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Hepatoprotective and Hematological Effect of Aqueous Extract of *Bombax costatum* in Wistar Rat

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

The aim of this study was to evaluate the hepatoprotective and hematological effects of aqueous extract of *Bombax costatum* in Wistar rats. Freshly harvested leaves of *Bombax costatum* washed with clean tap water were air dried at room temperature. The dried leaves were subsequently ground into fine powder with the aid of an electric blender. Exactly 500 g of powdered plant sample was processed into extract. Thirty (30) adult male Wistar rats were divided into six groups of five rats each. Group I was the normal control administered 2 ml of distilled water. Group II was the negative control i.e., induced liver damage without treatment. Groups III-V were administered 100, 200 and 400 mg/kg of extract of *B. constatum* respectively prior to induction of hepatic damage. Treatment lasted for 10 days after which animals were sacrificed and blood sample collected.

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Qualitative phytochemical screening, biochemical and hematological parameters were performed using standard procedures. While anthraquinone was reportedly absent, saponin was the most abundant of the phytochemicals reportedly present. The activity of the liver enzymes was high in the negative control which was induced hepatic damage without treatment. However, it was significantly (P<0.05) lower in groups which were pre-treated with aqueous extract of *Bombax constatum* prior to induction attempt. It was also observed that Packed Cell Volume (PCV), Hemoglobin concentration (Hb) and Red Blood Cell (RBC) reported for the negative control were significantly (P<0.05) lower than that reported for the normal control. However, in groups which were administered with the said extract before induction attempt, PCV, Hb, and RBC were significantly (P<0.05) higher than those reported for group II which were induced hepatic damage without treatment. In conclusion, aqueous extract of *B. costatum* has hepato and hemato protective effects.

Keywords: Liver; phytochemicals; bombax costatum; blood; hemoglobin.

1. INTRODUCTION

The liver is the largest solid organ as well as the most vital organs of the human body. It is saddled with the task of metabolizing nutrients and excretion of the resulting metabolic waste products [1]. The indispensability of the liver is evident by the fact that a total loss of its vitality can translate to death in a matter of minutes [2]. Blood is another important life determinant. It is actively involved in conveying essential life supporting materials such as nutrients and oxygen. Its crucial metabolic role is also seen in the detoxification of metabolic waste products.

The liver is under constant threat by both metabolic and external insults ranging from pollution from an individual's immediate environment to toxic substances inherent in some poorly processed or even contaminated food stuffs available to the populace which wield the potential upon ingestion and metabolism to stimulate the lipid peroxidative system and consequent generation of lipid peroxide which can attack and destroy the red blood cell. The use of synthetic drugs to protect the liver from injuries may not stand the test of time as it may predispose the organ to damage over a prolonged consumption period [3].

An estimated 80% of the word population depends on plant based therapeutic substances to meet their health goals. This is evident by the usefulness of plants since prehistoric times. Plants have been developed into different formulations for ease of use, storability, acceptability, digestibility, etc. factors which have further made them a more attractive option. Several parts of *Bombax costatum* such as the stem bark, roots and leaves have been used in the treatment of diverse human illnesses such as skin diseases, epilepsy, insanity, yellow fever and headache etc. [4]. The fruits of *Bombax costatum* is a viable source of antioxidants [5]. Thus, it is imperative to probe the ability of its leaf to protect the liver and blood from external injuries.

2. MATERIALS AND METHODS

2.1 Collection of Plant

Bombax costatum leaves were harvested from the botanical garden of the Department of Biological Sciences, Bayero University Kano. The plant was subsequently identified at the herbarium unit of the Department of Biological Ahmadu Bello University, Zaria.

2.2 Animals

Thirty adults male Wistar rats weighing 140-150 grams were procured from the animal house of the department of Science Laboratory Technology, Akanu Ibiam Federal Polytechnic Unwana Afikpo Ebonyi State. The rats were also maintained under standard conditions of humidity, temperature and 12 h light/dark cycle and were acclimatized for two weeks prior to the start of the study. Animals were handled in line with stipulated guidelines on the care and handling of laboratory animals [6].

2.3 Extraction

2.3.1 Preparation of aqueous methanol extracts of BCE

Dried leaves of *B. costatum* was ground into fine powder with the aid of an electric blender. Then, one gram of powdered plant sample was refluxed with aqueous methanol (70%W/V). The resulting solutions were pooled together, filtered, and subsequently concentrated using a rotary evaporator under reduced pressure in order to eliminate methanol. This was followed by the lyophilization of the extract to get rid of excess water, yielding 100 g of crude dark brown sticky BCE which was stored at −20 °C.

2.3.2 Phytochemical Analysis

Phytochemical analysis was performed on the extract in accordance with the method described by Harbone [7]. Basic phytochemical screening was carried out by simple chemical tests to detect the presence of secondary plant constituents such as alkaloids, tannins, flavonoids, saponins, sterols, phenols, cardiac glycoside, anthraquinones and soluble carbohydrate in the sample.

