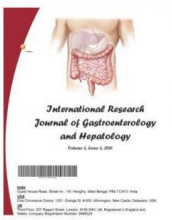

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The Potency of *Bombax costatum* Methanol Stem-bark Extract as a Hepato-curative Agent on Acetominophen Induced Hepato Toxicity in Wistar Albino Rats

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Author's contribution

The sole author designed, analysed, interpreted and prepared the manuscript.

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ABSTRACT

Background: The main thrust of the study was investigate the curative potentials of stem bark extract of *Bombax costatum* in acetaminophen induced hepatotoxicity in experimental animals.
Methods: Thirty experimental animals (Wistar rats) were grouped into six. Group III is the negative treatment hepato-toxified by sub chronic oral administration of acetaminophen at a dosage of 250 mg/kgbw, Groups IV, V and VI were hepato-toxified as in III and thereafter, followed up with treatment with 70% methanol stem bark extract of *Bombax costatum* at a dosage of 200, 400 and 600 mg/Kgbw on daily basis for another three weeks (20 days).
Results: There was significant decrease (P≤ 0.05) in both haematological and serum biochemical parameters of induced animals compared to the placebo in the first stanza while a significant increase (P≤ 0.05) was thereafter observed in the haemoglobin (HB), Packed cell volume (PCV), Mean copsucular volume (MCV), Red blood count (RBC) and Total white blood count (TWBC) with a corresponding decrease ((P≤ 0.05) in the platelets count in the treated groups. Similarly,

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significant decrease (P≤ 0.05) in the serum Aspartate transferase (AST), Alanine transferase (ALT), Alkaline phosphatase (ALP), Total protein, direct and indirect biluribin and Isocitrate dehydrogenase (ICDH) with a concomitant decrease ((P≤ 0.05) in Glutamate dehydrogenase (GDH) was also observed in the treated groups compared to the negative control.

Conclusion: The inadequacy of herbs used in curing of liver diseases and other dysfunctions caused by allopathic drugs is enough reason to focus on systematic scientific research to evaluate some species of plants that are traditionally claimed to possess hepato-curative activities.

Keywords: Acetominophen; hepatotoxicity, sub-chronic; Bombax costatum; haematological indices; biochemical indices; wistar albino rats.

1. INTRODUCTION

Liver disease has been one of the most risk factors threatening human health. With heart disease and stroke leading the chart, Liver disease is ranked as the fifth most common cause of death worldwide [1]. It comes in variety of forms mainly as alcoholic liver disease (ALD), non-alcoholic fatty liver disease (NAFLD), chronic viral hepatitis (e.g., hepatitis B virus and hepatitis C virus infections), autoimmune hepatitis (AIH), hepatic schistosomiasis (HS), liver cirrhosis (LC), hepatocellular carcinoma (HCC), and so forth [2].

NAFLD, with a reported prevalence of 6–35% world wide [3], is often associated with the metabolic syndrome. At present, NAFLD has become an important cause of chronic liver disease in developed countries, and its incidence has been increasing significantly in recent years. HCC has also been reported to accounts for almost 75% of liver cancer cases [4]. It is one of the most common malignant tumors in the world, especially in Asia, Africa, and Europe. According to World Health Organization (WHO) statistics, the mortality rate of HCC was as high as 95% in 2012. Moreover, report has it that, at least 2 and 150 million people worldwide are affected by hepatitis B virus (HBV) and hepatitis C virus (HCV) infections respectively [2].

Plants have been an important source of medicine for thousands of years. Even today, the World Health Organization (WHO) estimates that up to 80% of people still rely primarily on traditional remedies such as herbs for their medicines. Since time immemorial medicinal plants are an integral part of the African healthcare system. Being a fundamental part of the culture of the people who use it and also due to the economic challenge, there has been growing interest in traditional medicine particularly in the Asian and African countries. The major driving force towards full acceptance and application of traditional complementary medicine are: On one side, the pharmaceutical drugs are not accessible to the poor and on the other side, the richness and diversity of the fauna and flora of Africa are an inexhaustible source of therapies for panoply of ailments [5]. However, as much as it is embraced and practiced by the people in those regions there is need for scientific and clinical evaluations to show that they are effective and safe for humans as well as animals. Without this information, users of traditional medicinal plants in Africa and elsewhere (particularly the educated elites) will remain sceptical about the value of such therapies. This tendency will in the long run deny people the freedom to choose plants that are potentially less costly and are more accessible. During the last few decades, it has become evident that there exists a plethora of plants with medicinal potential and it is increasingly being accepted that the African traditional medicinal plants might offer potential template molecules in the drug discovery process.

