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TOXICOLOGICAL EVALUATION OF THE STEM BARK OF *BURKEA AFRICANA* HOOK. (CAESALPINIACEAE) IN WISTAR RATS

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ABSTRACT

Burkea africana Hook. (Caesalpiniaceae) is used traditionally to treat ulcers, headaches, skin disease and tumors. The study investigated the acute, sub-acute and chronic toxicity profiles of the ethanolic extract of *Burkea africana* stem bark. Rats of either sexes were used in this study (n=10). For acute toxicity, a single dose of 5,000 mg/kg was administered while for the sub-acute and chronic toxicity study, three doses (40, 200 and 1000 mg/kg) of the extract were administered orally for 28 and 90 days respectively. At the end of each study, the biochemical, hematological and histological parameters were evaluated. No mortality or behavioral changes were observed in the acute toxicity study. Extract caused significant changes in the hematological parameters after the sub-acute toxicity study. In the chronic toxicity study, the extract caused significant increase in the white blood cell count of the 200 mg/kg group. There was significant increase in the platelet count of treated groups compared to control in the sub-acute and chronic toxicity studies, with an observed total mortality of all the animals in the 1000 mg/kg group on the 44th day. No adverse pathology was observed in the organs examined. The extract elicited a hematological response and short term consumption of the extract at low doses might be relatively safe. However, long term consumption at high doses should be discouraged.

INTRODUCTION

Herbal medicines are gaining considerable interest worldwide mainly due to the perception that herbal medicines are devoid of adverse effects and a cheaper alternative when compared with conventional orthodox medicines. There has been a rapid acceptance of herbal medicines in both developed and developing countries, with more people resorting to them for treatment of health challenges [1, 2]. Presently, about 25% of the world's medicines are derived from medicinal plants, as these plants offer unlimited resources for the discovery of new drugs. The use of medicinal plants in the treatment and management of diseases has currently been on the increase, with majority of the population especially in third world countries relying on herbal preparations as alternative medicine [3, 4]. As the global market for herbal products continues to grow with many new products introduced into the market, there has been corresponding increase in concerns and public health issues regarding the consumption of such herbal remedies. This increase in use and the professed safety of herbal drugs has necessitated scientists to investigate the toxicity profiles of herbal remedies.

Burkea africana Hook. (Caesalpiniaceae) is a medium sized, deciduous, flat topped tree that grows in Western and Southern Africa. Traditionally, the stem bark is used in the treatment of cough and colds in Senegal and for the relief of headaches, hard abscesses and stomach aches in Southern and Central Africa [5]. The hepatoprotective and anti-microbial activities of the plant have been investigated [6, 7]. Previous studies also revealed the presence of pro-anthocyanidins in the semi-polar fractions of the stem bark [8]. We have earlier reported the anti-proliferative activity and caspase dependent apoptosis of the ethanolic extract [9]. The aim of this study was to investigate the acute, sub-acute and chronic toxicity of the ethanolic extract of *B. africana* stem bark.

RESULTS AND DISCUSSION

Acute toxicity

After administration of the extract at 5000 mg/kg, there were no observed behavioural changes and no mortality was recorded after 7 days.

Body and organ weight measurement

B. africana extract caused a decrease in the body weight of animals across all treatment groups and was statistically significant in the 1000 mg/kg group after the sub-acute toxicity study and the 200 mg/kg group after the chronic toxicity study (Table 1). The extract also caused significant increase in the weight of the liver in the 1000 mg/kg group after the sub-acute toxicity study and significant increase in the weight of the spleen of both the 40 mg/kg and 200 mg/kg was observed after the chronic study (Table 2).

