http://dx.doi.org/10.4314/ajtcam.v10i6.20

EVALUATION OF THE TOXICOLOGICAL PROFILE OF THE LEAVES AND YOUNG TWIGS OF *CAESALPINIA BONDUC* (LINN) ROXB

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Abstract

Acute and sub-acute toxicological effects of ethanolic extract of the leaves and young twigs of *Caesalpinia bonduc* were carried out on albino rats. Single extract doses from 2000 to 5000 mg/kg body weight were administered orally and monitored for 14 days in acute study, while extract doses from 200 to 1600 mg/kg body weight were orally administered daily for 28 days in sub-acute study and recovery was assessed 14 days after dosing. Biochemical, haematological and histopathological examinations were carried out. There was no mortality in the experimental animals in all acute treatment doses. However, there were significant alterations in the biomarkers and induced cellular damage to the liver in all acute treatment doses. In the sub-acute toxicity treatment, the assessed biomarkers were unaffected at extract dose of 200 mg/kg body weight compared to control, while significant changes were observed in rats administered with extract doses of 400 mg/kg body weight and above. No significant difference was observed between the tested groups and the recovery groups in the sub-acute toxicity study. In conclusion, the ethanolic extract of *C. bonduc* could be toxic to selected organs of the rat body in acute and sub-acute treatments.

Keywords: *Caesalpinia bonduc*, acute toxicity, sub-acute toxicity, biochemical, haematological and histopathological parameters

List of non-standard abbreviations

Forestry Research Institute of Nigeria (FRIN), Institute of Medical Research (NIMR), Organization for Economic Cooperation and Development (OECD), Ethylenediaminetetraacetic acid (EDTA), total protein (PTP), urea (PU), creatinine (PCT), uric acid (PUA), glucose (PG), triglyceride (PTG), aspartate aminotransferase (AST), alanine aminotransferase (ALT), cholesterol (PC), total bilirubin (PTB), direct bilirubin (PDB) and indirect bilirubin (PIB), packed cell volume (PCV), white blood cells (WBC), neutrophil (N), eosinophil (E), lymphocyte (L) and monocyte (M)

Introduction

Indigenous knowledge of medicinal plants, have made them a valuable tool in the treatment of several ailments. The knowledge about the various functions of plants is communicated from generation to generation through frequent usage and by oral tradition (Sagar and Vidyasagar, 2010). The traditional use of medicinal plants in the treatment of different diseases is popular in Nigeria and other developing countries. Natural products are conceived to be a major source of chemical substances with possible therapeutic activity. World Health Organization (WHO) reported that 80 % of the world's population use medicinal plants as their main primary health care source in the treatment of diseases (Ajose, 2007). Some herbal drugs are associated with both harmful as well as beneficial effects to the biological system (Sagar and Vidyasagar, 2010)..

Caesalpinia bonduc, (family: *Caesalpiniaceae*, genus *Fabaceae*), commonly known as Gray Nicker nut (English) and Ayòó (Yoruba, Nigeria), is a prickly shrub with grey, hard, globular shaped seeds and a smooth shining surface (Nadkarni, 1954). It is a medicinal plant predominantly distributed in the tropical and subtropical regions of Africa, Asia and the Caribbean (Gupta *et al*., 2003). The pharmacological screening of the plant extracts reveals their anticancer, antioxidant, antimalarial, antihyperglycemic, anti-inflammatory, antirheumatic, antipyretic, anticonvulsant, antiameaobic, anti-estrogenic and abortifacient activities (Adesina, 1982; Datté *et al.*, 1998; Gupta *et al*., 2003; Chakrabarti *et al*. 2003; Gupta *et al*., 2004; Sonibare *et al*., 2009). Recently, Jäger and Saaby (2011) reported the anti-depressant, anti-anxiety, memory inducer and relaxing enhancer of *C. bonduc*. The phytochemical analysis of the plant shows that it contains saponins, alkaloids, flavonoids, triterpenoids, diterpenoids, tannins and steroids (Kumar *et al*., 2005).

The leaves and young twigs of *C. bonduc* are used as an antimalarial decoction (Innocent *et al*., 2009), as well as for other ethno-medicinal uses in Nigeria. There is paucity of information on the toxicity profile of the ethanolic extract of the leaves and twigs of *C. bonduc*. Consequently, detailed toxicity studies of *C. bonduc* using animal model are required to avoid the potential adverse effects of the plant. The present work was designed to study the acute and sub-acute toxicity profile of the ethanolic extract of leaves and young twigs of *C. bonduc* (L) Robx using methods developed by Organization for Economic Community and Development (OECD, 1995; OECD, 2001). The toxicity studies of ethanolic extract of the leaves and young twigs of *C. bonduc* using OECD methods have not been reported before in published work. Hence, the work was designed and reported herein for the first time.

http://dx.doi.org/10.4314/ajtcam.v10i6.20

Materials and Methods

Plant material:

Young twigs and leaves of *C. bonduc* were collected from Forestry Research Institute of Nigeria (FRIN), Ibadan, Oyo State. Plant identification was done by Dr. Conrad Omonhinmi, Biological Sciences, College of Science and Technology, Covenant University, Ota, Ogun State. The authentication and voucher referencing were carried out at FRIN with voucher specimen no SHI108408 deposited in the FRIN Herbarium.

Preparation of extract

The leaves and young twigs of the plant were air-dried at room temperature (25^{o}C) and powdered. Three hundred grams of the plant were extracted with 75% (v/v) ethanol by maceration using three successive cold extractions (25^{o}C) for 72 hours. The total filtrate was concentrated to dryness on a rotary evaporator at 50^{o}C.