Bombax costatum is a deciduous tree up to 25 m high in the savannah region; might be just 6 m in the sahel region. It is locally called *Kuryaa* or *Gurjiiyaa* and *Joohi* in “Hausa” and “Fulfulde” languages, respectively [6]. It is a fire resisting tree of the savannah and dry woodlands from Senegal to Central Africa, from Guinea across Ghana and Nigeria, Niger to Southern Chad. Crown structure is the common feature in young trees becoming irregular and sturdy in older trees. It prominently features a thick bark with a grey brown and corky with typical conical stout and sharp pointed spines on the stem and branches. The leaves are digitately compound, with 5-7 leaflets, 8-15 cm long on long petioles. Leaflets partly ovate, partly acuminate at both ends, with 8-10 pairs of lateral nerves. It flowers after leaf fall in November to February. Fructifies according to site and conditions, from the sixth year on, but very irregularly [7]. Medicinally, the bark is used for the treatment of skin diseases,

yellow fever and headache. The leaves and immature fruit are used as an ammolient. Various parts are used are equally used for fever or to promote lactation and as tonic for fatigue.

The main thrust of this work is to investigate the hepato-curative potentials of this of this plant species on experimentally drug induced liver injury (DILI) in Wistar albino rats.

2. MATERIALS AND METHODS

2.1 Experimental Site

The research was conducted at the Biochemistry and Nutrition teaching and research laboratory of the Federal College of Wildlife Management, New Bussa, Niger State, Middle belt region of Nigeria. The experimental station (New Bussa) is located between longitude 4° 31' and latitude 7.3°N and 10°N [8]. The research work was carried between the Months of May to July (early part of rainy season in that geo-political zone of Nigeria).

2.2 Plant Materials

The ethno-botanical survey was carried out in the surrounding villages namely, Old/New Awuru, Koro, Popo, Kere, Lubaruru and Dogongari villages around New-Bussa in Borgu local government area of Niger State. The main aim was to ascertain from the local people (particularly the elderly ones), the plant species commonly utilised in the traditional management of liver diseases. Part(s) utilised, method of preparation and period of harvest were also enquired from the interviewees. The identity of the plant was confirmed by Mr Musa Idris in the Department of Forestry, Federal College of Wildlife Management, New Bussa, Nigeria. The plant was deposited at the Forestry Research Institute Herbarium with an assigned voucher number FIH/Garba/NBS/1467.

2.3 Preparation of the Extract

The crude extract was prepared based on the method described by Garba et al. [9]. Briefly, fifty gram of the dried sample was pulverised to powdered form and cold extracted by placing the powdered sample into a 1000 ml capacity conical flask to which was added 400 ml of 70% v/v (methanol/water mixture at 70:30 ratio). Tin foil was used properly cover the mouth of the flask with occasional shaking at intervals. When there is observed deepening of colour of the solvent, the extract is filtered using a muslin cloth into an empty 1000 ml flask and another volume of 400 ml of the solvent is added to the marc. Extraction lasted for 48 h. and the solvent was removed and recovered using rotary evaporator. The extract was then transferred into a sterile universal bottle and stored at 4°C until required for use. The yield of the extract was 6.63 g/50 g or 13.2% of the whole sample extracted.

2.4 Phytochemical Analysis

The phytochemical analysis of the extract from stem bark of *B. costatum* was carried out based on coloration and precipitation test as described by Trease and Evans [10] and Sofowara [11].

