The Potency of *Bombax costatum* Methanol Stem-bark Extract As a Hepato-curative Agent On Acetominophen Induced Hepato Toxicity In Wistar Albino Rats.

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 ($P \leq 0.05$) in both haematological and serum biochemical parameters of induced animals compared to the placebo in the first stanza while a significant increase ($P \leq 0.05$) 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 (($P \leq 0.05$) in the platelets count in the treated groups. Similarly, significant decrease ($P \leq 0.05$) 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 (($P \leq 0.05$) 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.

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

1.0 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

UNDER PEER REVIEW

40 years. HCC has also been reported to accounts for almost 75% of liver cancer cases.[4] t is
41 one of the most common malignant tumors in the world, especially in Asia, Africa, and
42 Europe. According to World Health Organization (WHO) statistics, the mortality rate of HCC
43 was as high as 95% in 2012. Moreover, report has it that, at least 2 and 150 million people
44 worldwide are affected by hepatitis B virus (HBV) and hepatitis C virus (HCV) infections
45 respectively.[2]

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

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

UNDER PEER REVIEW

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.

2.0 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^{o}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 1000ml 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

UNDER PEER REVIEW

103 mouth of the flask with occasional shaking at intervals. When there is observed deepening of
104 colour of the solvent, the extract is filtered using a muslin cloth into an empty 1000ml flask
105 and another volume of 400ml of the solvent is added to the marc. Extraction lasted for 48 h.
106 and the solvent was removed and recovered using rotary evaporator. The extract was then
107 transferred into a sterile universal bottle and stored at 4°C until required for use. The yield of
108 the extract was 6.63 g/50 g or 13.2% of the whole sample extracted.

109 **2.4 Phytochemical analysis**

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

113 **2.5 Experimental animals**

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

122 **2.6 Acute toxicity studies**

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

128 **2.7 Drugs**

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

UNDER PEER REVIEW

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

135 **2.8 Experimental design**

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

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

141 20 days post toxification.

142 Group III (negative treatment) hepato-toxified by sub chronic oral administration of
143 acetaminophen at a dosage of 250 mg/kgbw without follow up treatment with standard drug
144 (silymarin).
145 Groups IV, V and VI were hepato-toxified by sub chronic oral administration of
146 acetaminophen at a dosage of 250 mg/kgbw daily for three weeks (21 days) and thereafter,
147 followed up with treatment with 70% methanol stem bark extract of *Bombax costatum* at a
148 dosage of 200, 400 and 600 mg/Kgbw on daily basis for another three weeks (21 days).
149 The trial of induced toxicity and follow-up treatments with both standard drug and the extract
150 were carried out separately on three weeks basis respectively. The trial lasted for a period of
151 six weeks.

152 **2.9 Blood collection and measurement of haematological and serum biochemical**
153 **parameters**
154

155 Blood samples from all the groups of the experimental animals and controls was collected at
156 end of the first stanza of the hepato-toxification exercise (21st day) from the saphenous vein
157 in a heparinised and non-heparinisedsample bottles for haematological and serum
158 biochemical analysis respectively. The haematological parameters were determined using the
159 automated haemato-analyser Sysmex kx21, (product of Sysmex corporation, Japan).
160 In the second stanza of the experiment, the haematological and serum biochemical
161 parameters in all the groups administered the extract (after the intoxication with
162 acetominophen) were also determine, but in this case, at five (5) days interval as the
163 treatment progress up till the 21st day.

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UNDER PEER REVIEW

165 **2.10 Calculation of absolute values**

166 The different absolute values such as, mean corpuscular volume (MCV), mean corpuscular
167 hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were
168 calculated from values of RBC, PCV and Hb as follows: MCV (millimicron) = PCV% ×10 /
169 RBC count (x million per mm3); MCH (picogram) =Hb g/dl × 10 / RBC count
170 (× million per mm3) and MCHC (picogram) = Hb g/dl × 100 / PCV %
171

172 **2.11 Determination of biochemical parameters**

173 The biochemical analyses were determined for Alkaline phosphatase (ALP) based on
174 methods of Tietz (1995)[14] and Gornall *et al.*, 1949).[15] Aspartate transaminase (AST),
175 Alanine transaminase (ALT), Gamma glutamyl transferase (γGT), and Isocitrate
176 dehydrogenases (ICDH), Direct billuribin and Indirect billuribin as described by Reitman and
177 Frankel (1957).[16] While the serum total protein concentration was estimated by Biuret
178 method as described by Gornall *et al.* [15], Total cholesterol was measured by cholesterol
179 CHOD-PAP method which is an enzymatic end point method [17], while the Glutamate
180 dehydrogenase (GDH), Isocitrate dehydrogenase (ICDH) and Serum albumin were
181 determined using the method described by Alaedein *et al.* (2013) [18]
182

