

# 1 The Potency of *Bombax costatum* Stem-bark Extract As a Hepato-curative 2 Agent On Acetaminophen Induced Hepato Toxicity In Wistar Albino Rats.

## 3 Abstract

4 **Background:** The main thrust of the study was investigate the curative potentials of  
5 stem bark extract of *Bombax costatum* in acetaminophen induced hepatotoxicity in  
6 experimental animals.

7 **Methods:** Thirty experimental animals (Wistar rats) were grouped into six. Group III is  
8 the negative treatment hepato-toxified by sub chronic oral administration of  
9 acetaminophen at a dosage of 250 mg/kgbw, Groups IV, V and VI were hepato-toxified  
10 as in III and thereafter, followed up with treatment with 70% methanol stem bark  
11 extract of *Bombax costatum* at a dosage of 200, 400 and 600 mg/Kgbw on daily basis for  
12 another three weeks (20 days).

13 **Results:** There was significant decrease ( $P \leq 0.05$ ) in both haematological and serum  
14 biochemical parameters of induced animals compared to the placebo in the first stanza  
15 while a significant increase ( $P \leq 0.05$ ) was thereafter observed in the haemoglobin (HB),  
16 Packed cell volume (PCV), Mean cospucular volume (MCV), Red blood count (RBC)  
17 and Total white blood count (TWBC) with a corresponding decrease ( $P \leq 0.05$ ) in the  
18 platelets count in the treated groups. Similarly, significant decrease ( $P \leq 0.05$ ) in the  
19 serum Aspartate transferase (AST), Alanine transferase (ALT), Alkaline phosphatase  
20 (ALP), Total protein, direct and indirect bilirubin and Isocitrate dehydrogenase  
21 (ICDH) with a concomitant decrease ( $P \leq 0.05$ ) in Glutamate dehydrogenase (GDH) was  
22 also observed in the treated groups compared to the negative control.

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

27 **Key words:** Acetaminophen, Hepatotoxicity, Sub-chronic, *Bombax costatum*, Haematological  
28 indices, Biochemical indices, Wistar Albino rats.

## 29 1.0 Introduction

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

36 NAFLD, with a reported prevalence of 6–35% world wide[3], is often associated with the  
37 metabolic syndrome. At present, NAFLD has become an important cause of chronic liver  
38 disease in developed countries, and its incidence has been increasing significantly in recent  
39 years. HCC has also been reported to accounts for almost 75% of liver cancer cases.[4] t is

40 one of the most common malignant tumors in the world, especially in Asia, Africa, and  
41 Europe. According to World Health Organization (WHO) statistics, the mortality rate of HCC  
42 was as high as 95% in 2012. Moreover, report has it that, at least 2 and 150 million people  
43 worldwide are affected by hepatitis B virus (HBV) and hepatitis C virus (HCV) infections  
44 respectively.[2]

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

64 *Bombax costatum* is a deciduous tree up to 25m high in the savannah region; might be just  
65 6m in the sahel region. It is locally called *Kuryaa* or *Gurjiyaa* and *Joohi* in “Hausa” and  
66 “Fulfulde” languages, respectively.[6] It is a fire resisting tree of the savannah and dry  
67 woodlands from Senegal to Central Africa, from Guinea across Ghana and Nigeria, Niger to  
68 Southern Chad. Crown structure is the common feature in young trees becoming irregular and  
69 sturdy in older trees. It prominently features a thick bark with a grey brown and corky with  
70 typical conical stout and sharp pointed spines on the stem and branches. The leaves are  
71 digitately compound, with 5-7 leaflets, 8-15cm long on long petioles. Leaflets partly ovate,  
72 partly acuminate at both ends, with 8-10 pairs of lateral nerves. It flowers after leaf fall in

73 November to February. Fructifies according to site and conditions, from the sixth year on, but  
74 very irregularly.[7] Medicinally, the bark is used for the treatment of skin diseases, yellow  
75 fever and headache. The leaves and immature fruit are used as an ammolient. Various parts  
76 are used are equally used for fever or to promote lactation and as tonic for fatigue.

