

Phytochemical and Toxicity Study of the Root of *Boscia senegalensis* Plant: With Indepth Testicular Histopathological Screening

Peter Ndukaku Osuala^{1*}, Raymond Ugochukwu Okolo², Emmanuel Udo Etuk¹,
Shaibu Oricha Bello¹, Maxwell Osaronowen Egua¹

¹Department of Pharmacology, College of Health Sciences, Usmanu Danfodiyo University, Sokoto, Nigeria

²Department of Anatomy, College of Health Sciences, Usmanu Danfodiyo University, Sokoto, Nigeria

Email: *osuala91@gmail.com

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Abstract

Beyond the efficacy of herbal remedies, there is always a serious concern for safety. A safety study was carried out on the root of *Boscia senegalensis* (per plant prior to its intended use in a bioassay. The root of *Boscia senegalensis* per (capparaceae) was collected from Tureta Local Government Area of Sokoto State in August, 2011 and pulverized into a dry powder. About 700 g of the powder was extracted with methanol for six hours using Soxhlet extractor. The phytochemical study of the plant material was conducted and the median lethal dose (LD₅₀) of the extract was determined in rats orally according to modified Lorke's method. A sub-acute toxicity study was carried out with male albino rats dosed 250, 500 and 750 mg/kg body weights daily for 28 days of the extract. The animals were sacrificed on the 29th day and blood collected through cardiac puncture for hematological and biochemical screening for renal and hepatic status. The testes, liver and kidney were collected and stored in 10 percent formalin for histopathological examination. The phytochemicals present in the root alcoholic extract of *Boscia senegalensis* were alkaloids, phytosteroids and triterpenoids in appreciable quantity, saponins anthraquinones and tannins in moderate quantity while flavonoids were absent. The LD₅₀ is equal to or above 5000 mg/kg body weight. Supportive of the safety profile of the plant, a 28-day oral administration of the extract produced no significant effect on the creatinine, albumin, total protein concentration at all doses and there was a significant reduction in liver enzymes. The hematological evaluation showed that the extract on a prolonged administration had no significant effect. The histology of the kidney and liver were normal. There was a significant increase in the relative weight of the testis, area of the interstitial space and the diameter of the seminiferous tubules when compared to the control group histologically. The plant root extract

may be relatively safe (6000 mg/kg body weight) following oral administration in white albino rats and possesses testicular histopathological effect probably due to the presence of alkaloids saponins, triterpenoids and anthraquinones.

Keywords

Phytochemical, Methanol, Lethal Dose, Safety, Toxicity

1. Introduction

The plant *Boscia senegalensis* pers (family: capparaceae) commonly called Hermet (Arabic), Anza (Hausa), Hanita (Bumbara), Diendoum (Wolof) is an evergreen shrub usually 1 - 2 m tall indigenous plant to Mauritania, Sudan, Burkina Faso, Niger and Nigeria. The plant is used for many purposes by the indigenous people; the leaves are used in granary for preservative purpose [1]; an infusion of leaves used to remove intestinal parasite from camels in Niger [2]; the roots and leaves mixed with millet flour taken each morning on an empty stomach are antihelminthic; draught from leaves or dried bark taken for schistosomiasis; Infusion of the leaves used as eye wash in Sudan and for pruritus of the eye due to syphilis in Senegal; Bark, twigs leaves and roots are used as natural coagulants for water purification in Sudan, Niger and Nigeria [3]. The plant has anti-bacteria activities against 25 hospital isolates of methicillin-resistant *Staphylococcus aureus* (MRSA) [4]. It has recently been demonstrated that the plant has anti-plasmodial activity in mice [5]. Leafless twigs have been found to be very toxic because of the presence of glucosinolates which can be hydrolyzed to mustard oils, which are highly toxic and an irritant to mucous membrane [6]. Leaves contain alkaloids L-stachydrine and hydroxyl-3-stachydrine. The plant is being investigated for further biological activity with regards to its effect on fertility in males where the locals dissolve it in alcohol as a solvent (In press); hitherto, the phytochemical and toxicity study of the root of *Boscia senegalensis* plant (Figure 1) with special interest on the toxicity in the testes has not been undertaken. And it is a clear fact that the phytochemical content of natural products is the reason for their biological activity and for their possible toxicity [6].