Table 1: Effect of *Burkea africana* stem bark extract on weights of animals in the sub-acute and chronic study

		Initial weight(g)	Final weight (g)	Difference in weight (g)
Sub-acute toxicity	Control	178. 17 ±17.14	171.45 ± 14.74	6.72
	40mg/kg	175.25 ± 21.54	167.67 ± 10.54	7.58
	200mg/kg	176.58 ± 17.80	163.89 ± 14.67	12.69
	1000mg/kg	172.50 ± 15.76	155.67 ± 14.15*	16.83
Chronic toxicity	Control	178. 17 ±17.14	173.33 ± 15.02	4.84
	40mg/kg	175.25 ± 21.54	160.83 ±16.00	14.42
	200mg/kg	176.58 ± 17.80	158.08 ± 16.80*	18.50
	1000mg/kg	172.50 ± 15.76	N.D	N.D

Values are expressed as mean ± S.D (n=10) *$p < 0.05$ significantly different from control (One-way ANOVA followed by Dunnett's test)

Key: N.D: Not done

Table 2: Effect of *Burkea africana* stem bark extract on organ weights (per 100 g body weight) in the sub-acute and chronic study.

	Organs	Control	40 mg/kg	200 mg/kg	1000 mg/kg
Sub- acute study	Heart	0.63 ± 0.06	0.52 ± 0.08	0.50 ± 0.07	0.63± 0.12
	Liver	5.67 ± 0.46	4.34± 0.73	4.44 ± 0.51	6.13 ± 0.06*
	Lungs	1.40 ± 0.17	1.42± 0.33	1.42 ± 0.35	1.33 ± 0.21
	Spleen	0.67 ± 0.12	0.60 ± 0.19	0.58 ± 0.13	0.6 ± 0.17
	Right kidney	0.60 ± 0.00	0.52 ± 0.08	0.54 ± 0.05	0.63 ± 0.06
	Left kidney	0.53 ± 0.06	0.48 ± 0.04	0.46 ± 0.05	0.60 ± 0.00
Chronic study	Heart	0.60 ± 0.06	0.73 ± 0.08	0.75 ± 0.18	N.D
	Liver	4.52 ± 0.74	4.98 ± 0.47	6.17 ± 1.78*	N.D
	Lungs	1.45 ± 0.19	1.57 ± 0.27	1.87 ± 0.49	N.D
	Spleen	0.38 ± 0.08	0.65 ± 0.19**	0.65 ± 0.14**	N.D
	Right kidney	0.48 ± 0.08	0.58 ± 0.04	0.57 ± 0.12	N.D
	Left kidney	0.48 ± 0.08	0.50 ± 0.06	0.56 ± 0.12	N.D

Values are expressed as mean ± S.D (n=10) *$p < 0.05$, **$p < 0.01$ significantly different from control (One-way ANOVA followed by Dunnett's test)

Key: N.D: Not done

Hematology

The extract had no adverse effect on the RBC and hemoglobin levels in the animals in both the 28th and 90th day study, although after the chronic toxicity study there was an observed significant ($p < 0.001$) increase in the level of WBC in the 200 mg/kg group (Table 3). There were also observed significant non-dose dependent increase in the MCV, MCH and decrease in the MCHC values after the sub-acute and chronic study. The platelet count across all groups in both the sub-acute and chronic toxicity studies was significantly higher than the control.

Table 3: Effect of *B. africana* on hematology parameters in rats after sub-acute and chronic toxicity study