Experimental animals

Seventy healthy adult female albino Wistar rats, purchased from the Institute of Medical Research (NIMR), Yaba, Lagos, Nigeria, weighing between 60 – 100 g were used for the sub-acute assessment, while rats weighing between 120 - 135 g were used for the acute determination. The rats were kept in standard cages in the animal house of Covenant University, Ota, Ogun State, Nigeria. They were allowed to acclimatize for two weeks and were given food and water *ad libitum*. The cages were cleaned daily and the animals were treated according to standard ethical guidelines.

Acute toxicity

Acute toxicity study was conducted with the standard method of the Organization for Economic Cooperation and Development (OECD) guidelines 423 (OECD, 2001) with the limit test dose of 5000 mg/kg body weight. Plant extract at fixed doses of 2000, 4000 and 5000 mg/kg body weight were administered by gastric intubation to three groups of five rats each and were compared with control group, given distilled water. Observations were made for signs of morbidity and mortality at 1, 2, 4, 6 hours after dosing and subsequently on 7th and 14th days after dosing. Body weight was also monitored and documented. The biochemical, haematological and histological markers of toxicological effects of extract on animals were evaluated after 14 days of dosing.

Sub-acute toxicity

Fifty female albino Wistar rats were selected by stratified randomization for the sub-acute toxicity study. They were divided into six groups. The five animals in group one were the normal control group; the five in group two were the vehicle control group. The four subsequent groups (groups three to six), each containing ten rats, were the extract treated groups. In each extract treated group, five rats were for the actual test and five were for the recovery test. The rats were weighed before the commencement of treatment. Thereafter, they were weighed weekly throughout the duration of the study. The rats were dosed as follows: Group 1 was the normal control, dosed with 1 ml distilled water; group 2 was the vehicle control, dosed with 1 ml (0.25 %) sodium carboxymethyl cellulose; group 3 was dosed with 200 mg extract/kg between; group 4 was dosed with 400 mg extract/kg between; group 5 was dosed with 800 mg extract/kg between; and group 6 was dosed with 1600 mg extract/kg between. The animals were dosed daily by gastric intubation.

The physical appearance and the daily activities of the rats, such as eating patterns and signs of abnormalities, were observed and recorded. On the 29th day after treatment, five treated animals in each group were put under light ether anaesthesia (Muto *et al.*, 2003). The recovery groups were kept, left untreated for additional 14 days and later sacrificed. Blood samples were collected by cardiac puncture into heparin and EDTA bottles. The kidney, liver, heart and spleen were also collected from the animals and washed in normal saline, weighed and stored in 10% formalin in plastic bottles. The biochemical, haematological and histopathological markers of organ toxicity were evaluated in the treated animals and compared with controls. The relative organ weights were also calculated and recorded.

Biochemical analysis

The blood samples in heparin tubes were centrifuged at 3000 rpm for 10 minutes to collect the blood plasma. The blood plasma was analysed for parameters such as total protein (PTP), urea (PU), creatinine (PCT), uric acid (PUA), glucose (PG), triglyceride (PTG), aspartate aminotransferase (AST), alanine aminotransferase (ALT), cholesterol (PC), total bilirubin (PTB), direct bilirubin (PDB) and indirect bilirubin (PIB) using Randox test kit (Randox Laboratories Ltd, UK).

Haematological analysis

For haematological study, the following parameters were evaluated according to the methods of Dacie and Lewis (1984): packed cell volume (PCV), white blood cells (WBC), neutrophil (N), eosinophil (E), lymphocyte (L) and monocyte (M).

Histopathological study

The histopathological study of kidney, liver, spleen and heart removed from the experimental animals were carried out according to the method of Godkar and Godkar (2003). Samples were embedded in paraplast wax after fixation with 10 % formalin, dehydration with methanol and clearing with xylene. Sections were made at 5 μm, stained with hematoxylin and eosin, and studied under the light microscope.

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Statistical analysis

Experimental values were expressed as mean ± Standard Error of Mean (SEM). Statistical analysis of the results was carried out by one way analysis of variance with the Statistical Package for the Social Sciences (SPSS) version 15.0 (SPSS Inc., Chicago, IL, USA). Test for statistical significance was carried out at 95% confidence limit.

Results

Acute toxicity study

Behavioral pattern, body and organ weights

Behavioural observation of the rats after one hour of dosing showed a drowsy effect on all the rats dosed with plant extract compared with control. Table 1 shows the body weights of rats in all groups. The body weights of rats increased progressively throughout the duration of the experiment. There were no significant changes in the relative liver and heart weights of the rats at all treatment doses. However, there were significant decreases in relative kidney and spleen weights in rats at all extract doses (Table 2). No mortality was recorded in any group throughout the duration of the experiment.

Table 1: Body weights of rats in acute toxicity study

Groups	Start (g)	Week 1 (g)	Week 2 (g)
Normal control	127.00±3.0	129.00±4.0	131.67±4.2
2000 mg/kg	135.00±1.0	138.00±2.5	140.33±3.2
4000 mg/kg	128.00±1.7	131.00±1.0	133.33±1.5
5000 mg/kg	131.67±7.2	135.33±8.5	137.00±9.6

Values are presented as mean ± SEM; % increase represents percentage increase in body weight.