2. Material and Method

2.1. Plant Collection and Identification

The root of *Boscia senegalensis* per (capparaceae) was collected from Tureta Local Government Area of Sokoto State in August, 2011. The plant was authenticated by Mallam Muhammed Musa of Biological Science Department, Ahmadu Bello University, Zaria with voucher number 900537 deposited at the departmental herbarium. The collected plant was brought to the Department of Pharmacology, College of Health Sciences, Usmanu Danfodiyo University, Sokoto, Nigeria, where the study was conducted.



Figure 1. *Boscia senegalensis* (per) Lam: showing the flowers, fruit, oblong leaves and hard stem.

2.2. Preparation of the Extracts

The plant root was washed, air dried to a constant weight and pulverized into a dry powder using mortar and pestle. About 700 g of the powder was extracted with methanol for six hours using Soxhlet extractor. The liquid extract was concentrated in an electric oven at 50°C until a semi-solid residue was obtained. The percentage yield of the extract was calculated.

2.3. Phytochemical Analysis

The phytochemical study of the plant material was conducted using method outlined by Trease and Evans [7] and Odebiyi and Sofowora [8]. The following phytochemicals were screened for Carbohydrate, Alkaloids, Glycosides, Saponins, Flavonoids, Tannins, Steroids and Terpenoids, Anthraquinones.

2.4. Experimental Animal

Sexually matured male albino rats (weighing between 180 - 200 g and about 12 - 30 weeks of age) were obtained from the animal unit of the Faculty of Pharmaceutical Sciences, Usmanu Danfodiyo University, Sokoto, Nigeria (UDUS). Animals were kept at a constant 12 hours light/dark cycle and maintained at 35°C for 2 weeks for acclimatization to the pharmacology laboratory. The animals were fed with rats pellets (vital feeds) and had free access to tap water ad libitum, before the commencement of the experiment.

2.5. Acute Toxicity Studies

The median lethal dose (LD₅₀) of the extract was determined in rats orally according to modified Lorke's method [9]. The study was carried out in two phases. In the first phase, nine rats were randomized into three groups of three rats each given 10, 100 and 1000 mg extract/kg body weight orally. The rats were kept under the same conditions and observed for signs of toxicity which include but not limited to paw-licking stretching, respiratory distress and mortality for the first critical 4 hours and thereafter daily for 7 days. In the second phase of

this study 2000, 4000 and 6000 mg/kg body weight orally respectively were administered to another fresh set of three groups of three rats each and observed as in the first phase.

2.6. Sub-Acute Toxicity Studies

The sub-acute toxicity study was carried out in accordance with the WHO [10] and OECD 407 [11] guidelines. Twenty male rats were deprived of food for twenty four hours and then randomly divided into four groups of five rats each. Group A which served as a control received normal saline while rats in groups B, C and D were given 250, 500 and 750 mg extract/kg body weight respectively daily per oral for 28 days. All the rats had free access to food and water throughout the duration of the experiment and were observed daily for general symptoms of toxicity and mortality. On the 29th day of the experiment, all the rats were sacrificed by cervical dislocation and blood samples were collected by cardiac puncture after opening up the rat surgically. One portion was collected into K + EDTA bottle for estimation of packed cell volume (PCV), Haemoglobin concentration (Hb) Red blood cell count (RBC), platelets, lymphocytes, white blood cell count (WBC), mean corpuscular haemoglobin concentration (MCHC) using an automated hematological machine (Cell-Dyn in Abbot, US). Another portion was dispensed into plane bottles, allowed to clot, and centrifuged at 3500 rpm for 10 minutes. The sera were separated and stored at -4°C and then used for evaluation of biochemical parameters which included, Alamine transaminases (ALT), Aspartate transaminase (AST), Alkaline phosphatase levels (ALP), total chollesterol, albumin, protein, urea and creatinine concentration were also determined and recorded.