183 **2.12 Statistical analysis**

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

188 **3.0 Results and Discussion**

189 The current and very disturbing trends of many marketed drugs having the potentials to cause
190 hepatotoxicity called drug induced liver Injury (DILI) are quite alarming. The common types
191 of drugs known to be notorious in causing DILI include but not limited to nonsteroidal anti-
192 inflammatory drugs (NSAIDs), anti-infective drugs (including antituberculosis drugs), anti-
193 cancer drugs, central nervous system drugs, cardiovascular system drugs, drugs used for
194 metabolic disorders, hormonal drugs, certain biological preparations, as well as Traditional
195 Chinese medicine, natural medicine, health products and dietary supplements. [19] [20] The

UNDER PEER REVIEW

196 cases of Herb induced liver injury (HILI) though previously neglected by both the herbs users
197 and the herbalist, has now come to the fore.[21] Phytochemcail screening of the extract reveal
198 the presence of polyphenols such as the flavonoids and tannins (Table 1) and is corroborated
199 by the findings of Nuhu *et al.* [6] Phenolics and flavonoids contained in the stem bark have
200 various biological activities, including antioxidant, anticarcinogenic, immunomodulatory,
201 antidiabetic, antiatherogenic, and hepatoprotective functions and the regulation of thyroid
202 status.

203
204
205
206
207 **Table 1:** Phytochemical constituents of methanol stem barkextract of *Bombax costatum.*
208

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

209 **+= Present, - = Absent**
210

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

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

UNDER PEER REVIEW

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.
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.

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

222 Pathogenesis of hematological changes is multifactorial, hence, by this study the correlation
223 between abnormalities in hematological indices with severity of the induced liver disease has
224 been revealed and future complications can be prevented by taking early steps. As revealed

UNDER PEER REVIEW

225 in Table 3, with the increasing severity of the induced toxicity, the MCV level was showing
226 the increasing trend with the decreasing mean PCV. The mean Hb level in the entire groups
227 also showed decreasing trend when compared with the placebo group. The MCH level and
228 MCHC level showed a statistically significant ($p \leq 0.05$) change in the induced groups when
229 also compared with the placebo. There was significant decrease ($P \leq 0.05$) in RBC in all the
230 induced groups compared with the placebo. It is pertinent to point out that, the platelet count
231 was normal in early stages but decreasing trend of platelet count was observed with the
232 severity of the induced hepatotoxicity. A significant decrease ($P \leq 0.05$) in the TWBC was
233 observed in the all the treatments except G_P and $G_{200.}$ While the placebo showed the higher
234 percentage composition of NEU and MON, G_{400} was observed to have higher value of the
235 TWBC. The observation made thus, agrees with the results reported by Das *et al.* [22] where
236 it was also well established that many haematological and biochemical abnormalities occur in
237 sub-acute and chronic liver diseases.

UNDER PEER REVIEW

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), $G_{200-600}$ = group to be treated with 200,400 and 600mg/kgbw of the extract

UNDER PEER REVIEW

244 When compared with the clinical pathology reference ranges of laboratory animals (Sprauge
245 Dawley rats) developed by Toshiaki *et al.*, (1993), [23] there is pathological increase ($P \leq$
246 0.05) in the Serum biochemical values when compared with the placebo (Table 4). This
247 observation is not unusual due to the fact that, Paracetamol (acetaminophen) when
248 administered in higher doses sub chronically, has been established to inhibit the activity of
249 multiple cytochrome P450 enzymes, including CYP2B6, CYP2C8, CYP2C19, CYP2D6, and
250 CYP3A, in human liver and intestinal microsomes. [24] In the case of rats, the activities of
251 hepatic microsomal cytochrome P450s were decreased, including those of CYP2C, CYP2E1
252 and CYP3A.[25] The mechanism by which over dosage with paracetamol leads to
253 hepatocellular injury and death involves its conversion to the toxic *N*-acetyl-*p*-
254 benzoquinoneimine (NAPQI) metabolite. This toxic metabolite accumulates as a result of
255 saturation of the glucuronide and sulfate conjugation pathways. In the setting of paracetamol
256 overdose, hepatocellular levels of GSH become depleted. The highly reactive NAPQI
257 metabolite binds covalently to cell macromolecules, leading to dysfunction of enzymatic
258 systems and structural and metabolic disarray. Furthermore, depletion of intracellular GSH
259 renders the hepatocytes highly susceptible to oxidative stress and apoptosis.[6]

UNDER PEER REVIEW

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), $G_{T\ 200-600}$ = group treated with 200,400 and 600mg/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