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

79

## 80 **2.0 Materials and Methods**

### 81 **2.1 Experimental site**

82 The research was conducted at the Biochemistry and Nutrition teaching and research  
83 laboratory of the Federal College of Wildlife Management, New Bussa, Niger State, Middle  
84 belt region of Nigeria. The experimental station (New Bussa) is located between longitude 4°  
85 31' and latitude 7.3°N and 10°N.[8] The research work was carried between the Months of  
86 May to July (early part of rainy season in that geo-political zone of Nigeria).

### 87 **2.2 Plant Materials**

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

### 97 **2.3 Preparation of the extract**

98 The crude extract was prepared based on the method described by Garba *et al.* (2015).[9]  
99 Briefly, fifty gram of the dried sample was pulverised to powdered form and cold extracted in  
100 400 ml of 70% v/v (methanol/water mixture). Extraction lasted for 48 h. The extract was  
101 filtered using muslin cloth and the solvent was removed and recovered using rotary  
102 evaporator. The extract was then transferred into a sterile universal bottle and stored at 4°C

103 until required for use. The yield of the extract was 6.63 g/50 g or 13.2% of the  
104 whole sample extracted.

#### 105 **2.4 Phytochemical analysis**

106 The phytochemical analysis of the extract from stem bark of *B. costatum* was carried out  
107 based on coloration and precipitation test as described by Trease and Evans (2002)[10] and  
108 Sofowara (1982).[11]

#### 109 **2.5 Experimental animals**

110 Thirty healthy albino Wistar rats (1;1 male to female ratio) of average weight 120-150g were  
111 purchased from animal house, University of Ibadan, Oyo State, Nigeria. The rats were housed  
112 in a rat Pen(s) measuring 3 m × 2 m × 2.5 m. The floor surface was overlaid with sawdust  
113 which was changed at three days intervals to prevent mould growth. They were properly fed  
114 with rat's pellets and water *ad libitum*. They were allowed twelve days to get properly  
115 acclimatised with our laboratory conditions. The handling of the animals in the course of  
116 experimental work was done strictly based on the Canadian Council on Animal Care  
117 guidelines (CCAC, 1999).[12]

#### 118 **2.6 Acute toxicity studies**

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

#### 124 **2.7 Drugs**

125 Acetaminophen (Glaxo Smithkline Ltd) was purchased from Na'uzo Pharmacy Ltd, Minna,  
126 Nigeria. Silymarin (Abbot Laboratories) was purchased from the Hepzibah Pharmacy Ltd,  
127 Minna, Nigeria. Diagnostic kits (Merck and DisSys Diagnostic systems, Germany) were  
128 purchased from the NAHCO Laboratory Equipments/Reagents Stores Ltd Minna, Nigeria.  
129 All other chemical and reagents used were of high analytical grade and were used without  
130 further modification.

#### 131 **2.8 Experimental design**

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

134 Group II was the standard treatment, hepato-toxified by sub chronic oral administration of  
135 acetaminophen at a dosage of 250 mg/kgbw on daily basis for 21 days **with** follow up  
136 treatment with the standard drug silymarin at 100 g/kgbw on daily basis for another period of  
137 20 days post toxification.

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

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

## 148 **2.9 Blood collection and measurement of haematological and serum biochemical** 149 **parameters**

150  
151 Blood samples from all the groups of the experimental animals and controls was collected at  
152 end of the first stanza of the hepato-toxification exercise (21<sup>st</sup> day) from the saphenous vein  
153 in a heparinised and non-heparinised sample bottles for haematological and serum  
154 biochemical analysis respectively. The haematological parameters were determined using the  
155 automated haemato-analyser Sysmex kx21, (product of Sysmex corporation, Japan).

156 In the second stanza of the experiment, the haematological and serum biochemical  
157 parameters in all the groups administered the extract (after the intoxication with  
158 acetaminophen) were also determine, but in this case, at five (5) days interval as the  
159 treatment progress up till the 21<sup>st</sup> day.