2.5 Experimental Animals

Thirty healthy albino Wistar rats (1;1 male to female ratio) of average weight 120-150 g were purchased from animal house, University of Ibadan, Oyo State, Nigeria. The rats were housed in a rat Pen(s) measuring 3 m × 2 m × 2.5 m. The floor surface was overlaid with sawdust which was changed at three days intervals to prevent mould growth. They were properly fed with rat's pellets and water *ad libitum*. They were allowed twelve days to get properly acclimatised with our laboratory conditions. The handling of the animals in the course of experimental work was done strictly based on the Canadian Council on Animal Care guidelines (CCAC) [12].

2.6 Acute Toxicity Studies

Acute toxicity studies of the extract on samples of the experimental animals were performed according to the Organisation of Economic Cooperation and Development guidelines [13]. Briefly, twenty (20) rats of average weight of 125-160 g were grouped into five (5) and simultaneously administered 400, 800, 1200, 1600 and 2000 mg/kgbw of the *Bombax costatum s*tem bark extract and then closely monitored for 24 hours.

2.7 Drugs

Acetominophen (Glaxo Smithkline Ltd) was purchased from Na'uzo Pharmacy Ltd, Minna, Nigeria. Silymarin (Abbot Laboratories) was purchased from the Hepzibah Pharmacy Ltd, Minna, Nigeria. Diagnostic kits (Merck and DisSys Diagnostic systems, Germany) were purchased from the NAHCO Laboratory Equipments/Reagents Stores Ltd Minna, Nigeria.

All other chemical and reagents used were of high analytical grade and were used without further modification.

2.8 Experimental Design

Thirty experimental animals (Wistar rats) were grouped into six of five rats each (n=5). Group I was the placebo.

Group II was the standard treatment, hepato-toxified by sub chronic oral administration of acetaminophen at a dosage of 250 mg/kgbw on daily basis for 21 days with follow up treatment with the standard drug silymarin at 100 g/kgbw on daily basis for another period of 20 days post toxification.

Group III (negative treatment) hepato-toxified by sub chronic oral administration of acetaminophen at a dosage of 250 mg/kgbw without follow up treatment with standard drug (silymarin).

Groups IV, V and VI were hepato-toxified by sub chronic oral administration of acetaminophen at a dosage of 250 mg/kgbw daily for three weeks (21 days) and thereafter, followed up with treatment with 70% methanol stem bark extract of *Bombax costatum* at a dosage of 200, 400 and 600 mg/Kgbw on daily basis for another three weeks (21 days). The trial of induced toxicity and follow-up treatments with both standard drug and the extract were carried out separately on three weeks basis respectively. The trial lasted for a period of six weeks.

2.9 Blood Collection and Measurement of Haematological and Serum Biochemical Parameters

Blood samples from all the groups of the experimental animals and controls was collected at end of the first stanza of the hepato-toxification exercise (21^{st} day) from the saphenous vein in a heparinised and non-heparinisedsample bottles for haematological and serum biochemical analysis respectively. The haematological parameters were determined using the automated haemato-analyser Sysmex kx21, (product of Sysmex corporation, Japan).

In the second stanza of the experiment, the haematological and serum biochemical parameters in all the groups administered the extract (after the intoxication with acetominophen) were also determine, but in this case, at five (5) days interval as the treatment progress up till the 21^{st} day.

2.10 Calculation of Absolute Values

The different absolute values such as, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were calculated from values of RBC, PCV and Hb as follows: MCV (millimicron) = PCV% ×10 / RBC count (x million per mm3); MCH (picogram) =Hb g/dl × 10 / RBC count (× million per mm 3) and MCHC (picogram) = Hb g/dl×100/PCV%.

2.11 Determination of Biochemical Parameters

The biochemical analyses were determined for Alkaline phosphatase (ALP) based on methods of Tietz (1995) [14] and Gornall et al., 1949) [15]. Aspartate transaminase (AST), Alanine transaminase (ALT), Gamma glutamyl transferase (γGT), and Isocitrate dehydrogenases (ICDH), Direct billuribin and Indirect billuribin as described by Reitman and Frankel (1957) [16]. While the serum total protein concentration was estimated by Biuret method as described by Gornall et al. [15], Total cholesterol was measured by cholesterol CHOD-PAP method which is an enzymatic end point method [17], while the Glutamate dehydrogenase (GDH), Isocitrate dehydrogenase (ICDH) and Serum albumin were determined using the method described by Alaedein et al. (2013) [18].