UNDER PEER REVIEW

268 The continuous daily administration of the stem bark methanol extract of *Bombax costatum*
269 at doses of 200, 400 and 600mg/kgbw to the hepatotoxic animals brings about a significant
270 improvement (P≤ **0.05)** in the haematological indices (Table 5). Of interest to note is the
271 improvement in the Hb, PCV and RBC indices that compares favourably (P≤ 0.05) with the
272 standard drug (Silymarin) while a continuous significant decrease (P≤ **0.05)** in these indices
273 was observed in the negative control group. Consequent upon reduction in the oxidative
274 stress that is possibly initiated by the phenols and flavonoids components of the extract, the
275 TWBC was significantly lower (P≤ 0.05**)** in both the groups treated with the extract and the
276 standard drug compared to the negative control (Table 5). Since the highly reactive NAPQI
277 metabolite resulting from acetaminophen overdose binds covalently to cell macromolecules
278 thus leading to dysfunction of enzymatic systems and structural and metabolic disarray that
279 may lead to GSH depletion, and Bombax costatum has been established to contain apart from
280 polyphenols and flavonoids, also some small molecules such as vitamins A, C and E, beta-
281 carotene.[24] , that reduce the reactivity of various reactive radicals as an auxiliary antioxidant
282 defense system.. Eugene *et al.* [26] also reported that, apart from the high Iron content
283 (23±2.1mg/100g)) observed in the methanol stem bark extract, Percentage inhibition of the
284 DPPH radicals was also as high as 54% . Therefore, the observed improvement in these
285 haematological indices might stemmed from these nutritional an anti-oxidative qualities
286 and probably some yet to be determined haematopoietic molecules present in the extract.

UNDER PEER REVIEW

287 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**

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

289 **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 600mg/kgbw of the**
290 **extract**

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UNDER PEER REVIEW

293 After the treatment regime in the hepato-toxified rats with both the standard drug and the
294 70% methanol extract, there was a significant decrease ($P \leq 0.05$) in the serum AST in the
295 groups treated with both extract and the standard drug (silymarin) compared with the
296 negative group (Table 6). Despite the fact that histopatholigical studies was not conducted in
297 this study, it suffice to state that, the significant decrease ($P \leq 0.05$) in the serum ALT in the
298 negative control compared to the groups treated with both the standard drug and the extract,
299 coupled with the observed significantly higher values ($P \leq 0.05$) of the serum enzyme GDH
300 in the same group when compared with both the standard and the extract-treated groups, the
301 extract has not only reversed the toxicity trend but has also prevented necrosis of the
302 hepatocytes [27] in the treated groups. Of interest is also the significantly lower values ($P \leq$
303 0.05) of the serum biomarker Isocitrate dehygrogenase (ICDH) observed in the negative
304 control when compared with both the standard and the treated groups, is a clear indication of
305 reversal of the inhibition of the antioxidants biomarkers (GSH, SOD and CAT) activities
306 caused by the reactive oxygen species (ROS), reactive nitrogen species (RNS) and other
307 metabolites generated by CYP450 inhibiting acetaminophen metabolites. As observed by
308 Rangboo *et al.* [28] ALP level significantly decrease due to necrotic liver damage,
309 hyperthyroidism, biliary tract disease, intestinal damage, hyperadrenocorticism,
310 corticosteroid administration, barbiturate administration, and generalized tissue damage
311 (including neoplasia). The result from this study (Table 6) showed no significant difference
312 ($P \leq 0.05$) between the standard group, extract treated groups and the placebo and all the three
313 groups significantly differs ($P \leq 0.05$) with the decreased values from the negative control
314 which at this point might be suspected to be necrotic due to sustained injury from the
315 acetaminophen metabolites. Other parameters such as γGT, Albumin, Cholesterol, Direct and
316 indirect billuribin were all found not to be significantly different ($p \leq 0.05$) from the positive
317 control but significantly different ($p \leq 0.05$) from the negative (Table 6). Levels of circulating
318 steroids and biliary disease that may be inherent in the animals within the negative group.
319 [29] There has not been any report on the hepatotoxicity of this plant with regards to the
320 inhibition or induction of the CYP450 enzymes. Of the hepatotocity of 52 plants (most of
321 which are of African origin) reviewed by Christopher and Taosheng. [30], mention has not
322 been made of *Bombax costatum.* This cannot be unconnected to its rich composition of
323 essential mineral elements, varieties of vitamins and also very low level of heavy metals such
324 as Lead and Cadmium as observed by Eugene *et al.* [26]

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UNDER PEER REVIEW

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 600mg/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

UNDER PEER REVIEW

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

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

355

356 **Ethical Approval:**

357

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

360 **Consent: NA**

361

362 **CONFLICT OF INTERESTS**
363 The authors have not declared any conflict of interests

364 COMPETING INTERESTS DISCLAIMER:

UNDER PEER REVIEW

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