Table 2: Relative organ weights of rats in acute toxicity study

Groups	Liver	Heart	Kidney	Spleen
Normal control	0.040±0.00	0.005±0.00	0.009±0.00	0.005±0.00
2000 mg/kg	0.040±0.00	0.004±0.00	0.006±0.00*	0.003±0.00*
4000 mg/kg	0.040±0.02	0.005±0.00	0.006±0.00*	0.003±0.00*
5000 mg/kg	0.050±0.01	0.005±0.00	0.006±0.00*	0.002±0.00*

Values are presented as mean ± SEM. Values with * are significantly different at P <0.05 compared with control.

Biochemical effect

There were significant (p <0.05) changes in the biochemical markers with significant increases in uric acids (PUA), urea (PU), creatinine (PCT), triglyceride (PTG), glucose (PG), cholesterol (PC), concentrations and Aspartate aminotransferases activity (AST) in rats treated with the highest dose of 5000 mg/kg bwt. However, an insignificant increase was observed with the activity of alkaline aminotransferases (ALT) and an insignificant decrease in the concentration of total plasma protein (PTP) as extract doses increased (Table 3).

Table 3: Biochemical parameters of acute toxicity study in rats

Groups	Normal control	2000 mg/kg	4000 mg/kg	5000 mg/kg
PUA (*mg/dl*)	7.24±1.70	9.04±1.56	14.80±1.74	54.57±28.20*
PU (*mg/dl*)	144.90±4.65	122.09±3.55	136.85±4.65	461.53±46.79*
PCT (*mg/dl*)	0.80±0.06	0.62±0.05	0.73±0.04	1.79±0.21*
PTG (*mg/dl*)	117.11±16.84	100.89±7.42	104.12±5.63	188.20±13.43*
AST (*U/I*)	11.47±2.37	11.67±2.10	40.89±4.07	97.77±23.53*
ALT (*U/I*)	84.58±6.07	87.11±7.26	93.53±13.11	161.78±64.68
PG (*mg/dl*)	131.89±1.73	274.17±16.65*	309.17±58.33*	412.50±52.20*
PTP (*g/dl*)	5.65±0.69	5.43±0.94	4.93±0.14	4.06±0.59
PC (*mg/dl*)	78.43±5.49	55.69±3.06*	90.90±3.06*	131.65±12.02*

Values are presented as mean±SEM of five replicates. Values with * are significantly different at P<0.05 compared with control. PUA, PU, PCT, PTG, AST, ALT, PG, PTP and PC represent Plasma uric acid, plasma urea, plasma creatinine, plasma triglycerides, aspartate aminotransferases, alanine aminotransferases, plasma glucose, plasma total protein and plasma cholesterol respectively.

Haematological effect

There were insignificant decreases in white blood cells (WBC), packed cell volume (PCV) and Neutrophil (N) counts, but general increase in lymphocyte counts in the rats as dosage increased (Table 4).

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Table 4: Haematological parameters of acute toxicity study in rats

Groups	PCV ($\times10^{12}$/L)	WBC ($\times10^{12}$/L)	sL (%)	N (%)
Normal control	39.00±1.00	6.90±0.50	45.00±7.64	55.00±7.64
2000 mg/kg	31.33±1.76	4.27±0.81	75.67±3.38	30.67±1.76
4000 mg/kg	30.00±6.08	3.87±0.63	75.33±3.18	28.67±3.18
5000 mg/kg	20.33±2.60	5.63±1.19	79.67±1.45	21.67±6.06

Values are presented as mean±SEM of five replicates. Packed cell volume (PCV), White blood cell count (WBC), percentage lymphocyte (% L) and percentage neutrophil (% N). PCV, WBC, L and N represent pack cell volume, white blood cell, lymphocyte and neutrophil counts

Histopathological effect:

There were no obvious histopathological alterations or remarkable changes in the internal organs of all treated groups in comparison to the control, except in the liver. The liver showed hepatic fatty changes in rats treated with extract dose of 2000 mg/kg bwt and hepatic fatty congestion in rats treated with higher extract doses of 4000 and 6000 mg/kg bwt (Plate I).