2.12 Statistical Analysis

The data are presented as mean ± S.E.M. All the data were analysed by one-way ANOVA and differences between the means were assessed with Duncan Multiple comparison test. Differences were considered significant at $P \leq 0.05$. All analyses were carried out using Statistical Package for the Social Science (SPSS) version 2.0 (USA).

3. RESULTS AND DISCUSSION

The current and very disturbing trends of many marketed drugs having the potentials to cause hepatotoxicity called drug induced liver Injury (DILI) are quite alarming. The common types of drugs known to be notorious in causing DILI include but not limited to nonsteroidal anti-inflammatory drugs (NSAIDs), anti-infective drugs (including antituberculosis drugs), anti-cancer drugs, central nervous system drugs, cardiovascular system drugs, drugs used for

metabolic disorders, hormonal drugs, certain biological preparations, as well as Traditional Chinese medicine, natural medicine, health products and dietary supplements [19,20]. The cases of Herb induced liver injury (HILI) though previously neglected by both the herbs users and the herbalist, has now come to the fore [21]. Phytochemcail screening of the extract reveal the presence of polyphenols such as the flavonoids and tannins (Table 1) and is corroborated by the findings of Nuhu et al. [6] Phenolics and flavonoids contained in the stem bark have various biological activities, including antioxidant, anticarcinogenic, immunomodulatory, antidiabetic, antiatherogenic, and hepatoprotective functions and the regulation of thyroid status.

The LD_{50} determined when the 70% methanol extract was orally administered to experimental rats was found to be 2000 mg/kgbw (Table 2). This finding however, differs greatly from the values reported by Nuhu et al [6]. The variation could not come as a surprise due to the fact that, the samples were collected from different locations in which the soil mineral composition and edaphic factors may greatly vary. For instance, recent study in India has shown that dried *Bombax costatum leaves* contain lead at very high values of 352.0 mg/L. This phenomenon may replay itself whenever the plant sample is harvested in any soil with high lead or any other heavy metal composition as is the case the area from where our sample was collected.

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

Phyto chemicals	Inference
Alkaloids	+
Anthrquinones	-
Flavonoids	+
Glycosides	+
Saponins	+
Terpenoids	+
Tannins	+
Phytosterols	+

+= Present, - = Absent

Table 2. Effects of administration of various doses of the crude extract to healthy rats

Dosage	No of Animals	T/D	Observations
Distilled H20 or Normal Saline	4	4/0	No sign of toxicity, animals remained active even after the administration.
400 mgkg-1bw	4	4/0	No sign of toxicity, animals remained active even after the administration.
800 mgkg-1bw	4	4/0	Looked a bit depressed, the breathing was slow and remained Sluggish for a short while became normal again.
1200 mgkg-1bw	4	4/0	Sluggishness was observed, the breathing was slow and there was closing of the eyes and the feathers stood erect but conditions returned to normal after about 24 h.
1600 mgkg-1bw	4	4/1	One death was recorded about 13 h after the administration of the fraction and it took almost 27 h before the animals recovered fully from the sluggishness, depressed breathing, and erected feather.
2000 mgkg-1bw	4	4/2	Two deaths were recorded about 17 h after administration of the extract and it took almost 48 h before the animals recovered fully from the sluggishness, depressed breathing, erect fur and closing of the eyes.

T/D = Death per total number of animals in a group

Table 3. Observed serum haematological parameters in acetaminophen induced and non-treated hepatotoxic rats