Study	Parameter	Control	Dose (mg/kg) 40	200	1000
Sub-acute	WBC	5.06±0.9	7.30 ±3.3	5.57 ±1.0	4.68 ±1.1
	RBC (10^{12}/L)	7.49 ±1.2	7.77 ±0.81	7.51 ±0.85	8.36 ±0.80
	HCT %	36.28 ±4.5*	48.00 ±5.1*	38.30 ±2.4	46.42 ±9.9
	MCV (FL)	48.34 ±2.3	63.93 ±4.5***	46.30 ±1.6	54.47 ±6.1
	MCH (pg)	19.12 ±0.5	17.28 ±0.3***	18.15 ±0.4	18.10 ±0.9*
	MCHC (g/dL)	39.50 ±2.2	25.67 ±2.2***	37.28 ±5.1	33.54 ±5.8
	PLT (10^9/L)	470.67 ±47.6	781.33 ±76.3***	504.33 ±66.7	764.67±23.0***
	HGB (g/dL)	15.28 ±1.5	13.48 ±1.6	14.23 ±1.5	15.16 ±1.1
Chronic	WBC	5.64 ±1.19	6.53 ±0.85	9.96±0.16***	N.D
	RBC (10^{12}/L)	8.21±0.77	8.43 ±0.54	8.34 ±0.91	N.D
	HCT %	49.42 ±4.23	51.98 ±1.87	51.28 ±6.07	N.D
	MCV (FL)	60.3 ±1.96	61.87 ±2.90	62.46 ±1.14	N.D
	MCH (pg)	18.18 ±0.59	17.77 ±0.43	18.38 ±0.44	N.D
	MCHC (g/dL)	30.25 ±0.84	28.85 ±0.90*	29.98 ±1.09	N.D
	PLT (10^9/L)	755.5 ±29.56	852.75±11.30**	798.75 ±37.46	N.D
	HGB (g/dL)	14.95 ±1.07	15.02 ±0.77	15.37 ±1.49	N.D

Values are expressed as mean ± S.D (n=10) *p < 0.05 **p < 0.01
***p < 0.001 statistically significant using one way ANOVA and Dunnett's test
Key: N.D: Not done

Biochemical analysis

Effect of *B. africana* on the biochemical analysis estimated in the serum after 28 and 90 days is shown in Table 4. There was a significant ($p < 0.01$) decrease in the urea levels at 40 mg/kg and 200 mg/kg (31.33 ± 3.88 and 30.75 ± 0.96) when compared to the control (41.0 ± 5.79). Also a significant ($p<0.05$) decrease in creatinine levels was observed at 40 mg/kg and 200 mg/kg (1.07 ± 0.18 and 1.12 ± 0.13) when compared to the control (1.36 ± 0.11). There was no significant change observed on the liver function test for AST, ALP and ALT indicating that the extract had no adverse effect on the liver enzymes. The albumin, conjugated and direct bilirubin levels also were not statistically different. The extract however, caused a significant increase in the total protein levels at the 40 mg/kg and 200 mg/kg (8.20 ± 0.11 and 8.20 ± 0.12) groups when compared with the control (7.93 ± 0.12).

Table 4a: Effect of *B. africana* on serum biochemistry parameters in rats after sub-acute toxicity study

Study	Parameter	Control	Dose in mg/kg 40	200	1000
Sub-acute	Urea (mg/dl)	41.00 ± 5.79	31.33 ± 3.88**	30.75 ± 0.96**	35.40 ± 4.98
	Creatinine (mg/dl)	1.36 ±0.11	1.07 ±0.18*	1.12 ±0.13*	1.22 ± 0.15
	Cholesterol (mg/dl)	150.00 ±6.96	118.80 ±3.42***	113.00 ±4.00****	132.75±19.00*
	HDL (mg/dl)	48.67 ±2.42	29.83 ±3.71****	25.75±1.71****	39.50± 11.70
	LDL (mg/dl)	77.75 ±4.99	74.00 ±2.45	69.00 ±2.92*	71.33 ±4.73
	Triglyceride (mg/dl)	72.75 ±12.45	73.75 ±11.93	96.33 ±2.52*	97.25 ±9.64*
	ALP (µ/L)	53.83 ±4.17	53.33 ±4.59	54.20 ±4.55	54.20 ±4.55
	AST (µ/L)	48.83 ±0.41	48.83 ±0.41	48.80 ±0.45	48.60 ±0.55
	ALT (µ/L)	20.67 ±1.97	22.60 ±1.67	22.60 ±2.19	22.60 ±1.67
	Total bilirubin (mg/dl)	0.92 ±0.08	0.80 ±0.06	0.86 ±0.11	0.82 ±0.08
	Conjugated bilirubin (mg/dl)	0.52 ±0.08	0.45 ±0.05	0.50 ±0.10	0.50 ±0.07
	Total protein (mg/dl)	7.93 ±0.12	8.2 ±0.11**	8.20 ±0.12**	8.10 ±0.07
	Albumin (mg/dl)	4.02 ±0.12	4.1 ±0.21	3.92 ±0.19	4.24 ±0.11