160

## 161 **2.10 Calculation of absolute values**

162 The different absolute values such as, mean corpuscular volume (MCV), mean corpuscular  
163 hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were  
164 calculated from values of RBC, PCV and Hb as follows:  $MCV \text{ (millimicron)} = PCV\% \times 10 /$   
165  $RBC \text{ count (x million per mm}^3\text{)}; MCH \text{ (picogram)} = Hb \text{ g/dl} \times 10 / RBC \text{ count}$

166 ( $\times$  million per mm<sup>3</sup>) and MCHC (picogram) = Hb g/dl  $\times$  100 / PCV %  
167

## 168 **2.11 Determination of biochemical parameters**

169 The biochemical analyses were determined for Alkaline phosphatase (ALP) based on  
170 methods of Tietz (1995)[14] and Gornall *et al.*, 1949).[15] Aspartate transaminase (AST),  
171 Alanine transaminase (ALT), Gamma glutamyl transferase ( $\gamma$ GT), and Isocitrate  
172 dehydrogenases (ICDH) as described by Reitman and Frankel (1957).[16] While the serum  
173 total protein concentration was estimated by Biuret method as described by Gornall *et al.*  
174 (1949).[15]

175

## 176 **2.12 Statistical analysis**

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

## 181 **3.0 Results and Discussion**

182 The current and very disturbing trends of many marketed drugs having the potentials to cause  
183 hepatotoxicity called drug induced liver Injury (DILI) are quite alarming. The common types  
184 of drugs known to be notorious in causing DILI include but not limited to nonsteroidal anti-  
185 inflammatory drugs (NSAIDs), anti-infective drugs (including antituberculosis drugs), anti-  
186 cancer drugs, central nervous system drugs, cardiovascular system drugs, drugs used for  
187 metabolic disorders, hormonal drugs, certain biological preparations, as well as Traditional  
188 Chinese medicine, natural medicine, health products and dietary supplements. [17][18] The  
189 cases of Herb induced liver injury (HILI) though previously neglected by both the herbs users  
190 and the herbalist, has now come to the fore.[19] Phytochemical screening of the extract reveal  
191 the presence of polyphenols such as the flavonoids and tannins (Table 1) and is corroborated  
192 by the findings of Nuhu *et al.* (2018).[6] Phenolics and flavonoids contained in the stem  
193 bark have various biological activities, including antioxidant, anticarcinogenic,  
194 immunomodulatory, antidiabetic, antiatherogenic, and hepatoprotective functions and the  
195 regulation of thyroid status.

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

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

202 += Present, - = Absent

203

204 The LD<sub>50</sub> determined when the 70% methanol extract was orally administered to  
205 experimental rats was found to be 2000mg/kgbw (Table 2). This finding however, differs  
206 greatly from the values reported by Nuhu et al. (2018).[6] The variation could not come as a  
207 surprise due to the fact that, the samples were collected from different locations in which the  
208 soil mineral composition and edaphic factors may greatly vary. For instance, recent study in  
209 India has shown that dried *Bombax costatum* leaves contain lead at very high values of  
210 352.0 mg/L. This phenomenon may replay itself whenever the plant sample is harvested in  
211 any soil with high lead or any other heavy metal composition as is the case the area from  
212 where our sample was collected.

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

Dosage	No of Animals	T/D	Observations
Distilled H <sub>2</sub> O or Normal Saline	4	4/0	No sign of toxicity, animals remained active even after the administration.
400mgkg-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 24h.
1600 mgkg-1bw	4	4/1	One death was recorded about 13 h after the administration of the fraction and it took almost 27h 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 48h before the animals recovered fully from the sluggishness, depressed breathing, erect fur and closing of the eyes.

