

# 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 The main thrust of the study was to investigate the curative potentials of stem bark  
5 extract of *Bombax costatum* in acetaminophen induced hepatotoxicity in experimental  
6 animals. Thirty experimental animals (Wistar rats) were grouped into six. Group III is  
7 the negative treatment hepato-toxified by sub chronic oral administration of  
8 acetaminophen at a dosage of 250 mg/kgbw, Groups IV, V and VI were hepato-toxified  
9 as in III and thereafter, followed up with treatment with 70% methanol stem bark  
10 extract of *Bombax costatum* at a dosage of 200, 400 and 600 mg/Kgbw on daily basis for  
11 another three weeks (20 days). There was significant decrease ( $P=0.05$ ) in both  
12 haematological and serum biochemical parameters of induced animals compared to the  
13 placebo in the first stanza while a significant increase ( $P \leq 0.05$ ) was thereafter observed  
14 in the HB, PCV, MCV, RBC and TWBC with a corresponding decrease ( $P \leq 0.05$ ) in  
15 the platelets count in the treated groups. Similarly, significant decrease ( $P \leq 0.05$ ) in the  
16 serum AST, ALT, ALP, Total protein, direct and indirect bilirubin and ICDH with a  
17 concomitant decrease ( $P \leq 0.05$ ) in GDH was also observed in the treated groups  
18 compared to the negative control. The inadequacy of herbs used in curing of liver  
19 diseases and other dysfunctions caused by allopathic drugs is enough reason to focus on  
20 systematic scientific research to evaluate some species of plants that are traditionally  
21 claimed to possess hepato-curative activities.

22 Key words: Acetaminophen, Hepatotoxicity, Sub-chronic, *Bombax costatum*, Haematological  
23 indices, Biochemical indices, CYP450 enzymes, Wistar Albino rats.

## 24 1.0 Introduction

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

31 NAFLD, with a reported prevalence of 6–35% world wide<sup>3</sup>, is often associated with the  
32 metabolic syndrome. At present, NAFLD has become an important cause of chronic liver  
33 disease in developed countries, and its incidence has been increasing significantly in recent  
34 years. HCC has also been reported to accounts for almost 75% of liver cancer cases.<sup>4</sup> It is  
35 one of the most common malignant tumors in the world, especially in Asia, Africa, and  
36 Europe. According to World Health Organization (WHO) statistics, the mortality rate of HCC  
37 was as high as 95% in 2012. Moreover, report has it that, at least 2 and 150 million people

38 worldwide are affected by hepatitis B virus (HBV) and hepatitis C virus (HCV) infections  
39 respectively.<sup>2</sup>

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

59 *Bombax costatum* is a deciduous tree up to 25m high in the savannah region; might be just  
60 6m in the sahel region. It is locally called *Kuryaa* or *Gurjiyyaa* and *Joohi* in “Hausa” and  
61 “Fulfulde” languages, respectively.<sup>6</sup> It is a fire resisting tree of the savannah and dry  
62 woodlands from Senegal to Central Africa, from Guinea across Ghana and Nigeria, Niger to  
63 Southern Chad. Crown structure is the common feature in young trees becoming irregular and  
64 sturdy in older trees. It prominently features a thick bark with a grey brown and corky with  
65 typical conical stout and sharp pointed spines on the stem and branches. The leaves are  
66 *digitate* compound, with 5-7 leaflets, 8-15cm long on long petioles. Leaflets are partly  
67 ovate, partly acuminate at both ends, with 8-10 pairs of lateral nerves. It flowers after leaf fall  
68 in November to February. Fructifies according to site and conditions, from the sixth year on,  
69 but very irregularly.<sup>7</sup> Medicinally, the bark is used for the treatment of skin diseases, yellow

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

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

74

## 75 **2.0 Materials and Methods**

### 76 **2.1 Experimental site**

77 The research was conducted at the Biochemistry and Nutrition teaching and research  
78 laboratory of the Federal College of Wildlife Management, New Bussa, Niger State, Middle  
79 belt region of Nigeria. The experimental station (New Bussa) is located between longitude 4°  
80 31' and latitude 7.3°N and 10°N.<sup>8</sup> The research work was carried between the Months of  
81 May to July (early part of rainy season in that geo-political zone of Nigeria).