Values are expressed as mean ± S.D (n=10)
*p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.001 statistically significant using one way ANOVA and Dunnett's test

Table 4b: Effect of *B. africana* on serum biochemistry parameters in rats after chronic toxicity study

Study	Parameter	Dose in mg/kg Control	40	200	1000
Chronic	Urea (mg/dl)	28.00 ±3.58	25.80 ±3.43	27.50 ±3.83	N.D
	Creatinine (mg/dl)	1.00 ±0.21	0.88 ±0.19	1.03 ±0.20	N.D
	Cholesterol (mg/dl)	90.40 ±6.02	103.50 ±3.02**	102.00 ±14.46*	N.D
	HDL (mg/dl)	30.00 ±2.00	33.17 ±2.23	35.33 ±5.20*	N.D
	LDL (mg/dl)	46.20 ±4.07	55.00 ±4.26	44.20 ±10.14	N.D
	Triglyceride (mg/dl)	63.40 ±10.38	83.50±10.13***	90.40±5.98****	N.D
	ALP (µ/L)	51.67 ±0.82	51.50 ±0.84	52.00 ±0.00	N.D
	AST (µ/L)	44.00 ±5.73	46.80 ±5.62	43.17 ±4.58	N.D
	ALT (µ/L)	20.67 ±5.23	21.80 ±4.89	19.80 ±4.46	N.D
	Total bilirubin (mg/dl)	0.77 ±0.08	0.72 ±0.08	0.70 ±0.09	N.D
	Conjugated bilirubin (mg/dl)	0.43 ±0.05	0.40 ±0.06	0.42 ±0.08	N.D
	Total protein (mg/dl)	8.10 ±0.68	8.80 ±0.21*	8.27 ±0.45	N.D
	Albumin (mg/dl)	3.87 ±0.25	4.10 ±0.18	3.95 ±0.10	N.D

Values are expressed as mean ± S.D (n=10)
*p < 0.05
**p < 0.01
***p < 0.001
****p < 0.001 statistically significant using one way ANOVA and Dunnett's test
Key: N.D: Not done

Histology

After microscopic evaluation of the liver, kidney, heart and lungs, no organ damage was observed after treatment with *B. africana* extract at all doses as shown in Figure 1.