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

215 Pathogenesis of hematological changes is multifactorial, hence, by this study the correlation  
 216 between abnormalities in hematological indices with severity of the induced liver disease has  
 217 been revealed and future complications can be prevented by taking early steps. As revealed  
 218 in Table 3, with the increasing severity of the induced toxicity, the MCV level was showing  
 219 the increasing trend with the decreasing mean PCV. The mean Hb level in the entire groups  
 220 also showed decreasing trend when compared with the placebo group. The MCH level and  
 221 MCHC level showed a statistically significant ( $p \leq 0.05$ ) change in the induced groups when  
 222 also compared with the placebo. There was significant decrease ( $P \leq 0.05$ ) in RBC in all the  
 223 induced groups compared with the placebo. It is pertinent to point out that, the platelet count  
 224 was normal in early stages but decreasing trend of platelet count was observed with the  
 225 severity of the induced hepatotoxicity. A significant decrease ( $P \leq 0.05$ ) in the TWBC was

226 observed in the all the treatments except G<sub>p</sub> and G<sub>200</sub>. While the placebo showed the higher  
227 percentage composition of NEU and MON, G<sub>400</sub> was observed to have higher value of the  
228 TWBC. The observation made thus, agrees with the results reported by Das et al. (2011) [20]  
229 where it was also well established that many haematological and biochemical abnormalities  
230 occur in sub-acute and chronic liver diseases.

UNDER PEER REVIEW

231 **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 $\times 10^6/\text{mm}^3$	PLC ( $\times 10^3/\text{mm}^3$ )	TWBC ( $\times 10^3/\text{mm}^3$ )	NEU (%)	LEU (%)	MON (%)
Placebo	12.5 $\pm$ 2.34c	48.0 $\pm$ 2.3b	5.3 $\pm$ 1.34a	1.4 $\pm$ 0.22a	26.04 $\pm$ 1.33a	8.9 $\pm$ 0.32c	1050 $\pm$ 22.45a	124 $\pm$ 3.23d	24.0 $\pm$ 1.34	50.0 $\pm$ 3.23	26.0 $\pm$ 1.32
<b>G<sub>P</sub>(Std drug)</b>	12.9 $\pm$ 2.35d	32.0 $\pm$ 1.21b	6.1 $\pm$ 0.55c	4.8 $\pm$ 1.12c	80.9 $\pm$ 1.23d	5.2 $\pm$ 0.23	2298 $\pm$ 21.14d	93.7 $\pm$ 2.32b	6.0 $\pm$ 1.23	81.0 $\pm$ 4.11d	13.0 $\pm$ 1.11
<b>G<sub>N</sub></b>	11.5 $\pm$ 2.57c	20.0 $\pm$ 3.56a	6.3 $\pm$ 0.21c	4.2 $\pm$ 0.63c	67.5 $\pm$ 1.14c	3.2 $\pm$ 0.22a	1287 $\pm$ 22.08c	104.0 $\pm$ 2.11c	9.0 $\pm$ 1.22b	74.0 $\pm$ 3.23	17.0 $\pm$ 1.23
<b>G<sub>200</sub></b>	9.4 $\pm$ 1.45b	23.0 $\pm$ 1.32a	5.8 $\pm$ 0.22b	2.4 $\pm$ 0.67b	40 $\pm$ 1.56b	4.0 $\pm$ 0.32b	1132 $\pm$ 22.89b	69.0 $\pm$ 2.32a	10.0 $\pm$ 1.23	72.0 $\pm$ 4.33	18.0 $\pm$ 2.32
<b>G<sub>400</sub></b>	7.9 $\pm$ 1.45b	21.0 $\pm$ 1.32a	6.0 $\pm$ 0.23c	2.3 $\pm$ 0.13b	37.6 $\pm$ 1.32b	3.5 $\pm$ 0.11b	1023 $\pm$ 21.13a	150.0 $\pm$ 3.22d	11.0 $\pm$ 1.45	72.0 $\pm$ 5.23	17.0 $\pm$ 2.12
<b>G<sub>600</sub></b>	5.8 $\pm$ 0.34a	29.0 $\pm$ 1.34a	6.4 $\pm$ 1.76c	67.0 $\pm$ 2.65d	99.0 $\pm$ 2.23d	4.5 $\pm$ 0.33b	1118 $\pm$ 31.22b	106.1 $\pm$ 3.14c	12.0 $\pm$ 0.12	66.0 $\pm$ 3.45	22.0 $\pm$ 2.32

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

233 **G<sub>Positive</sub>** = group to be treated with standard drug, **G<sub>Negative</sub>** = group not to be treated (Negative control), **G<sub>200-600</sub>** = group to be  
 234 treated with 200,400 and 600mg/kgbw of the extract