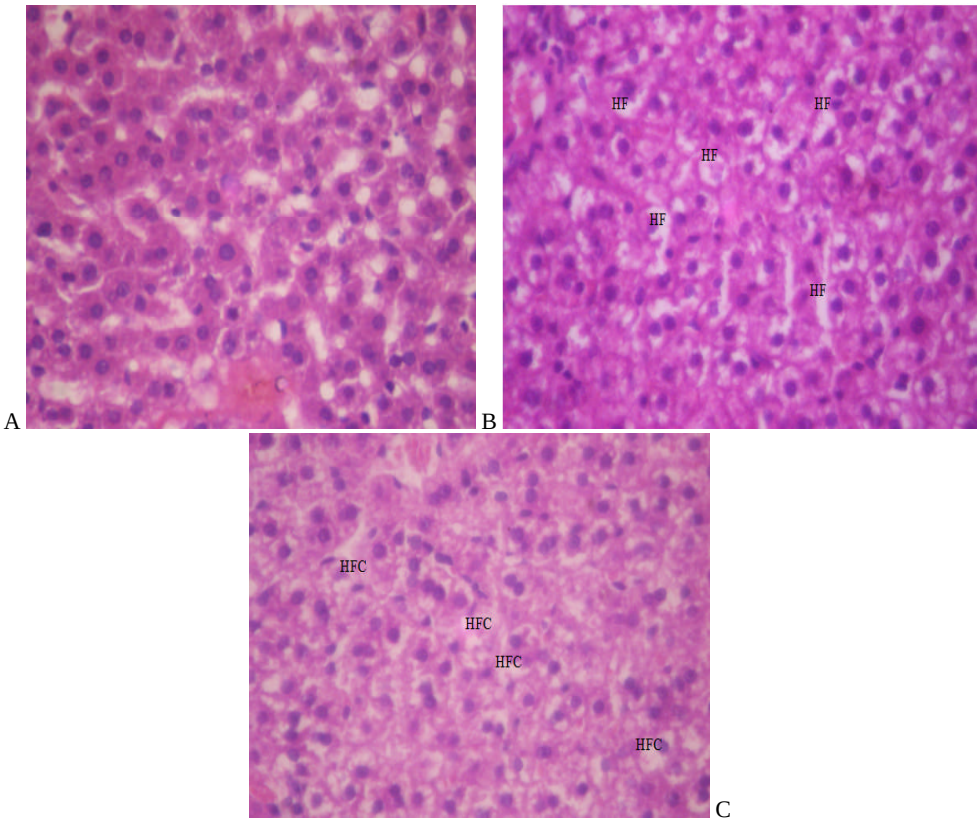

Plate I: Hematoxylin and Eosin-stained cross-sectional liver views. (A) Normal histology of control group; (B) Histology of group orally administered 2000 mg/kg of *C. bonduc* (single dose) showing arrow areas of hepatic fatty changes (HF); (C) Histology of group orally administered 5000 mg/kg of *C. bonduc* showing areas of hepatic fatty congestion (Magnification ×400).

Sub-acute toxicity study
Effect on body and organ weights

Body weights of all experimental animals increased progressively throughout the duration of the experiment (Table 5). No significant changes in the relative organ weights were noticed in rats treated with extract at dose of 200 mg/kg between compared with the control groups. However, there was significant increase in relative organ weights in rats treated with higher doses and in the recovery test groups (Table 6).

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Table 5: Sub-acute toxicity assessment on body weights of rats

Groups	Start (g)	s Week 1 (g)	Week 2 (g)	Week 3 (g)	Week 4 (g)
NC	100.40±32.48	119.40±27.73	122.80±27.52	133.2±25.87	146.80±29.79
VC	88.40±21.84	115.60±23.30	122.00±24.33	135.20±22.57	151.20±26.44
200 mg/kg	96.57±14.55	116.00±15.01	129.43±11.87	142.29±16.91	158.00±12.91
200R mg/kg	93.33±233.86	109.33±22.74	124.67±17.24	144.67±11.37	154.00±17.09
400 mg/kg	82.29±16.91	106.29±15.11	116.29±15.85	131.14±17.62	146.29±16.14
400R mg/kg	78.00±12.49	102.67±15.28	113.33±12.22	132.67±13.01	146.00±16.37
800 mg/kg	103.43±21.09	119.43±18.68	125.71±18.31	130.86±26.93	152.00±24.60
800R mg/kg	88.00±8.00	117.33±9.24	124.67±9.87	135.67±8.14	153.33±9.45
1600 mg/kg	73.67±23.85	94.33±22.43	106.67±23.96	113.67±20.10	125.00±20.27
1600R mg/kg	57.33±15.28	73.33±8.33	84.00±10.39	94.33±10.62	103.33±9.87

Values are presented as mean±SEM of five replicates. NC, VC and R represent normal control, vehicle control and recovery groups respectively.

Table 6: Sub acute toxicity assessment on relative organ weight of rats

Groups	Liver (g)	Heart (g)	Kidney (g)	Spleen (g)
N C	0.033±0.003	0.003±0.001	0.003±0.001	0.003±0.001
V C	0.031±0.003	0.003±0.000	0.003±0.001	0.003±0.001
200 mg/kg	0.035±0.005	0.003±0.001	0.003±0.001	0.003±0.001
200R mg/kg	0.031±0.080	0.003±0.000	0.003±0.001	0.004±0.001
400 mg/kg	0.036±0.0057	0.003±0.0002	0.003±0.0003	0.004±0.000
400R mg/kg	0.033±0.0027	0.003±0.0003	0.003±0.0009	0.004±0.000
800 mg/kg	0.043±0.0089*	0.004±0.0008*	0.004±0.0016*	0.006±0.001*
800R mg/kg	0.049±0.0018*	0.004±0.0003*	0.004±0.0004*	0.005±0.001*
1600 mg/kg	0.049± 0.0010*	0.004±0.0003*	0.004±0.0001*	0.005±0.001*
1600R mg/kg	0.054±0.0003*	0.004±0.0005*	0.004±0.0002*	0.005±0.001*

Values are presented as mean±SEM of five replicates. NC, VC and R represent normal control, vehicle control and recovery groups respectively. Values with * are significantly different at $P<0.05$ compared with normal control

Biochemical effect

Table 7 shows that there was insignificant differences in the concentrations of ALT, AST, PTB, PDB, PIB in rats treated with extract dose of 200 mg/kg body weight in comparison with normal control rats. However, there were significant ($p< 0.05$) increases in the activities of ALT and AST and the concentrations of PTB and PIB at higher extract doses. There was no significant effect on PDB as extract doses increased in comparison with normal control. Table VIII shows that there were insignificant changes in the concentrations of PU, PCT and PTG at extract dose of 200 mg/kg body weight in comparison with control rats, but there was significant increase in PUA. Nevertheless, there were significant increases in the concentrations of PUA, PU and PTG, but insignificant increase in the concentration of PCT in rats treated with higher extract doses of 400, 800 and 1600 mg/kg between in comparison with rats treated with distilled water (control) and with low extract dose of 200 mg/kg between.