2.7. Histopathological Studies

The testes removed from the rats were weighed individually and then fixed in 10% formal saline for at least 48 hours. They were processed routinely and the tissues were embedded in paraffin wax. Histological sections of the testis were cut at 5 - 6 μm . The sections were put distilled water, the nuclei were stained with alum haematoxylin and rinsed with tap water, differentiated with 0.3% acid alcohol and then rinsed in a running tap water. It was then rinsed in a Scott's tap water and again rinsed in a tap water and stained with eosin for two minutes, dehydrated cleared and mounted. These were then examined by a histopathologist. The testicular effects of the root of *Boscia senegalensis* per were observed, recorded as a measure of changes in the mean seminiferous tubules diameter and mean area of the interstitial cells of the leydig. These measurements were carried out using a Digital microscope. Finally, the photomicrographs of representative testicular lesions were taken at various magnifications.

2.8. Statistical Analysis

The data was analysed using a one way analysis of variance (ANOVA) and the

comparison between the control and the experimental/groups was done using the turkey krammars test. The level of significance was set of $P < 0.05$. The data were expressed as mean \pm SD.

3. Results

3.1. Yield from Plant Material

The extraction of *B. senegalensis* with methanol for six hours produced an extract with a peculiar pleasant smell. The Percentage yield of crude root extract of *Boscia senegalensis* was 17.18 w/w.

3.2. Phytochemical Screening

The phytochemicals present in the root alcoholic extract of *Boscia senegalensis* were alkaloids, phytosteroids and triterpenoids in appreciable quantity, saponins anthraquinones and tannins in moderate quantity while flavonoids were absent as shown in **Table 1**.

3.3. Acute Toxicity Study

The oral administration of the doses 10, 100 and 1000 mg/kg body weights of the extract in the first phase and 2000, 4000 and 6000 mg/kg body weight of the extract in the second phase did not produce any mortality or obvious signs of adverse effect in the rat during the period of the observation hence the LD₅₀ is equal to or above 5000 mg/kg body weight.

3.4. Sub Acute Toxicity Study

3.4.1. Biochemical Assay

In the repeated dose (Sub acute toxicity) study, oral administration of the extract for 28 days at doses 250,500 and 750 mg/kg body weights produced no significant difference ($p > 0.05$) in the creatinin (crt), total serum protein (TP), serum albumin (Alb) values between the control groups and the treated groups at all dose levels. Also there were no significant difference ($P > 0.05$) in the serum Aspartate amino transferease (AST) and Alkaline Phosphatase (ALP) values between the treated and the control groups at 250 and 500 mg/kg body weight. However, there was a significant decrease ($p < 0.01$) in the serum aspartate amino transferease concentration in the 750 mg/kg body weight treated group (56.80 ± 12.80) when compared to the control group (96 ± 2.5). Also, a significant increase ($p < 0.01$) in the serum (ALP) was observed between the control group (9.40 ± 1.14) and 750 mg/kg body weight treated group (44.00 ± 25.33). The serum ALT of the treated groups 500 and 750 mg/kg body weight showed a significant ($p < 0.05$) raised level (21.20 ± 1.30 and 21.60 ± 3.05 respectively) of enzyme compared to the control group (27.0 ± 1.58). Whereas a significant increase ($p < 0.05$) occurred in the serum urea and cholesterol concentration only at the 250 mg/kg dose (41.00 ± 1.58 and 124.00 ± 9.67 respectively) as compared to the control group (34.00 ± 0.71 and 79.60 ± 8.05 respectively) (**Table 2**).

Table 1. Phytochemical screening of *Boscia senegalensis*.

Phytochemical group	Result
Carbohydrates	+++
Alkaloids	+++
Glycosides	+++
Saponins	++
Flavonoids	-
Tannins	++
Anthraquinone	++
Steroids and triterpenoids	+++
Alkalinity	-
Acidity	-

- Absence, ++ moderate, +++ heavy.

Table 2. Serum biochemical indices of rats after 28 days of oral administration of methanol extract of *B. senegalensis*.