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236

237 When compared with the clinical pathology reference ranges of laboratory animals (Sprague  
238 Dawley rats) developed by Toshiaki *et al.*, (1993), [21] there is pathological increase ( $P \leq$   
239 0.05) in the Serum biochemical values when compared with the placebo (Table 4). This  
240 observation is not unusual due to the fact that, Paracetamol (acetaminophen) when  
241 administered in higher doses sub chronically, has been established to inhibit the activity of  
242 multiple cytochrome P450 enzymes, including CYP2B6, CYP2C8, CYP2C19, CYP2D6, and  
243 CYP3A, in human liver and intestinal microsomes (Misaka et al., 2013).[22] In the case of  
244 rats, the activities of hepatic microsomal cytochrome P450s were decreased, including those  
245 of CYP2C, CYP2E1 and CYP3A.[23] The mechanism by which over dosage with  
246 paracetamol leads to hepatocellular injury and death involves its conversion to the toxic *N*-  
247 acetyl-*p*-benzoquinoneimine (NAPQI) metabolite. This toxic metabolite accumulates as a  
248 result of saturation of the glucuronide and sulfate conjugation pathways. In the setting of  
249 paracetamol overdose, hepatocellular levels of GSH become depleted. The highly reactive  
250 NAPQI metabolite binds covalently to cell macromolecules, leading to dysfunction of  
251 enzymatic systems and structural and metabolic disarray. Furthermore, depletion of  
252 intracellular GSH renders the hepatocytes highly susceptible to oxidative stress and  
253 apoptosis.[6]

254 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 $\mu$ mol/L	IDBIL $\mu$ mol/L
Placebo	72.5 $\pm$ 2.34c	40.0 $\pm$ 4.3b	283.0 $\pm$ 3.34d	30 $\pm$ 2.22a	75 $\pm$ 1.33b	10.5 $\pm$ 1.32b	6.5 $\pm$ 0.45b	3.2 $\pm$ 0.23a	6.4 $\pm$ 1.34a	5.0 $\pm$ 0.23a	10.0 $\pm$ 1.32c
<b>G<sub>P</sub></b> (Std drug)	115.9 $\pm$ 10.35a	62.0 $\pm$ 4.21d	136.0 $\pm$ 5.55b	32.0 $\pm$ 2.12a	80.9 $\pm$ 1.23c	17.8 $\pm$ 1.23d	9.8 $\pm$ 0.14c	4.3 $\pm$ 1.32b	9.1 $\pm$ 0.23b	11.0 $\pm$ 5.11c	9.0 $\pm$ 0.11b
<b>G<sub>N</sub></b>	103.5 $\pm$ 12.57a	150.0 $\pm$ 3.56a	183.0 $\pm$ 4.21c	41.0 $\pm$ 2.63b	67.5 $\pm$ 1.14a	9.3 $\pm$ 1.22a	5.8 $\pm$ 0.08a	4.0 $\pm$ 0.11b	12.4 $\pm$ 1.22d	12.0 $\pm$ 1.23d	17.0 $\pm$ 2.23d
<b>G<sub>200</sub></b>	119.4 $\pm$ 2.45b	109.0 $\pm$ 1.32b	106.0 $\pm$ 4.22a	50.0 $\pm$ 2.67c	97.0 $\pm$ 1.56d	20.4 $\pm$ 1.32e	11.3 $\pm$ 1.89d	6.20 $\pm$ 1.32d	10.0 $\pm$ 1.23c	8.5.0 $\pm$ 0.33b	8.0 $\pm$ 1.32a
<b>G<sub>400</sub></b>	127.9 $\pm$ 9.45b	80.0 $\pm$ 1.32c	153.0 $\pm$ 3.23b	46.0 $\pm$ 2.13b	89.5 $\pm$ 0.32bc	14.4 $\pm$ 1.11c	8.2.3 $\pm$ 0.63c	5.0 $\pm$ 0.22c	11.0 $\pm$ 1.45c	10.0 $\pm$ 0.23c	11.0 $\pm$ 1.42c
<b>G<sub>600</sub></b>	109.8 $\pm$ 2.34a	132.0 $\pm$ 1.34a	146.0 $\pm$ 5.76b	67.0 $\pm$ 2.65d	99.0 $\pm$ 1.23d	9.4 $\pm$ 0.73a	6.8 $\pm$ 0.22b	6.2 $\pm$ 1.14d	12.0 $\pm$ 0.12d	11.6.0 $\pm$ 3.45c	9.4 $\pm$ 2.32b