Table 7: Sub acute toxicity assessment on liver function markers of rats

Groups	ALT (U/I)	AST (U/I)	PTB (mg/dl)s	PDB (mg/dl)	PIB (mg/dl)
NC	243.06±23.68	21.19±2.80	0.17±0.04	0.09±0.01	0.08±0.03
VC	246.46±51.04	25.08±0.58	0.14±0.02	0.07±0.02	0.07±0.00
200 mg/kg	242.86±44.51	25.67±1.16	0.17±0.03	0.07±0.03	0.10±0.02
200R mg/kg	247.60±28.86	28.00±1.01	0.19±0.03	0.10±0.03	0.09±0.05
400 mg/kg	334.83±11.69*^{c}	32.67±1.78*	0.24±0.02*^{c}	0.05±0.01	0.19±0.03*^{c}
400R mg/kg	323.17±12.63*^{c}	34.22±3.39*	0.22±0.04*^{c}	0.06±0.01	0.16±0.05*^{c}
800 mg/kg	320.83±19.75*^{c}	33.25±0.58*	0.27±0.03*	0.11±0.03	0.16±0.04*^{c}
800R mg/kg	310.92±20.32*^{c}	33.06±1.56*	0.25±0.03*^{c}	0.11±0.00	0.14±0.03*^{c}
1600 mg/kg	320.06±13.14*^{c}	31.31±2.45*	0.29±0.03*^{c}	0.11±0.04	0.18±0.03*
1600R mg/kg	318.31±20.09*^{c}	32.96±0.17*	0.30±0.04*^{c}	0.09±0.03	0.21±0.06*

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Values are presented as mean±SEM of five replicates. NC, VC and R represent normal control, vehicle control and recovery groups respectively. Values with * and superscript c '[c]' are significantly different at $P<0.05$ compared with normal control group and 200 mg/kg group respectively.

Table 8: Sub acute toxicity assessment on kidney function markers of rats

Groups	PUA (mg/dl)	PU (mg/dl)	PCT (mg/dl)	PTG (mg/dl)
NC	0.39±0.03[c]	93.92±7.10	1.08±0.06	64.18±3.46
VC	1.50±0.17[c]	84.53±4.03	0.88±0.10	72.42±3.16
200 mg/kg	4.56±0.40*	85.87±14.20	0.94±0.09	71.95±17.22
200R mg/kg	4.60±0.35*	83.18±11.70	0.89±0.26	75.01±26.17
400 mg/kg	6.89±0.99*[c]	112.70±12.30*[c]	1.08±0.05	116.90±30.35*[c]
400R mg/kg	9.00±0.35*[c]	117.33±7.10*[c]	1.15±0.01	123.01±4.37*[c]
800 mg/kg	8.56±0.95*[c]	120.70±4.65*[c]	1.07±0.16	144.13±37.97*[c]
800R mg/kg	11.60±0.40*[c]	118.07±2.68*[c]	1.10±0.03	149.26±8.59*[c]
1600 mg/kg	10.00±1.39*[c]	185.15±44.82*[c]	1.23±0.29	154.46±42.42*[c]
1600R mg/kg	13.60±0.80*[c]	187.83±9.67*[c]	1.16±0.05	141.59±3.86*[c]

Values are presented as mean±SEM of five replicates. NC, VC and R represent normal control, vehicle control and recovery groups respectively. Values with * and superscript c '[c]' are significantly different at $P<0.05$ compared with normal control group and 200 mg/kg group respectively.

The results also show that there were insignificant differences in the concentrations of PTP, PC and PG in rats treated with extract dose of 200 mg/kg between in comparison with normal control rats (Table IX). On the contrary, there was significant increase in the concentration of PC and significant decrease in the concentration of PTP in rats treated with higher extract doses of 800 and 1600 mg/kg between in comparison with normal control rats (Table 9). It is worthy to note that the levels of glucose (PG), direct bilirubin (PDB) and creatinine (PCT) were not significantly changed in rats treated with extract doses of 400, 800, 1600 mg/kg.

Table 9: Sub acute toxicity assessment on other biochemical markers of rats

Groups	PTP (g/dl)	PG (mg/dl)	PC (mg/dl)
NC	9.04±1.45	354.87±5.96	54.44±4.04
VC	9.56±2.16	365.00±1.92	50.64±10.15
200 mg/kg	8.62±1.70	222.82±55.34	60.53±1.08
200R mg/kg	8.38±0.13	183.61±10.97	66.27±3.74
400 mg/kg	7.45±0.63	318.97±41.43	70.35±4.09
400R mg/kg	7.67±0.15	292.22±14.19	70.59±13.58
800 mg/kg	6.71±0.41*	322.59±58.85	77.71±4.09*[c]
800R mg/kg	6.72±0.07*	318.06±126.35	79.12±4.13*[c]
1600 mg/kg	6.55±0.48*	330.17±12.14	85.89±7.08*[c]
1600R mg/kg	6.51±0.41*	335.17±12.14	81.37±8.22*[c]

Values are presented as mean±SEM of five replicates. NC, VC and R represent normal control, vehicle control and recovery groups respectively. Values with * and superscript c '[c]' are significantly different at $P<0.05$ compared with normal control group and 200 mg/kg group respectively.