2.3.3 Median lethal dose 50% (LD50%)

Nine rats divided into three groups of three rats were used to achieve the first phase of the LD 50%. Subsequently, three groups were orally administered with 10, 100 and 1000 mg/kg of extract respectively. Animals were observed for 24 h to for signs of toxicity. Upon confirmation of absence of mortality in any of the groups, another set of three groups of one rat per group was set up and each administered with 1600, 2900 and 5000 mg/kg of extract separately. The animals were observed for 48 h for signs of toxicity. Lorke" [8].

2.3.4 Animal grouping

Group I: (Normal control) administered 2 ml of distilled water only
Group II: (Negative control) CCl_4
Group III: rats administered 100 mg/Kg BCE+ CCl_4
Group IV: rats administered 200 mg/Kg BCE+ CCl_4
Group V: rats administered 400 mg/Kg BCE+ CCl_4
Group V1: rats administered 100 mg/Kg silymarin + CCl_4

2.3.5 Blood collection

Diethyl ether was employed to anaesthetize the animals. Heart puncture was performed with the aid a 5 ml disposable syringe. Exactly 2 ml of blood was drawn gently and slowly. The blood collected was transferred immediately to clean dried centrifugation tubes, allowed to clot and serum was separated by centrifugation at 3000 rpm for 15 min. The resulting serum was separated, and then preserved at -20°C in the freezer until analysis.

2.3.6 Hematological evaluation

Hematological parameters (Red Blood Cells, Hemoglobin concentration and packed cell volume) were determined with the aid of an automatic hematological analyzer (Coulter STKS, Beckman) [9]. Mean Cell Haemoglobin (MCH), Mean Cell Volume (MCV) and Mean Cell Haemoglobin Concentration was calculated using the following equations respectively:

$$MCH\ (pg) = hemoglobin \times \frac{10}{RBC}$$

$$MCV(fl) = \frac{hematocrit}{10} \times RBC$$

$$MCHC\left(\frac{g}{dL}\right) = Hemoglobin \times PCV \times 100\%$$

2.4 Biochemical Analysis

2.4.1 Determination of ALP activity

The approach of Bassey et al. [10] which was modified by Wright et al. [11] "Substrate solution (3 ml) was incubated at 37^0C for 15 min and then 0.5 ml of a sample was added. This was mixed well and immediately 0.05 ml of the mixture was removed and mixed with 9.5 ml of 0.085 N NaOH. This corresponded to zero-time assay (blank). The remaining solution (substrate+ enzyme) was incubated for 15 min at 37^0C and then 0.5 ml was drawn and mixed with 9.5ml of 0.085 N NaOH. Absorbance was measured at 405 nm against the reference blank. Specific activities were expressed as µM of p-nitrophenol formed per min of sample Serum Alkaline phosphatase Activity (IU/L) = A/min x Factor Where F = 2713

2.4.2 Determination of ALT activity

The sample reagent (1 ml) was added to five (5) test tubes. Sample (500 µl) was added to the test sample and 50 µl of the standard reagent to the standard and non to the blank. The tubes were incubated at room temperature for 20 min and was mixed immediately. The first absorbance of test exactly at 1 min was read and thereafter at 30 ,60, 90 and 120 secs were read at 340 nm. The mean change in absorbance per minute was determined and the test results was calculated. Serum ALT activity (IU/L) = Change in A/min × F, where F= 3376 [12].

2.4.3 Determination of AST

Similar procedure adopted for ALT was applied in the determination of the activity of AST, except that the AST reagent was used instead. The sample reagent (1 ml) was added to five (5) test tubes. Sample (500 µl) was added to the test sample and 50 µl of the standard reagent to the standard and non to the blank. The tubes were incubated at room temperature for 20 min and was mixed immediately. The first absorbance of test exactly at 1 minute was read and thereafter at 30 ,60, 90 and 120 secs were read at 340 nm. The mean change in absorbance per minute was determined and the test results was calculated. Serum AST activity (IU/L) = Change in A/min × F, where F= 3376

2.5 Statistical Analysis

Data were analyzed using SPSS (Statistical Package for the Social Sciences) version 9.05 software (USA). All values were expressed as the mean ± SD. Significant differences between the groups were statistically analyzed by one way analysis of variance (ANOVA) followed by Turkey's multiple comparison post hoc test. A statistical difference of $P < 0.05$ was considered significant.