255 Values are mean  $\pm$ SEM of 3 determinations. The values along the column with different superscripts are significantly different ( $p \leq 0.05$ ).  
 256

257 **G<sub>PT</sub>** = group treated with standard drug, **G<sub>N</sub>** = group not treated (Negative control), **G<sub>T 200-600</sub>** = group treated with 200,400 and  
 258 **600mg/kgbw** of the extract

259 AST = Aspartate transaminase, ALT = Alanine transaminase, ALP = Alkaline phosphatase, GDH= Glucose dehydrogenase, TP= Total protein,  
 260  $\gamma$ GT = Gamma glutamyl transferase, ICD = Isocitratatedehydrogenase. DBIL= Direct bilirubin, IDBIL= Indirect bilirubin, TP= Total protein,  
 261 ALBN=Albumin, CHTRL= cholesterol

262 The continuous daily administration of the stem bark methanol extract of *Bombax costatum*  
263 at doses of 200, 400 and 600mg/kgbw to the hepatotoxic animals brings about a significant  
264 improvement ( $P \leq 0.05$ ) in the haematological indices (Table 5). Of interest to note is the  
265 improvement in the Hb, PCV and RBC indices that compares favourably ( $P \leq 0.05$ ) with the  
266 standard drug (Silymarin) while a continuous significant decrease ( $P \leq 0.05$ ) in these indices  
267 was observed in the negative control group. Consequent upon reduction in the oxidative  
268 stress that is possibly initiated by the phenols and flavonoids components of the extract, the  
269 TWBC was significantly lower ( $P \leq 0.05$ ) in both the groups treated with the extract and the  
270 standard drug compared to the negative control (Table 5). Since the highly reactive NAPQI  
271 metabolite resulting from acetaminophen overdose binds covalently to cell macromolecules  
272 thus leading to dysfunction of enzymatic systems and structural and metabolic disarray that  
273 may lead to GSH depletion, and *Bombax costatum* has been established to contain apart from  
274 polyphenols and flavonoids, also some small molecules such as vitamins A, C and E, beta-  
275 carotene.<sup>24</sup>, that reduce the reactivity of various reactive radicals as an auxiliary antioxidant  
276 defense system.. Eugene et al.( 2018) [25] also reported that, apart from the high Iron  
277 content ( $23 \pm 2.1 \text{ mg}/100\text{g}$ ) observed in the methanol stem bark extract, Percentage inhibition  
278 of the DPPH radicals was also as high as 54% . Therefore, the observed improvement in  
279 these haematological indices might stemmed from these nutritional an anti-oxidative  
280 qualities and probably some yet to be determined haematopoietic molecules present in the  
281 extract.

282 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 <sup>6</sup> /mm <sup>3</sup>	PLC (x10 <sup>3</sup> /mm <sup>3</sup> )	TWBC (x10 <sup>3</sup> /mm <sup>3</sup> )	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
<b>G<sub>PT</sub></b> (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
<b>G<sub>N</sub></b>	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
<b>G<sub>T200</sub></b>	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
<b>G<sub>T400</sub></b>	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
<b>G<sub>T600</sub></b>	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

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

284 **G<sub>PT</sub>** = group treated with standard drug, **G<sub>N</sub>** = group not treated (Negative control), **G<sub>T 200-600</sub>** = group treated with 200,400 and 600mg/kgbw of the  
 285 extract