### 82 **2.2 Plant Materials**

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

### 92 **2.3 Preparation of the extract**

93 The crude extract was prepared based on the method described by Garba *et al.* (2015).<sup>9</sup>  
94 Briefly, fifty gram of the dried sample was pulverised to powdered form and cold extracted in  
95 400 ml of 70% v/v (methanol/water mixture). Extraction lasted for 48 h. The extract was  
96 filtered using muslin cloth and the solvent was removed and recovered using rotary  
97 evaporator. The extract was then transferred into a sterile universal bottle and stored at 4°C  
98 until required for use. The yield of the extract was 6.63 g/50 g or 13.2% of the  
99 whole sample extracted.

## 100 **2.4 Phytochemical analysis**

101 The phytochemical analysis of the extract from stem bark of *B. costatum* was carried out  
102 based on coloration and precipitation test as described by Trease and Evans (2002)<sup>10</sup> and  
103 Sofowara (1982).<sup>11</sup>

## 104 **2.5 Experimental animals**

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

## 113 **2.6 Acute toxicity studies**

114 Acute toxicity studies of the extract on samples of the experimental animals were performed  
115 according to the Organisation of Economic Cooperation and Development guidelines  
116 (OECD, 2000).<sup>13</sup> Briefly, twenty (20) rats of average weight of 125-160g were grouped into  
117 five (5) and simultaneously administered 400, 800, 1200, 1600 and 2000mg/kg bw of the  
118 *Bombax costatum* stem bark **methanolic** extract and then closely monitored for 24 hours.

## 119 **2.7 Drugs**

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

## 126 **2.8 Experimental design**

127 Thirty experimental animals (Wistar rats) were grouped into six of five rats each (n=5).

128 Group I was the placebo

129 Group II was the standard treatment, hepato-toxified by sub chronic oral administration of  
130 acetaminophen at a dosage of 250 mg/kgbw on daily basis for 21 days **without follow up**  
131 **treatment with the standard drug silymarin at 100 g/kgbw on daily basis for another period of**  
132 **20 days post toxification.**

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

136 Groups IV, V and VI were hepato-toxified by sub chronic oral administration of  
137 acetaminophen at a dosage of 250 mg/kgbw daily for three weeks (21 days) and thereafter,  
138 followed up with treatment with **70% methanol** stem bark extract of *Bombax costatum* at a  
139 dosage of 200, 400 and 600 mg/Kg bw on daily basis for another three weeks (**20 days**).  
140 The trial of induced toxicity and follow-up treatments with both standard drug and the extract  
141 were carried out separately on three weeks basis respectively. The trial lasted for a period of  
142 six weeks.

## 143 **2.9 Blood collection and measurement of haematological and serum biochemical** 144 **parameters**

145  
146 Blood samples from all the groups of the experimental animals and controls was collected at  
147 end of the first stanza of the hepato-toxification exercise (21<sup>st</sup> day) from the saphenous vein  
148 in a heparinised and non-heparinised sample bottles for haematological and serum  
149 biochemical analysis respectively. The haematological parameters were determined using the  
150 automated haemato-analyser Sysmex kx21, (product of Sysmex corporation, Japan).