It was also observed that there was no significant change in the biochemical parameters of the rats treated with various extract doses in the test groups compared with the rats in the recovery test groups (Tables 7, 8 & 9).

Haematological effect

There was no significant change in the number of white blood cells and in neutrophils, lymphocytes, monocytes counts as well as the packed cell volume in the rats treated with extract dose of 200 mg/kg bwt compared with control. However, there were significant decreases in white blood cells (WBC) and neutrophil (N) counts as well as a significant ($P<0.05$) increase in lymphocyte (L) counts. There was a general decrease in packed cell volume (PCV) and monocyte (M) counts in rats treated with high extract doses of 400, 800 and 1600 mg/kg between in comparison with rats treated with distil water (normal control) (Table 10).

Histopathological effect

Compared with the rats in the control groups, no remarkable changes on the morphology of the experimental rats treated with low extract dose of 200 mg/kg bwt were noticed on gross examinations. The histological section of the heart and spleen in all groups showed preserved normal architecture of their cellular components. On the other hand, liver of rats treated with high extract doses of 400 mg/kg bwt and above showed noticeable cellular alterations such as the presence of lipid-filled hepatocytes (hepatic fatty changes) in rats treated with extract dose of 400 mg/kg bwt (Plate IIB), prominent hepatic sinusoids which are engorged with red blood cells (sinusoidal congestion), central hepatic venous congestion which are

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engorged with fatty vacuoles (fatty congestion) in rats treated with extract dose of 800 mg/kg bwt (Plate IIC), and aggregation of dead cells, inflammatory cells and amorphous debris (hepatic fatty necrosis) in rats treated with extract dose of 1600 mg/kg bwt (Plate IID).

Table 10: Sub acute toxicity assessment on haematological markers of rats

Groups	PCV ($\times10^{12}$/L)	WBC ($\times10^{12}$/L)	L (%)	N (%)	M (%)
NC	38.33±5.36	9.63±1.72	45.67±2.91	53.00±3.2	1.67±0.33
VC	37.50±6.50	10.00±2.00	42.00±4.00	57.00±3.00	2.00±0.00
200 mg/kg	42.33±4.70	9.13±3.08	47.67±2.60	51.00±2.65	1.97±0.33
200R mg/kg	41.00±1.15	7.03±0.83	46.33±5.93	48.67±4.10	1.67±0.33
400 mg/kg	38.67±1.45	6.47±0.08	64.33±12.25*^{c}	35.67±12.25*	1.63±0.33
400R mg/kg	38.00±1.15	4.37±1.28*^{c}	60.00±5.77*^{c}	40.00±2.89*^{c}	1.33±0.33
800 mg/kg	37.33±2.60	5.87±1.52*^{c}	64.33±11.05*	39.33±11.46*^{c}	1.73±1.33
800R mg/kg	34.00±0.58	3.47±0.49*^{c}	63.33±6.01*^{c}	35.00±5.77*^{c}	0.67±0.67
1600 mg/kg	31.00±3.21	4.23±0.55*^{c}	74.33±2.96*^{c}	25.67±2.96*^{c}	0.38±0.33
1600R mg/kg	32.67±2.03	4.80±0.59*^{c}	70.33±5.78*^{c}	29.00±1.15*^{c}	0.42±0.33

Values are presented as mean±SEM of five replicates. NC, VC and R represent normal control, vehicle control and recovery groups respectively. Values with * and superscript c '^{c}' are significantly different at $P<0.05$ compared with normal control group and 200 mg/kg group respectively.