3. RESULTS AND DISCUSSION

The liver is the principal organ saddled with the task of metabolizing numerous substances in the body such as breaking down and synthesis of fats, proteins and most especially carbohydrate to release energy, bile production and excretion as well as enzyme activation [13]. Table 1 shows the phytochemical composition of aqueous extract of *Bombax costatum* indicating the presence of alkaloids, flavonoids, tannins, saponins, phenols, carbohydrates and anthraquinones. "While cardiac glycoside was absent, saponin was the most abundant phytochemical. Hepatic damage causes impairment to liver function which may have deleterious effect on human health [14]. Table 2 shows indices of hepatic functions in rats pretreated with aqueous extract of *Bombax costatum* prior to attempt to induce experimental hepatic damage. Successful induction of hepatic damage significantly ($P<0.05$) increased the activities of alanine transaminase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP).However, the activities of the aforementioned enzymes were significantly reduced in groups which had been pretreated with the extract before exposure to the liver damaging agent to levels which though were significantly ($P<0.05$) higher than that reported for the normal control which in turn were not significantly ($P>0.05$) different from that reported for the standard control administered silymarin. The hepatoprotective effect of the extract could be attributed to the phytochemical composition of the leaf of *B. costatum*. The valuable compounds offer potent antioxidant and hypolipidemic properties of *Bombax ceiba* flower extract in liver tissue [15]. This observation is in tandem with the finding of Arafa et al. [16] which showed that treatment with the extract of the flower of *Bombax ceiba* a member of the *Bombacacae* ameliorates hepatosteatosis induced by ethanol and relatively moderate fat diet in rats

Table 1. Qualitative phytochemical composition of aqueous extract leaf of *Bombax costatum*

Phytoconstituents	Composition
Alkaloids	++
Flavonoids	+
Glycosides	+
Tannins	+
Saponins	+++
Cardiac glycosides	-
Phenols	+
Carbohydrate	+
Antraquinones	++

Key: + = Present and - = Absent, ++= abundant, +++ = very abundant

Table 2. Indices of Hepatic Functions in Mice with Hepatic Damage Pretreated with Aqueous Extract of *Bombax costatum*

Groups	ALT (U/L)	AST (U/L)	ALP (U/L)
Normal control	62.26 ± 2.20^a	171.10 ± 0.62^a	225.01 ± 1.20^a
Negative control	140.27 ± 2.20^e	328.23 ± 1.30^e	315.82 ± 2.60^d
AEBC 100 mg/kg	113.23 ± 2.00^d	258.12 ± 1.70^d	289.22 ± 2.30^c
AEBC 200 mg/kg	104.10 ± 2.10^c	246.12 ± 2.40^c	270.32 ± 2.10
AEBC 400 mg/kg	73.26 ± 2.20^b	205.25 ± 0.45^b	234.25 ± 2.75^b
Silymarin 100 mg/kg	63.02 ± 0.23^a	173.03 ± 0.47^a	227.09 ± 3.76^a

Results are expressed as mean± standard deviation. Values with the different superscript in a column are significantly different at P<0.05

Table 3. Haematological Indices of Wistar rats with Impaired Hepatic Health Pretreated Aqueous extract of *Bombax costatum*

Grouping	PCV (%)	Hb (g/dL)	RBC($\times 10^{12}$/L)	MCV(FL)	MCHC(g/dL)
Normal control	25.02 ± 0.45^{d}	11.44 ± 0.53^{d}	4.94 ± 0.45^{d}	56.57 ± 1.98^{c}	45.76 ± 3.02^{d}
Negative control	16.05 ± 1.02^{a}	5.91 ± 0.56^{a}	2.81 ± 0.78^{a}	48.92 ± 1.34^{a}	36.94 ± 2.89^{a}
AEBC 100 mg/kg	22.08 ± 2.04^{b}	8.58 ± 0.65^{b}	3.98 ± 0.89^{b}	55.30 ± 2.22^{bc}	39.00 ± 1.98^{b}
AEBC 200 mg/kg	22.06 ± 0.98^{b}	10.78 ± 0.78^{c}	3.97 ± 1.23^{b}	55.52 ± 2.43^{bc}	40.03 ± 2.98^{bc}
AEBC 400 mg/kg	24.02 ± 1.20^{c}	10.98 ± 0.98^{c}	4.00 ± 2.04^{bc}	54.21 ± 2.32^{b}	43.12 ± 2.32^{c}
Silymarin 100 mg/kg	25.67 ± 0.97^{d}	11.59 ± 2.34^{d}	4.67 ± 1.89^{d}	57.01 ± 2.09^{c}	44.08 ± 1.98^{c}

Results are expressed as mean± standard deviation. Values with the different superscript in a column are significantly different at P<0.05

family to which *Bonax costatum* belongs. Cellular destruction causes direct tissue damage and this may have haematological basis [17]. Table 3 shows the hematological indices of rats pretreated with *B. costatum* extract prior to exposure to attempt to induce hepatic damage by chemical means indicating that the PCV, Hb and RBC reported for the negative control were significantly (P<0.05) lower than those reported for the normal control. However, the values reported for PCV, Hb and RBC on groups pretreated with extract were higher. Similarly, the MCV and MCHC reported for the normal control were significantly (P<0.05) higher than that reported for the negative control. while the values reported for the pretreated groups were significantly (P<0.05) higher than that reported for the negative control. The hemato-protective effect of the extract observed on the some of the groups could be as a result of the presence of flavonoids. This observation is consistent with the finding of Asgary [18] which showed that rutin, a flavonoid impressively inhibited hemolysis.

4. CONCLUSION

It can be deduced from this study that aqueous extract of *Bombax costatum* has the potential to protect the liver and blood against external insult. However, observations made on this study implies that that achieving optimal protection against damage would ultimately depend on the dose administered.

CONSENT AND ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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