Treatment	HB(g/dl)	PCV (%)	MCV (mmicron)	MCH (pg)	MCHC (g/L)	RBC $x10^6/mm^3$	PLC $(x10^3/mm^3)$	TWBC $(x10^3/mm^3)$	NEU (%)	LEU (%)	MON (%)
Placebo	12.5±2.34d	48.0±2.3c	5.3±1.34a	1.4±0.22a	26.04±1.33a	8.9±0.32d	1050±22.45a	124±3.23d	24.0±1.34d	50.0±3.23a	26.0±1.32d
G_P(Std drug)	12.9±2.35d	32.0±1.21b	6.1±0.55c	4.8±1.12c	80.9±1.23d	5.2±0.23c	2298±21.14d	93.7±2.32b	6.0±1.23a	81.0±4.11d	13.0±1.11a
G_N	11.5±2.57c	20.0±3.56a	6.3±0.21c	4.2±0.63c	67.5±1.14c	3.2±0.22a	1287±22.08c	104.0±2.11c	9.0±1.22b	74.0±3.23c	17.0±1.23b
G_{200}	9.4±1.45b	23.0±1.32a	5.8±0.22a	2.4±0.67b	40±1.56b	4.0±0.32b	1132±22.89b	69.0±2.32a	10.0±1.23b	72.0±4.33b	18.0±2.32b
G_{400}	7.9±1.45b	21.0±1.32a	6.0±0.23c	2.3±0.13b	37.6±1.32b	3.5±0.11a	1023±21.13a	150.0±3.22d	11.0±1.45c	72.0±5.23c	17.0±2.12b
G_{600}	5.8±0.34a	29.0±1.34b	6.4±1.76c	67.0±2.65d	99.0±2.23d	4.5±0.33b	1118±31.22b	106.1±3.14c	12.0±0.12c	66.0±3.45b	22.0±2.32c

Values are mean ±SEM of 3 determinations. The values along the column with different superscripts are significantly different ($p \leq 0.05$)

$G_{Positive}$ = group to be treated with standard drug, $G_{Negative}$ = group not to be treated (Negative control), $G_{200\text{-}600}$ = group to be treated with 200,400 and 600 mg/kgbw of the extract

Table 4. Observed serum biochemical parameters in acetaminophen induced and non-treated hepatotoxic rats

Treatment	AST U/L	ALT U/L	ALP U/L	GDH U/L	ICDH U/L)	TP g/L	ALBN g/L	CHTRL (mmol/L)	Urea mmol/L	DBIL µmol/L	IDBIL µmol/L
Placebo	72.5±2.34a	40.0±4.3b	283.0±3.34d	30±2.22a	75±1.33b	10.5±1.32b	6.5±0.45b	3.2.±0.23a	6.4±1.34a	5.0±0.23a	10.0±1.32b
G_P (Std drug)	115.9±10.35c	62.0±4.21d	136.0±5.55b	32.0±2.12a	80.9±1.23c	17.8±1.23d	9.8±0.14c	4.3.±1.32b	9.1±0.23b	11.0±5.11c	9.0±0.11a
G_N	103.5±12.57b	150.0±3.56a	183.0±4.21c	41.0±2.63b	67.5±1.14a	9.3±1.22a	5.8±0.08a	4.0±0.11b	12.4±1.22d	12.0±1.23d	17.0±2.23d
G_{200}	119.4±2.45c	109.0±1.32b	106.0±4.22a	50.0±2.67c	97.0±1.56d	20.4±1.32e	11.3±1.89d	6.20±1.32d	10.0±1.23c	8.5.0±0.33b	8.0±1.32a
G_{400}	127.9±9.45d	80.0±1.32c	153.0±3.23b	46.0±2.13b	89.5±0.32bc	14.4±1.11c	8.2.3±0.63c	5.0.±0.22c	11.0±1.45c	10.0±0.23c	11.0±1.42c
G_{600}	109.8±2.34b	132.0±1.34a	146.0±5.76b	67.0±2.65d	99.0±1.23d	9.4±0.73a	6.8±0.22b	6.2±1.14d	12.0±0.12d	11.6.0±3.45d	9.4±2.32b

Values are mean ±SEM of 3 determinations. The values along the column with different superscripts are significantly different ($p \leq 0.05$).

G_{PT} = group treated with standard drug, G_N = group not treated (Negative control), $G_{T\ 200\text{-}600}$ = group treated with 200,400 and 600 mg/kgbw of the extract

AST = Aspartate transaminase, ALT = Alanine transaminase, ALP = Alkaline phosphatase,GDH= Glucose dehydrogenase, TP= Total protein, γGT = Gamma glutamyl transferase, ICD = Isocitratedehydrogenase. DBIL= Direct bilirubin, IDBIL= Indirect bilirubin, TP= Total protein, ALBN=Albumin, CHTRL= Cholesterol