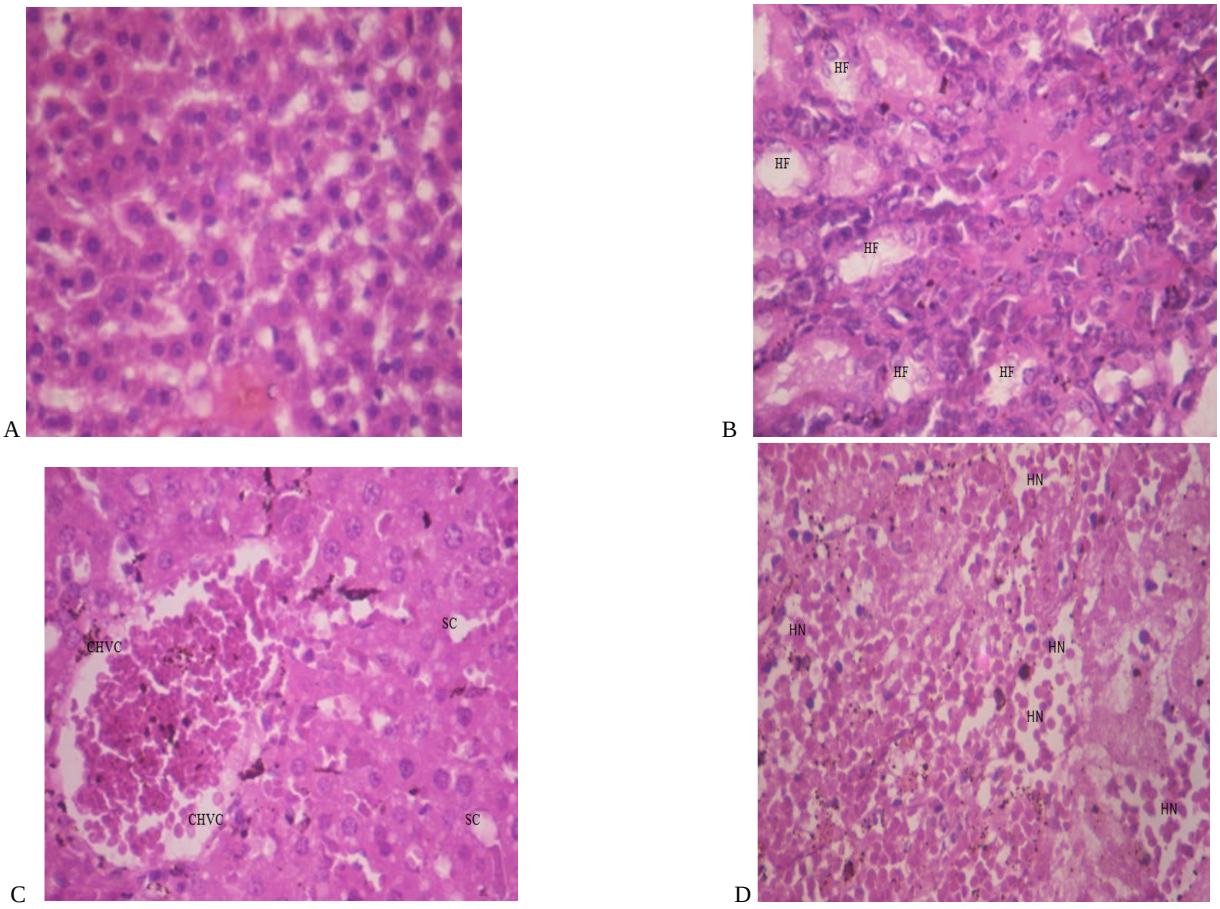

Plate II: Hematoxylin and Eosin-stained cross-sectional liver views
(A) Normal histology of control group. (B) Histology of a group orally administered 400 mg/kg of *C. bonduc* for 28 days showing areas of gross hepatic fatty changes (HF), (C) Histology of a group orally administered 800 mg/kg of *C. bonduc* for 28 days showing areas of central hepatic venous congestion (CHVC) and sinusoidal congestion (SC), (D) Histology of a group orally administered 1600 mg/kg of *C. bonduc* for 28 days showing areas of hepatic necrosis (HN) (Magnification ×400).

A gross examination of the kidney showed kidney tubular necrosis and glomerular congestion (gross dilatation, engorgement or distension of blood vessels) in rats at high extract doses of 400, 800 and 1600 mg/kg bwt (Plate IIIB).

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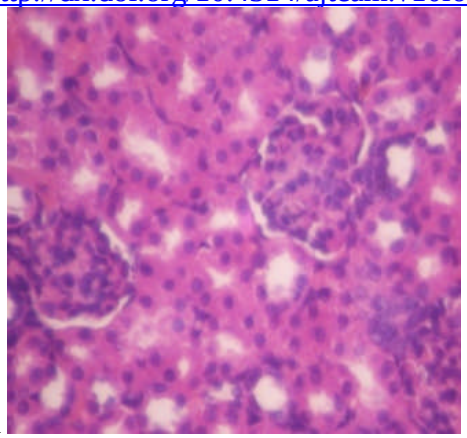
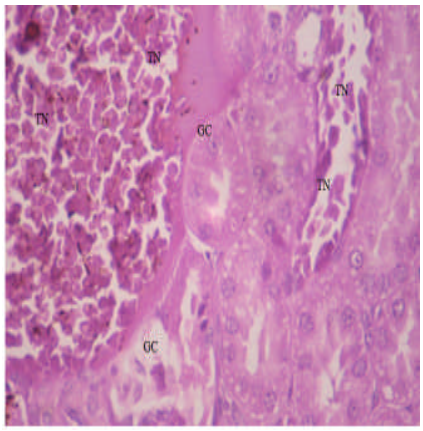
A  B 

Plate III: Hematoxylin and Eosin-stained cross-sectional kidney views
(A) Normal histology of control group. (B) Histology of a group orally administered 1600 mg/kg of *C. bonduc* for 28 days showing areas of tubular necrosis (TN) and glomerular congestion (GC) (Magnification ×400).

Discussion

In relation to the parameters tested in this study, it appears that treatment with low doses of 200 mg/kg body weight of the ethanolic extract of *C. bonduc* was not toxic to the rats. This is in agreement with other published works (Kumar *et al*., 2005; Sagar and Vidyasagar, 2010). However, higher extract treatment doses of 400 to 1600 mg/kg bwt were toxic to the experimental animals as there were significant increases in relative organ weights (Table VI) as well as in the activities of alanine and aspartate aminotransferases (Table VII), and in the concentrations of plasma total bilirubin, plasma indirect bilirubin, plasma uric acid, plasma urea, plasma triglyceride and plasma cholesterol (Tables VII, VIII & IX). This also is in agreement with published works (Gupta *et al*., 2004; Kumar *et al*., 2005; Sagar and Vidyasagar, 2010).

Feron *et al*. (1973) reported that in sub-acute toxicity experiments, the relative organ weight is a useful index of toxicity. Monitoring the body weight during treatment provides an index of the general health status of the animals. Such information may be important for gauging their health (Sharma *et al*., 2009). There was a progressive increase in the body weight of all animals throughout the duration of the experiment. So, it could be suggested that the treated rats were generally in good health despite the toxic effects of the extract. At the high treatment doses, there was a general increase in the relative organ weights of tested rats compared to the control (Tables VI). It could be suggested that the extract at these high doses induced toxic effects on some of the tested organs. The liver, being the detoxification organ of mammals, and the kidney, being the most important excretory organ in the body, might be susceptible to the toxic effects of extracts of *C. bonduc* at higher doses. This is supported by the report of Kumar *et al*. (2005), who reported the associated toxicity of anticancer agents and plants rich in flavonoids on the liver and kidney.

