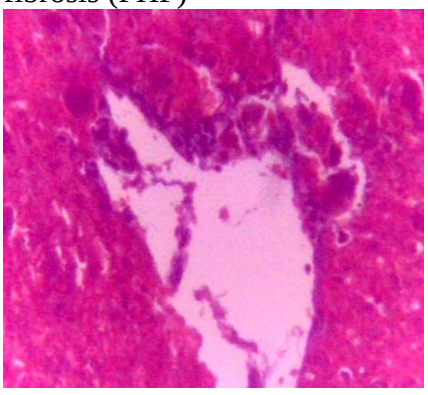
(Ic) CCl_4 + Observation: Moderate porto-hepatic fibrosis (PHF)



(Id) SLY100: Moderate porto-hepatic fibrosis (PHF)



(Ie) BC31.25: Moderate portal fibrosis and slight hepatic fibrosis (PHF)



(If) BC62.5: Slight portal fibrosis (PF)

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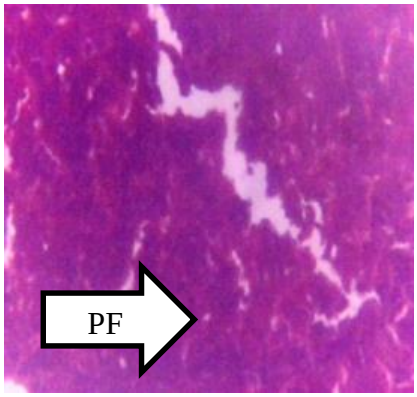

(Ig) BC125: Slight portal fibrosis (PF)

Plate I: Liver Histopathological Slides Showing Effect of Methanol Stem Bark Extract of *B. costatum* on CCl_4-Induced Liver Fibrosis in mice. (Masson's Trichrome ×250)
OO: Olive oil, SLY: Silymarin, BC: Methanol stem bark of *Bombax costatum*, O: Observation, CCl_4 : Carbon tetrachloride

CONCLUSION

The results of this study showed that methanol extract of *Bombax costatum* stem bark possesses anti-hepatofibrotic and *in vivo* antioxidant activities against CCl_4-induced liver fibrosis in mice. The reversal of liver fibrosis by BC could be due of its antioxidant activity and its ability to down regulate $TGF\beta_1$ signaling pathway.

Conflict of Interest

The authors declared 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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List of Abbreviation

ANOVA = Analysis of variance
BC = Methanol stem bark of Bombax costatum
CAT= Catalase
CCl4 = Carbon tetrachloride EASL= European Association for the Study of the Liver
GSH = Reduced glutathione
LD50 = Median lethal dose
MSBE= Methanol stem bark extract
OECD= Organisation for Economic Co-operation and Development
ROS = Reactive oxygen species
SEM = Standard error of mean
SLY = Silymarin
TNFα = Tumour Necrosis Factor α
TGFβ = Transforming Growth Factor β
WHO = World Health Organisation

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