Table 5. Observed serum haematological parameters in drug induced liver injury and treated hepatotoxic rats

Treatment	HB(g/dl)	PCV (%)	MCV (mmicron)	MCH (pg)	MCHC (g/L)	RBC $x10^6/mm^3$	PLC $(x10^3/mm^3)$	TWBC $(x10^3/mm^3)$	NEU (%)	LEU (%)	MON (%)
Placebo	12.5±2.34b	46.0±4.3b	5.1±1.34b	1.4±0.22b	27.7±1.33a	8.9±0.32d	950±32.45a	104±13.23a	24.0±1.34	50.0±3.23a	26.0±2.32
G_{PT} (Std drug)	15.9±2.35d	42.0±4.21b	5.8±0.55b	2.2±1.12d	37.8±1.23d	7.2±1.23b	698±21.14d	73.7±2.32e	6.0±1.23a	81.0±5.11d	13.0±2.11a
G_N	10.3±2.57a	29.0±3.56a	5.5±0.21b	1.9±0.63c	35.5±2.14c	5.2±0.22a	787±22.08c	94.0±2.11c	9±1.22b	74.0±7.23	17.0±2.23
G_{T200}	14.2±1.45c	43.0±1.32b	5.4±1.22b	1.7±0.67c	33.0±1.96b	8.0±0.32c	832±22.89b	89.0±1.32d	10.0±1.23b	72.0±4.33c	18.0±2.32b
G_{T400}	12.3±1.45b	47.0±1.32b	4.4±3.23a	1.2±0.13a	26.2±1.32ba	10.5±1.11e	923±21.13a	100.0±2.22b	11.0±1.45	72.0±5.23c	17.0±2.12b
G_{T600}	14.1±2.34c	40.0±1.34b	4.3±5.76a	1.5±0.65b	35.3±2.23c	9.2±0.33d	818±31.22b	96.1±2.14c	12.0±0.12c	66.0±3.45b	22.0±2.32c

Values are mean ±SEM of 3 determinations. The values along the column with different superscripts are significantly different ($p \leq 0.05$)

G_{PT} = group treated with standard drug, G_N = group not treated (Negative control), $G_{T\ 200\text{-}600}$ = group treated with 200,400 and 600 mg/kgbw of the extract

Table 6. Observed serum biochemical parameters in drug induced liver injury treated hepatotoxic rats

Treatment	AST U/L	ALT U/L	ALP U/L	GDH U/L	ICDH U/L)	TP g/L	ALBN g/L	T.CHTRL (mg/dl)	Urea mg/dl	DBIL µmol/L	IDBIL µmol/L
Placebo	112.0±3.34d	40.0±4.3b	252.1±3.34c	40.6±2.22e	95±1.33c	7.0±0.32a	3.6±0.45a	6.1.±0.23d	4.3±0.34a	6.2±0.23d	7.0±1.32c
G_{PT} (Std drug)	105.9±2.35c	41.2±1.21b	236.0±4.55d	52.0±2.12d	90.9±1.23d	6.8.0±0.23a	3.4±0.14a	5.4.±0.32a	6.5±1.23b	6.0±5.11c	7.0±0.11d
G_N	103.5±3.57b	39.7±1.56a	183.0±3.21e	61.0±1.63b	67.5±1.14d	3.2±3.22d	1.7±0.08c	5.8±1.11e	10.4±1.22c	12.6±1.23a	16.0±1.23a
G_{T200}	109.4±2.65c	43.2±1.32a	246.0±4.22c	58.2±2.67c	97.0±1.56b	6.0±3.32c	3.2±12.89a	7.1±0.32b	7.3±1.23a	6.5.0±0.33b	7.8±1.32c
G_{T400}	97.9±2.45a	40.0±1.32a	293.0±3.23a	56.0±2.13c	103.5±2.32a	6.4.0±0.11b	3.3±7.13a	6.5±1.82c	3.2±1.45b	6.4±0.23c	7.0±1.42b
G_{T600}	104.8±2.74b	38.4±1.34a	266.0±5.76b	53.0±2.68a	99.0±2.23b	6.5±4.33b	3.8±3.22b	7.1±1.94b	5.3±1.12a	5.6±0.45d	7.6±1.32c

Values are mean ±SEM of 3 determinations. The values along the column with different superscripts are significantly different ($p \leq 0.05$).