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

155

## 156 **2.10 Calculation of absolute values**

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

161 ( $\times$  million per mm<sup>3</sup>) and MCHC (picogram) = Hb g/dl  $\times$  100 / PCV %  
162

### 163 **2.11 Determination of biochemical parameters**

164 The biochemical analyses were determined for Alkaline phosphatase (ALP) based on  
165 methods of Tietz (1995)<sup>14</sup> and Gornall *et al.*, 1949).<sup>15</sup> Aspartate transaminase (AST), Alanine  
166 transaminase (ALT), Gamma glutamyl transferase ( $\gamma$ GT), and Isocitrate dehydrogenases  
167 (ICDH) as described by Reitman and Frankel (1957).<sup>16</sup> While the serum total protein  
168 concentration was estimated by Biuret method as described by Gornall *et al.* (1949).<sup>15</sup>  
169

### 170 **2.12 Statistical analysis**

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

## 175 **3.0 Results and Discussion**

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

196 += Present, - = Absent

197

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

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

Dosage	No of Animals	T/D	Observations
Distilled H2O 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.

208

209 Pathogenesis of hematological changes is multifactorial, hence, by this study the correlation  
210 between abnormalities in hematological indices with severity of the induced liver disease has  
211 been revealed and future complications can be prevented by taking early steps. As revealed  
212 in Table 3, with the increasing severity of the induced toxicity, the MCV level was showing  
213 the increasing trend with the decreasing mean PCV. The mean Hb level in the entire groups  
214 also showed decreasing trend when compared with the placebo group. The MCH level and  
215 MCHC level showed a statistically significant ( $p \leq 0.05$ ) change in the induced groups when  
216 also compared with the placebo. There was significant decrease ( $P \leq 0.05$ ) in RBC in all the  
217 induced groups compared with the placebo. It is pertinent to point out that, the platelet count  
218 was normal in early stages but decreasing trend of platelet count was observed with the  
219 severity of the induced hepatotoxicity. A significant decrease ( $P \leq 0.05$ ) in the TWBC was  
220 observed in the all the treatments except  $G_p$  and  $G_{200}$ . While the placebo showed the higher  
221 percentage composition of NEU and MON,  $G_{400}$  was observed to have higher value of the  
222 TWBC. The observation made thus, agrees with the results reported by Das et al. (2011)<sup>20</sup>  
223 where it was also well established that many haematological and biochemical abnormalities  
224 occur in sub-acute and chronic liver diseases.



225 **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±2.34c	48.0±2.3b	5.3±1.34a	1.4±0.22a	26.04±1.33a	8.9±0.32c	1050±22.45a	124±3.23d	24.0±1.34	50.0±3.23	26.0±1.32
G <sub>P</sub> (Std drug)	12.9±2.35d	32.0±1.21b	6.1±0.55c	4.8±1.12c	80.9±1.23d	5.2±0.23	2298±21.14d	93.7±2.32b	6.0±1.23	81.0±4.11d	13.0±1.11
G <sub>N</sub>	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±3.23	17.0±1.23
G <sub>200</sub>	9.4±1.45b	23.0±1.32a	5.8±0.22b	2.4±0.67b	40±1.56b	4.0±0.32b	1132±22.89b	69.0±2.32a	10.0±1.23	72.0±4.33	18.0±2.32
G <sub>400</sub>	7.9±1.45b	21.0±1.32a	6.0±0.23c	2.3±0.13b	37.6±1.32b	3.5±0.11b	1023±21.13a	150.0±3.22d	11.0±1.45	72.0±5.23	17.0±2.12
G <sub>600</sub>	5.8±0.34a	29.0±1.34a	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±0.12	66.0±3.45	22.0±2.32

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

227 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  
 228 treated with 200,400 and 600mg/kgbw of the extract

229

230

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

247 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

248 Values are mean  $\pm$ SEM of 3 determinations. The values along the column with different superscripts are significantly different ( $p \leq 0.05$ ).  
 249

250 **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  
 251 600mg/kgbw of the extract