The depletion in the total plasma protein (TPP) level observed in the present assessment (Table IX) may be due to impaired protein synthesis in the damaged liver or to the altered nutritional status of the animals (Sharma *et al.*, 2009). It has been reported that the synthesis of plasma proteins in the liver was quantitatively and qualitatively affected during liver damage (Kumar *et al*., 2005). The decrease in plasma albumin and in the total plasma protein indicated hepatocellular dysfunction or liver disease (Kumar *et al.*, 2005). Elevated levels of ALT and AST observed in this study (Table VII) may be due to pathological changes such as necrosis of hepatocytes, which caused an increase in the permeability of the cell membranes, resulting in the release of aminotransferases into the blood stream (Ali *et al*., 2008). ALT has been reported to be a marker with a high specificity for acute hepatocellular injury (Friedman *et al.*, 1996).

The significant high level of plasma cholesterol observed in rats at higher extract doses (Table IX) might be an indication of obstructive jaundice or chronic hepatitis (Albrink *et al*., 1950). The significant increase in plasma urea concentration at high extract doses (Table VIII) may be as a result of nephrotoxicity. Since the kidney eliminates the waste products of metabolism from the body, an elevated plasma urea level has been linked to elevated non-protein nitrogen in diseases associated with nephrotoxicity (Varley *et al*., 1980). In renal failure, there is an increase in metabolic waste products, especially nitrogenous substances like plasma urea and uric acid. In addition, the significant increase in plasma triglyceride in the rats at higher extract doses (Table VIII) may also be a useful biomarker in the prediction of renal dysfunction (Muntner *et al*., 2000). Nevertheless, plasma creatinine concentration, the least variable nitrogenous constituent of the blood, was found to be insignificant (Table VIII). Contrarily, increased plasma creatinine has been reported in renal injury subsequent to trauma or anuria in traumatic injuries to the muscle and in muscular dystrophy (Srisawat *et. al*., 2010). The findings in this study corroborate the report of Gupta *et al*. (2004) and Kumar *et al*. (2005) on the 14 days and 12 weeks intraperitoneal toxicity study of the methanolic extract of *C. bonduc* respectively, with a significant increase in plasma urea and enzymes (ALT and AST) in the experimental animals at high extract doses.

Blood is a sensitive index of the physiological changes in response to any environmental pollutant in an animal. It has been documented that toxicant or potentially toxic substances induce conspicuous and significant changes in the haematological parameters (Jain *et al*., 2009). The significant decreases in the neutrophil and white blood cell counts and an increase in the lymphocyte count at high *C. bonduc* extract doses (Table X) were in agreement with the findings of Gupta *et al*. (2004), Kumar *et al*. (2005) and Sagar and Vidyasagar (2010).

Kramp *et al*. (1974) opined that functional studies in toxicology should be coupled with the appropriate histological studies because appropriate morphological studies are useful, especially during the anatomical localization of the action of a toxin. Based on this, the results of histopathological study of the effect of ethanolic extract of *C. bonduc* on organ of the rats were important. It was observed that there were gross alterations in the cellular architecture of the liver and the kidney at higher extract doses (Plates II & III). These histopathological damages are probably responsible for the alterations in the biochemical and haematological markers of the liver and kidney functions.

Histopathological biomarkers are indices of induced organ toxicity or injury. This finding corroborates the reports of Kumar *et al*. (2005) and Sagar and Vidyasagar (2010) who observed changes in cellular architecture of the liver of animals treated with 400 and 250 mg/kg between of a

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methanolic and ethyl acetate extracts of *C. bonduc* respectively. On the other hand, the methanolic extract of *C. bonduc* seeds orally administered to mice at a dose of 400 mg/kg body weight for 28 days has been reported to be non-toxic to the experimental animals (Pillaia and Suresh, 2011). The observed differences in the toxicity profiles of the leaves and young twigs of *C. bonduc* and its seeds could be due to the presence of some phytochemicals such as flavonoids that are dominant in the leaves of *C. bonduc*.

The lack of significant changes in the biochemical, haematological and histopathological parameters in the recovery groups (200R, 400R, 800R and 1600R mg/kg between) and the extract tested groups (Tables VI, VII, VIII & IX) may be due to the irreversibility in the effects of toxicity measured. It is therefore suggested that the induced alterations in rats caused by the high extract doses might be irreversible after two weeks of dosing.

The fact that there was no mortality in the experimental animals at all the extract treatment doses, indicates that concentrations used were not lethal but only toxic causing significant alterations in the biochemical and histopathological markers at high extract doses especially increases in cholesterol, glucose, uric acid, triglyceride, urea and creatinine concentrations, in aspartate aminotransferase levels and induced cellular damage in the liver of animals at higher doses (Table III; Plate I).

It can be concluded that the daily administration of crude ethanolic extract of *C. bonduc* at 400 mg/kg body weight and above, as well as the fixed dose of *C. bonduc* at 2000 mg/kg body weight is toxic to experimental animals, even though there were no observed mortality at all the tested doses. Consequently, extreme care should be taken concerning the concentration of the extracts of the plant to be used in the treatment of malaria.

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