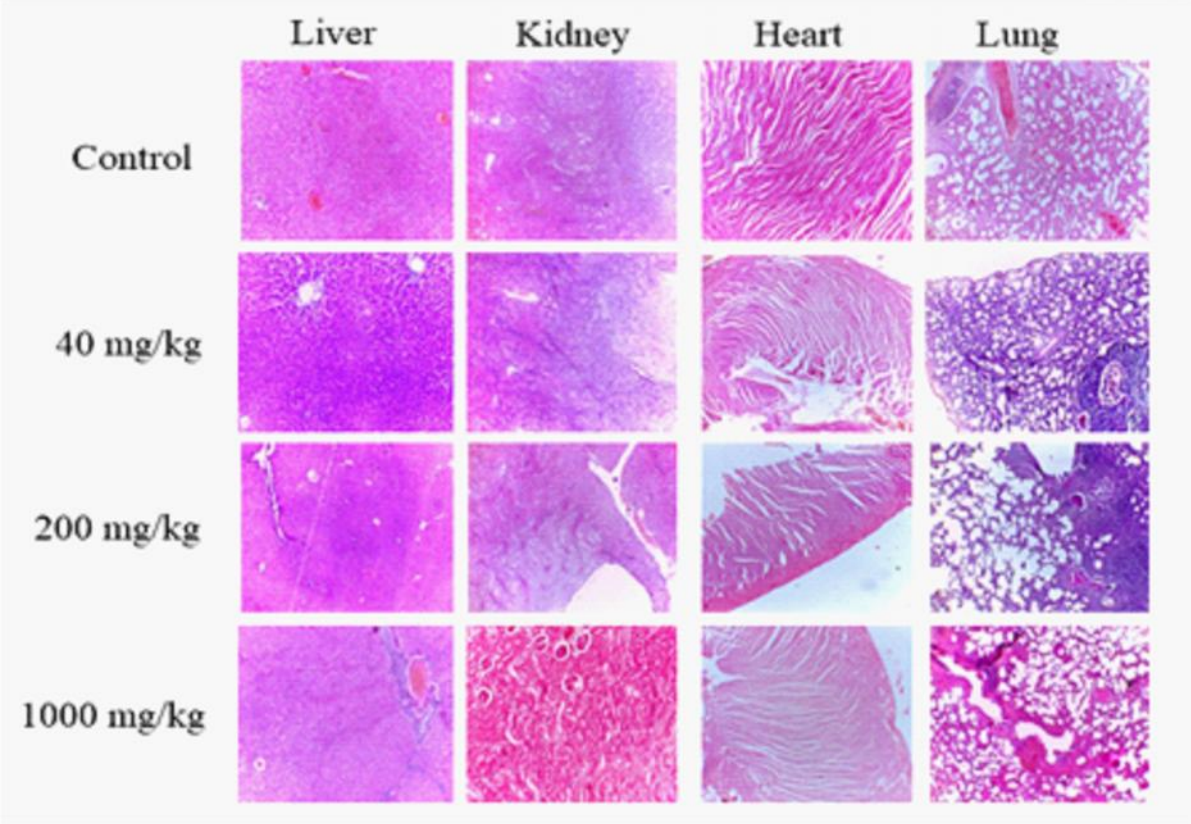

Figure 1

DISCUSSION

Medicinal plants have been widely adapted for therapeutic use in the treatment of various diseases worldwide [11, 12]. The first step to approve the use of a medicinal plant in treatment is an evaluation of the toxicity potential, as toxicity testing provides information on the safety of herbal products [13]. The acute toxicity of *Burkea africana* was done at a single dose of 5000 mg/kg to check for immediate adverse effects. After close observation for 24 hours and 7 days, there were no observed changes in the behaviour of the animals and no mortality was recorded.

Changes in animal and organ weights are critical in toxicity studies, as these changes may indicate acute injury, physiological perturbations and enzyme stimulations [4, 14]. Significant reduction of these two parameters serves as a sensitivity index of toxicity [13] After the sub-acute and chronic toxicity studies, there was an observed decrease in the weight of animals across all treatment groups when compared to the control, this difference was statistically significant at in the 1000 mg/kg group after the sub-acute toxicity study and the 200 mg/kg group after the chronic toxicity study. Similar observations have reported weight loss induced by the administration of *Alstonia scholaris* stem bark extract and *Annona muricata* leaf extract in Wistar albino rats [15-16]. The decrease observed was also noticed in the control group although it was not statistically significant and may be due to differences in food consumption. There was an observed statistically significant increase in the weight of the liver in the 1000 mg/kg group when compared to the control in the sub-acute toxicity study. An increase in the liver weight of the 200 mg/kg group was also statistically significant when compared to the control in the chronic study; the extract also caused significant increase in the weight of the spleen in the 40 mg/kg and 200 mg/kg group. However, it should be noted that no animal from the 1000 mg/kg group survived till the last day of this study, as 100% mortality was recorded in this group at day 44.

Assessments of hematological parameters were evaluated to obtain further information on the health status that may not have been visible during physical examination of the organs [13]. The hematopoietic system is very sensitive to the ingestion of toxic substances and its evaluation provides information about bone marrow activity, intravascular effects as well as revealing abnormalities in the body's metabolic processes [17-18]. There were no significant differences observed in the red blood cell (RBC) and hemoglobin (HGB) in both the sub-acute and chronic toxicity studies. This indicates that the extract does not affect RBC morphology and formation.