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

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

274 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

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

276 **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  
 277 extract

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280 After the treatment regime in the hepato-toxified rats with both the standard drug and the  
281 70% methanol extract, there was a significant decrease ( $P \leq 0.05$ ) in the serum AST in the  
282 groups treated with both extract and the standard drug (silymarin) compared with the  
283 negative group (Table 6). Despite the fact that histopathological studies was not conducted in  
284 this study, it suffice to state that, the significant decrease ( $P \leq 0.05$ ) in the serum ALT in the  
285 negative control compared to the groups treated with both the standard drug and the extract,  
286 coupled with the observed significantly higher values ( $P \leq 0.05$ ) of the serum enzyme GDH  
287 in the same group when compared with both the standard and the extract-treated groups, the  
288 extract has not only reversed the toxicity trend but has also prevented necrosis of the  
289 hepatocytes<sup>26</sup> in the treated groups. Of interest is also the significantly lower values ( $P \leq$   
290  $0.05$ ) of the serum biomarker Isocitrate dehydrogenase (ICDH) observed in the negative  
291 control when compared with both the standard and the treated groups, is a clear indication of  
292 reversal of the inhibition of the antioxidants biomarkers (GSH, SOD and CAT) activities  
293 caused by the reactive oxygen species (ROS), reactive nitrogen species (RNS) and other  
294 metabolites generated by CYP450 inhibiting acetaminophen metabolites. As observed by  
295 Rangboo et al. (2016),<sup>27</sup> ALP level significantly decrease due to necrotic liver damage,  
296 hyperthyroidism, biliary tract disease, intestinal damage, hyperadrenocorticism,  
297 corticosteroid administration, barbiturate administration, and generalized tissue damage  
298 (including neoplasia). The result from this study (Table 6) showed no significant difference  
299 ( $P \leq 0.05$ ) between the standard group, extract treated groups and the placebo and all the three  
300 groups significantly differs ( $P \leq 0.05$ ) with the decreased values from the negative control  
301 which at this point might be suspected to be necrotic due to sustained injury from the  
302 acetaminophen metabolites. Other parameters such as  $\gamma$ GT, Albumin, Cholesterol, Direct and  
303 indirect billuribin were all found not to be significantly different ( $p \leq 0.05$ ) from the positive  
304 control but significantly different ( $p \leq 0.05$ ) from the negative (Table 6). Levels of circulating  
305 steroids and biliary disease that may be inherent in the animals within the negative group.<sup>28</sup>  
306 There has not been any report on the hepatotoxicity of this plant with regards to the inhibition  
307 or induction of the CYP450 enzymes. Of the hepatotocity of 52 plants (most of which are of  
308 African origin) reviewed by Christopher and Taosheng (2017)<sup>29</sup>, mention has not been made  
309 of *Bombax costatum*. This cannot be unconnected to its rich composition of essential mineral  
310 elements, varieties of vitamins and also very low level of heavy metals such as Lead and  
311 Cadmium as observed by Eugene et al. (2018).<sup>25</sup>

313

314 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

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

316

317 **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  
318 **600mg/kgbw** of the extract319 **AST** = Aspartate transaminase, **ALT** = Alanine transaminase, **ALP** = Alkaline phosphatase, **GDH**= Glucose dehydrogenase, **TP**= Total protein,  
320  **$\gamma$ GT** = Gamma glutamyl transferase, **ICD** = Isocitrate dehydrogenase. **DBIL**= Direct bilirubin, **IDBIL**= Indirect bilirubin, **TP**= Total protein,  
321 **ALBN**=Albumin

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

#### 339 **CONFLICT OF INTERESTS**

340 The authors have not declared any conflict of interests

#### 341 **COMPETING INTERESTS DISCLAIMER:**

342

343 **Authors have declared that no competing interests exist. The products used for this research**  
344 **are commonly and predominantly use products in our area of research and country. There is**  
345 **absolutely no conflict of interest between the authors and producers of the products because**  
346 **we do not intend to use these products as an avenue for any litigation but for the advancement**  
347 **of knowledge. Also, the research was not funded by the producing company rather it was**  
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