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288 After the treatment regime in the hepato-toxified rats with both the standard drug and the  
289 70% methanol extract, there was a significant decrease ( $P \leq 0.05$ ) in the serum AST in the  
290 groups treated with both extract and the standard drug (silymarin) compared with the  
291 negative group (Table 6). Despite the fact that histopathological studies was not conducted in  
292 this study, it suffice to state that, the significant decrease ( $P \leq 0.05$ ) in the serum ALT in the  
293 negative control compared to the groups treated with both the standard drug and the extract,  
294 coupled with the observed significantly higher values ( $P \leq 0.05$ ) of the serum enzyme GDH  
295 in the same group when compared with both the standard and the extract-treated groups, the  
296 extract has not only reversed the toxicity trend but has also prevented necrosis of the  
297 hepatocytes [26] in the treated groups. Of interest is also the significantly lower values ( $P \leq$   
298  $0.05$ ) of the serum biomarker Isocitrate dehydrogenase (ICDH) observed in the negative  
299 control when compared with both the standard and the treated groups, is a clear indication of  
300 reversal of the inhibition of the antioxidants biomarkers (GSH, SOD and CAT) activities  
301 caused by the reactive oxygen species (ROS), reactive nitrogen species (RNS) and other  
302 metabolites generated by CYP450 inhibiting acetaminophen metabolites. As observed by  
303 Rangboo et al. (2016), [27] ALP level significantly decrease due to necrotic liver damage,  
304 hyperthyroidism, biliary tract disease, intestinal damage, hyperadrenocorticism,  
305 corticosteroid administration, barbiturate administration, and generalized tissue damage  
306 (including neoplasia). The result from this study (Table 6) showed no significant difference  
307 ( $P \leq 0.05$ ) between the standard group, extract treated groups and the placebo and all the three  
308 groups significantly differs ( $P \leq 0.05$ ) with the decreased values from the negative control  
309 which at this point might be suspected to be necrotic due to sustained injury from the  
310 acetaminophen metabolites. Other parameters such as  $\gamma$ GT, Albumin, Cholesterol, Direct and  
311 indirect billuribin were all found not to be significantly different ( $p \leq 0.05$ ) from the positive  
312 control but significantly different ( $p \leq 0.05$ ) from the negative (Table 6). Levels of circulating  
313 steroids and biliary disease that may be inherent in the animals within the negative group.  
314 [28] There has not been any report on the hepatotoxicity of this plant with regards to the  
315 inhibition or induction of the CYP450 enzymes. Of the hepatotocity of 52 plants (most of  
316 which are of African origin) reviewed by Christopher and Taosheng (2017) [29], mention  
317 has not been made of *Bombax costatum*. This cannot be unconnected to its rich composition  
318 of essential mineral elements, varieties of vitamins and also very low level of heavy metals  
319 such as Lead and Cadmium as observed by Eugene et al. (2018). [25]