MCV, MCH and MCHC values are used in identifying anaemia or potential preliminary immunotoxicity caused by folate deficiency and liver disease. Increase in MCV, MCH and MCHC values are indicators of red blood cell swellings and could be a response to stress related conditions, while observed decrease usually might be the result of the release of young erythrocytes containing low hemoglobin into circulation [19, 20]. The significant increase and decrease observed in MCV, MCH and MCHC parameters indicates that the extract may interfere with the normal hemoglobin production and may have the potential to induce anemia [21].

Examination of WBC is used to assess the effect of test substances on the immune systems [18]. Increase in white blood cell (WBC) in animals indicates that the immune system has been compromised eliciting an immune response in the treated animal (leucocytosis), while a decrease in WBC indicates a decline in the animal's ability to fight off infections (leucopenia) [22, 23]. The ethanolic extract of the stem bark of *B. africana* did not affect the production of WBC in the sub-acute toxicity studies, although a statistically significant increase in the WBC count was observed after the chronic toxicity studies in the 200 mg/kg group. This observed increase implies that the extract elicited an immune response after chronic administration. Further research needs to be done to investigate the underlying cause of this increase.

Thrombocytosis is an abnormal increase in the number of circulating platelets [12, 24]. The plant extract caused statistically significant increase in the platelet count after both the sub-acute and chronic toxicity studies across all treatment groups. This increase observed may be due to underlying secondary infections in the body or the result of a bone marrow deficiency [25, 26]. The observed increase in the platelet count was confirmed by blood clots found during the autopsy of animals in the 1000 mg/kg group that died before the completion of the 90 day study. Although no blood clot was observed on the completion of the chronic study in the animals from the 40 and 200 mg/kg group. This observed increase is similar to the findings in literature [27] where treatment of Wistar rats with vinca alkaloids led to elevated platelet counts. Prolonged treatment with cytotoxic drugs is often accompanied by bone marrow depression with peripheral cytopenia [28, 29]. Therefore, caution needs to be applied when administering *B. africana* for a prolonged period.

Liver and kidney function tests are used to evaluate the effect of a drug on the hepatic and renal function of the animals as elevated levels of the enzymes in the serum produced by the liver and nitrogenous wastes to be excreted by the kidney might be an indication of tissue necrosis [24]. The liver is one of the most important organs involved in the body's metabolism as it is the first point of call for every ingested substance and any abnormal change in its enzymes would affect metabolism completely [4, 11, 12]. The enzymes investigated in this study include alanine aminotransferase (ALT), alkaline phosphatase (ALP), aspartate aminotransferase (AST), direct and conjugated bilirubin and albumin. Injury to the liver cells (hepatocytes) lead to increase of these enzymes in the blood stream [30]. ALT is one of the most specific enzyme biomarker in liver function tests as it is mainly found in hepatocytes as against AST that is found in the muscle of the heart, kidney and brain tissues. The sub-acute and chronic administration of the ethanolic extract of *B africana* did not cause any significant change in any of the liver function enzymes, suggesting that the extract does not have any toxic effect on the liver.

The kidney is involved in the excretion and removal of waste substances from the blood stream. Increase in serum creatinine and urea shows the inability of the kidney to properly dispose of the waste materials from the blood stream indicating organ malfunction, as increased values of creatinine and urea are the most common cause of acute and chronic renal failure [31, 32]. After the sub-acute study, the extract significantly lowered urea

levels in the 40 mg/kg and 200 mg/kg group, although the observed decrease in the creatinine and urea levels was not significant after the chronic toxicity studies. The results indicate that the extract does not have any adverse effect on the kidney.

Histological assessment of major organs was carried out to observe for any deleterious damage to the organs (liver, kidney, heart and lungs). No inflammation, hemorrhage, fluid accumulation or morphological damage was observed in any of the treated animal organs when compared with the control, indicating that oral administration of *B. africana* extract after 90 days has no harmful effect on the organs morphologically.

CONCLUSION

Overall, the sub-acute and chronic toxicity studies of the ethanolic extract of *Burkea africana*, showed no signs of alterations and injury on the examined organs. There were no observed negative effects on the renal and hepatic functions. However, due to the significant increase in the platelet count and the total mortality observed in the 1000 mg/kg group on day 44, more studies need to be conducted to understand the effect of *B. africana* on the hematopoietic system and caution needs to be taken when administering *B. africana* at high doses for prolonged period of time.