G_{PT} = group treated with standard drug, G_N = group not treated (Negative control), $G_{T\ 200\text{-}600}$ = group treated with 200,400 and 600 mg/kgbw of the extract

AST = Aspartate transaminase, ALT = Alanine transaminase, ALP = Alkaline phosphatase, GDH= Glucose dehydrogenase, TP= Total protein, γGT = Gamma glutamyl transferase, ICD = Isocitratedehydrogenase. DBIL= Direct bilirubin, IDBIL= Indirect bilirubin, TP= Total protein, ALBN=Albumin

Pathogenesis of hematological changes is multifactorial, hence, by this study the correlation between abnormalities in hematological indices with severity of the induced liver disease has been revealed and future complications can be prevented by taking early steps. As revealed in Table 3, with the increasing severity of the induced toxicity, the MCV level was showing the increasing trend with the decreasing mean PCV. The mean Hb level in the entire groups also showed decreasing trend when compared with the placebo group. The MCH level and MCHC level showed a statistically significant ($p \leq 0.05$) change in the induced groups when also compared with the placebo. There was significant decrease ($P \leq 0.05$) in RBC in all the induced groups compared with the placebo. It is pertinent to point out that, the platelet count was normal in early stages but decreasing trend of platelet count was observed with the severity of the induced hepatotoxicity. A significant decrease ($P \leq 0.05$) in the TWBC was observed in the all the treatments except G_P and G_{200}. While the placebo showed the higher percentage composition of NEU and MON, G_{400} was observed to have higher value of the TWBC. The observation made thus, agrees with the results reported by Das et al. [22]. Where it was also well established that many haematological and biochemical abnormalities occur in sub-acute and chronic liver diseases.

When compared with the clinical pathology reference ranges of laboratory animals (Sprauge Dawley rats) developed by Toshiaki et al., (1993), [23] there is pathological increase ($P \leq 0.05$) in the Serum biochemical values when compared with the placebo (Table 4). This observation is not unusual due to the fact that, Paracetamol (acetaminophen) when administered in higher doses sub chronically, has been established to inhibit the activity of multiple cytochrome P450 enzymes, including CYP2B6, CYP2C8, CYP2C19, CYP2D6, and CYP3A, in human liver and intestinal microsomes [24]. In the case of rats, the activities of hepatic microsomal cytochrome P450s were decreased, including those of CYP2C, CYP2E1 and CYP3A [25]. The mechanism by which over dosage with paracetamol leads to hepatocellular injury and death involves its conversion to the toxic *N*-acetyl-*p*-benzoquinoneimine (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 [6].

The continuous daily administration of the stem bark methanol extract of *Bombax costatum* at doses of 200, 400 and 600 mg/kgbw to the hepatotoxic animals brings about a significant improvement ($P \leq 0.05$) in the haematological indices (Table 5). Of interest to note is the improvement in the Hb, PCV and RBC indices that compares favourably ($P \leq 0.05$) with the standard drug (Silymarin) while a continuous significant decrease ($P \leq 0.05$) in these indices was observed in the negative control group. Consequent upon reduction in the oxidative stress that is possibly initiated by the phenols and flavonoids components of the extract, the TWBC was significantly lower ($P \leq 0.05$) in both the groups treated with the extract and the standard drug compared to the negative control (Table 5). Since the highly reactive NAPQI metabolite resulting from acetaminophen overdose binds covalently to cell macromolecules thus leading to dysfunction of enzymatic systems and structural and metabolic disarray that may lead to GSH depletion, and Bombax costatum has been established to contain apart from polyphenols and flavonoids, also some small molecules such as vitamins A, C and E, beta-carotene.[24], that reduce the reactivity of various reactive radicals as an auxiliary antioxidant defense system. Eugene et al. [26] also reported that, apart from the high Iron content (23±2.1 mg/100 g)) observed in the methanol stem bark extract, Percentage inhibition of the DPPH radicals was also as high as 54%. Therefore, the observed improvement in these haematological indices might stemmed from these nutritional an anti-oxidative qualities and probably some yet to be determined haematopoietic molecules present in the extract.