320

321

322 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 $\mu$ mol/L	IDBIL $\mu$ mol/L
Placebo	112.0 $\pm$ 3.34d	40.0 $\pm$ 4.3b	252.1 $\pm$ 3.34c	40.6 $\pm$ 2.22e	95 $\pm$ 1.33c	7.0 $\pm$ 0.32a	3.6 $\pm$ 0.45a	6.1 $\pm$ 0.23d	4.3 $\pm$ 0.34a	6.2 $\pm$ 0.23d	7.0 $\pm$ 1.32c
<b>G<sub>PT</sub></b> (Std drug)	105.9 $\pm$ 2.35c	41.2 $\pm$ 1.21b	236.0 $\pm$ 4.55d	52.0 $\pm$ 2.12d	90.9 $\pm$ 1.23d	6.8.0 $\pm$ 0.23a	3.4 $\pm$ 0.14a	5.4 $\pm$ 0.32a	6.5 $\pm$ 1.23b	6.0 $\pm$ 5.11c	7.0 $\pm$ 0.11d
<b>G<sub>N</sub></b>	103.5 $\pm$ 3.57b	39.7 $\pm$ 1.56a	183.0 $\pm$ 3.21e	61.0 $\pm$ 1.63b	67.5 $\pm$ 1.14d	3.2 $\pm$ 3.22d	1.7 $\pm$ 0.08c	5.8 $\pm$ 1.11e	10.4 $\pm$ 1.22c	12.6 $\pm$ 1.23a	16.0 $\pm$ 1.23a
<b>G<sub>T200</sub></b>	109.4 $\pm$ 2.65c	43.2 $\pm$ 1.32a	246.0 $\pm$ 4.22c	58.2 $\pm$ 2.67c	97.0 $\pm$ 1.56b	6.0 $\pm$ 3.32c	3.2 $\pm$ 12.89a	7.1 $\pm$ 0.32b	7.3 $\pm$ 1.23a	6.5.0 $\pm$ 0.33b	7.8 $\pm$ 1.32c
<b>G<sub>T400</sub></b>	97.9 $\pm$ 2.45a	40.0 $\pm$ 1.32a	293.0 $\pm$ 3.23a	56.0 $\pm$ 2.13c	103.5 $\pm$ 2.32a	6.4.0 $\pm$ 0.11b	3.3 $\pm$ 7.13a	6.5 $\pm$ 1.82c	3.2 $\pm$ 1.45b	6.4 $\pm$ 0.23c	7.0 $\pm$ 1.42b
<b>G<sub>T600</sub></b>	104.8 $\pm$ 2.74b	38.4 $\pm$ 1.34a	266.0 $\pm$ 5.76b	53.0 $\pm$ 2.68a	99.0 $\pm$ 2.23b	6.5 $\pm$ 4.33b	3.8 $\pm$ 3.22b	7.1 $\pm$ 1.94b	5.3 $\pm$ 1.12a	5.6 $\pm$ 0.45d	7.6 $\pm$ 1.32c

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

324

325 **G<sub>PT</sub>** = group treated with standard drug, **G<sub>N</sub>** = group not treated (Negative control), **G<sub>T 200-600</sub>** = group treated with 200,400 and  
326 600mg/kgbw of the extract327 **AST** = Aspartate transaminase, **ALT** = Alanine transaminase, **ALP** = Alkaline phosphatase, **GDH**= Glucose dehydrogenase, **TP**= Total protein,  
328  **$\gamma$ GT** = Gamma glutamyl transferase, **ICD** = Isocitrate dehydrogenase. **DBIL**= Direct bilirubin, **IDBIL**= Indirect bilirubin, **TP**= Total protein,  
329 **ALBN**=Albumin

330 Though, in this study only qualitative phytochemical analysis was carried out, and the  
331 phytochemicals were not characterised. It could still be hypothesised that, of the  
332 Phytochemicals contained in the *Bombax costatum* stem bark, may contain some ligand-like  
333 molecules similar to rifamycin and many other compounds which interact with the Pregnane  
334 X receptor (PXR) and act as agonists to the ligand binding domain of the PXR to enable the  
335 recruitment of co-activating proteins to trigger the transcriptional activation of genes for the  
336 expression of cytochrome P450 enzymes (CYPs) CYP3A4, CYP2B6, CYP2C9, and  
337 CYP2C19; phase II enzymes, including UDP-glucuronosyltransferases and sulfotransferases;  
338 and transporters, including ATP-binding cassette transporter ABCB1 (also known as MDR1)  
339 multiple organic anion transporters, and multidrug-resistance protein3 (MRP3) [30] [31]  
340 which in similar fashion helps to restore the normal integrity of the hepatocytes as revealed  
341 by Tables 5 and 6. Alternatively, the probable agonist may act as a Constitutive Androstane  
342 Receptor (CAR) activator in a similar fashion to Phenobarbital and hence induces CAR's  
343 dephosphorylation, which indirectly activates CAR and increases such target genes as  
344 CYP2B6, the CYP2C subfamily, and CYP3A4 that are involved in drug metabolism and  
345 transport which might ultimately lead to the significant conversion of the excess  
346 acetaminophen metabolite into less toxic and excretable moieties.

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

350

351 **Ethical Approval:**

352

353 **As per international standard or university standard ethical approval has been collected**  
354 **and preserved by the authors.**

355

356 **CONFLICT OF INTERESTS**

357 The authors have not declared any conflict of interests

358 **COMPETING INTERESTS DISCLAIMER:**

359

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