MATERIALS AND METHODS

Collection and preparation of extract

B. africana stem bark was obtained from Bauchi State, Nigeria and identified at the Forest Herbarium Jos, Nigeria in November 2015 with voucher specimen number FHJ 233. The stem bark was oven dried at 50°C and pulverized to powder and extracted using absolute ethanol for 72 hours. The filtrate was concentrated using a rotary evaporator (Buchi, Switzerland) at 40°C. The extract was stored at 4°C until needed for studies. The ethanolic extract was reconstituted in distilled water for daily animal administration.

Experimental animals

Wistar albino rats and Swiss albino mice of either sex were obtained from the Laboratory Animal Centre of the National Agency for Food and Drug Administration and Control (NAFDAC), Yaba, Lagos, Nigeria for this study and were randomly distributed into 4 groups of 20 rats each (control, 40 mg/kg, 200 mg/kg and 1000 mg/kg). The animals were housed at the Animal House of Faculty of Pharmacy, College of Medicine Campus, University of Lagos, provided with standard animal diets (Livestock Feeds PLC, Ibadan, Oyo state, Nigeria), allowed access to water *ad libitum* and maintained a 12 hour light and darkness cycle. Ethical approval for this study was obtained from College of Medicine, University of Lagos, Health Research Ethics Committee (CMUL/HREC) with approval number CM/HREC/03/16/004. Animals were allowed to acclimatize for one week before administration of the extract.

Acute toxicity

Six Swiss albino mice weighing 25-30 g were administered a single dose of 5000 mg/kg of the ethanolic extract of *B. africana*. The animals were observed hourly for four hours after administration and then for 7 days for behavioral changes or mortality [10].

Sub-acute and chronic toxicity study

Wistar albino rats weighing between 150-200 g were randomly distributed into four groups of twenty rats each (10 males and 10 females). The ethanolic extract of *B. africana* was administered orally for 28 and 90 consecutive days at 40, 200 and 1000 mg/kg. This treatment doses represent one-fifth of the pharmacologically active dose, pharmacologically active dose, and five times the pharmacological active dose respectively. Distilled water was administered daily to the control group during the period of the study. The animals were weighed weekly and observed daily for behavioral changes. On the 29th and 91st day, 10 animals (5 males and 5 females) each were fasted overnight. The animals were anesthetized with diethylether and blood samples were collected by retro-orbital technique in heparinized and non-heparinized tubes for hematology and biochemistry tests. Vital organs (kidney, liver, lungs, spleen and heart) were collected, cleansed of adhering tissues and stored in 10% formalin for histological studies

Hematology and biochemical analysis

Blood sample was collected in di-amine tetra acetic acid (EDTA) tubes for hematology and tubes without EDTA for biochemical analysis. The hematological parameters, white blood cell count (WBC), red blood cell count (RBC), platelet count (PLT), mean corpuscular hemoglobin (MCH), mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), and hemoglobin (HGB) were analyzed using a BC-3200 auto hematology analyzer. Standard biochemical kits (Randox, laboratory limited) were used for the analysis of various biochemical parameters including: urea, creatinine, alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), total protein, albumin, total bilirubin, conjugated bilirubin, cholesterol, triglyceride, high density lipoprotein (HDL) and low density lipoptotein (LDL) as per manufacturer's instruction.

Histology

The organs collected (heart, liver, kidney and lungs) from the control and treatment animals were fixed in 10% buffered formalin. The organs were processed, embedded in paraffin wax and sectioned at 3-5µm. The tissue sections were stained with haematoxylin and eosin for histological observation.

Statistical analysis

Data are presented as mean ± standard deviation (SD). One way analysis of variance (ANOVA) was used for statistical comparison followed by Dunnett's test on GraphPad prism®. Differences were considered significant at $p < 0.05$.

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