After the treatment regime in the hepato-toxified rats with both the standard drug and the 70% methanol extract, there was a significant decrease ($P \leq 0.05$) in the serum AST in the groups treated with both extract and the standard drug (silymarin) compared with the negative group (Table 6). Despite the fact that histopatholigical studies was not conducted in this study, it suffice to state that, the significant decrease ($P \leq 0.05$) in the serum ALT in the negative control compared to the groups treated

with both the standard drug and the extract, coupled with the observed significantly higher values (P≤ 0.05) of the serum enzyme GDH in the same group when compared with both the standard and the extract-treated groups, the extract has not only reversed the toxicity trend but has also prevented necrosis of the hepatocytes [27] in the treated groups. Of interest is also the significantly lower values (P≤ 0.05) of the serum biomarker Isocitrate dehygrogenase (ICDH) observed in the negative control when compared with both the standard and the treated groups, is a clear indication of reversal of the inhibition of the antioxidants biomarkers (GSH, SOD and CAT) activities caused by the reactive oxygen species (ROS), reactive nitrogen species (RNS) and other metabolites generated by CYP450 inhibiting acetaminophen metabolites. As observed by Rangboo et al. [28] ALP level significantly decrease due to necrotic liver damage, hyperthyroidism, biliary tract disease, intestinal damage, hyperadrenocorticism, corticosteroid administration, barbiturate administration, and generalized tissue damage (including neoplasia). The result from this study (Table 6) showed no significant difference (P≤ 0.05) between the standard group, extract treated groups and the placebo and all the three groups significantly differs (P≤0.05) with the decreased values from the negative control which at this point might be suspected to be necrotic due to sustained injury from the acetaminophen metabolites. Other parameters such as γGT, Albumin, Cholesterol, Direct and indirect billuribin were all found not to be significantly different (p≤ 0.05) from the positive control but significantly different (p≤ 0.05) from the negative (Table 6). Levels of circulating steroids and biliary disease that may be inherent in the animals within the negative group [29]. There has not been any report on the hepatotoxicity of this plant with regards to the inhibition or induction of the CYP450 enzymes. Of the hepatotocity of 52 plants (most of which are of African origin) reviewed by Christopher and Taosheng. [30], mention has not been made of *Bombax costatum.* This cannot be unconnected to its rich composition of essential mineral elements, varieties of vitamins and also very low level of heavy metals such as Lead and Cadmium as observed by Eugene et al*.* [26].

Though, in this study only qualitative phytochemical analysis was carried out, and the phytochemicals were not characterised. It could still be hypothesised that, of the Phytochemicals contained in the Bombax costatum stem bark, may contain some ligand-like molecules similar to rifamficin and many other compounds which interact with the Pregnane X receptor (PXR) and act as agonists to the ligand binding domain of the PXR to enable the recruitment of co-activating proteins to trigger the transcriptional activation of genes for the expression of cytochrome P450 enzymes (CYPs) CYP3A4, CYP2B6, CYP2C9, and CYP2C19; phase II enzymes, including UDP-glucuronosyltransferases and sulfotransferases; and transporters, including ATP–binding cassette transporter ABCB1 (also known as MDR1) multiple organic anion transporters, and multidrug-resistance protein3 (MRP3) [31,32] which in similar fashion helps to restore the normal integrity of the hepatocytes as revealed by Tables 5 and 6. Alternatively, the probable agonist may act as a Constitutive Androstane Receptor (CAR) activator in a similar fashion to Phenobarbital and hence induces CAR's dephosphorylation, which indirectly activates CAR and increases such target genes as CYP2B6, the CYP2C subfamily, and CYP3A4 that are involved in drug metabolism and transport which might ultimately lead to the significant conversion of the excess acetaminophen metabolite into less toxic and excretable moieties.

4. CONCLUSION

From the result summed of together, it could be observed that the *Bombax costatum* stembark methanol extract has the potency to be employed as a curative phyto-agent against liver toxicity.

CONSENT

It is not applicable.

ETHICAL APPROVAL

As per international standard or university standard ethical approval has been collected and preserved by the author.

COMPETING INTERESTS

Author has declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of

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