Biochemical indices	Dose (mg/kg)			
	Control	250 mg/kg	500 mg/kg	750 mg/kg
AST IU/l	96 ± 2.5	92.0 ± 9.03	81.20 ± 26.58	56.80 ± 12.80*
ALT IU/l	27.0 ± 1.58	29.00 ± 1.58	21.20 ± 1.30*	21.60 ± 3.05 *
ALP IU/l	9.40 ± 1.14	24.40 ± 1.14	32.40 ± 7.96	44.00 ± 25.33*
Cholesterol mg/dl	79.60 ± 8.05	124.00 ± 9.67*	96.40 ± 9.40	93.60 ± 24.06
TP g/dl	6.86 ± 0.63	7.26 ± 038	7.14 ± 0.80	7.04 ± 0.5
Alb g/dc	3.38 ± 0.13	3.42 ± 032	3.98 ± 0.77	3.78 ± 0.79
Urea mg/dc	34.00 ± 0.71	41.00 ± 1.58*	31.40 ± 2.41	3480 ± 4.66
Creatinine mg/dc	1.34 ± 0.30	1.52 ± 0.41	1.14 ± 0.19	1.24 ± 0.17

Values are expressed as mean ± SD, * = significantly different from control (P < 0.05).

3.4.2. Haematological Assay

The haematological examination revealed that there were no significant difference (P > 0.5) between the effect of the extract on red blood cell, white blood cell, lymphocytes, haemoglobin and mean corpuscular haemoglobin concentration when compared to the control group. However, there was a significant decrease (P < 0.05) in the platelet concentration of the treated group of 750 mg/kg body weight (296.80 ± 24.07) when compared to the control group (4560.6 ± 37.91) (Table 3).

3.4.3. Histopathological Assay

The histological examination of the liver and the kidney revealed normal architecture of these organs (Figure 2 and Figure 3 respectively). There was a significant increase (P < 0.05) in relative weight of testis (Table 4). The histology of

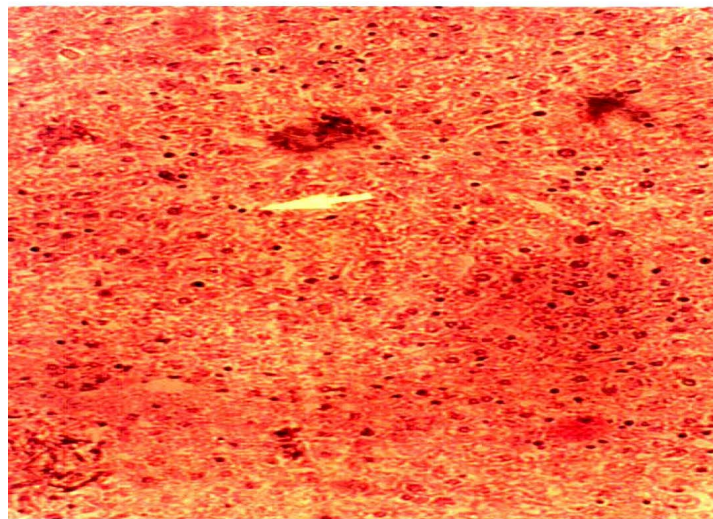
Table 3. Haematological values for Albino rats orally treated with various doses of root extract of *Boscia senegalensis* for 28 days.

Haematological indices	Dose (mg/kg)			
	Control	250 mg/kg	500 mg/kg	750 mg/kg
WBC ($\times 10^3$ /ul)	4.30 \pm 0.75	5.06 \pm 0.46	4.96 \pm 1.09	3.44 \pm 0.29
RBC ($\times 10^6$ /ul)	5.83 \pm 0.36	5.52 \pm .022	5.60 \pm 0.55	5.13 \pm 0.50
LY ($\times 10^3$ /uI)	2.46 \pm 0.47	2.52. \pm 0.53	3 \pm 0.65	2.24 \pm 0.21
HGB (g/dl)	11.72 \pm 0.58	11.44 \pm 0.32	11.22 \pm 1.48	11.08 \pm 0.36
PLT ($\times 10^3$ /ul)	4560.6 \pm 37.91	403.00 \pm 35.09	437.40 \pm 158.91	296.80 \pm 24.07*
MCHC (g/dl)	3340 \pm 0.41	32.46 \pm 1.41	32.88 \pm 1.74	32.36 \pm 1.14

Values are expressed as mean \pm SD, * = significantly different from control ($p < 0.05$), n = 5.

Table 4. The effect of crude extract of *Boscia senegalensis* on the relative weight of testis of male albino rat after a 28 day oral administration.

Group	Mean weight (mg)
Control	0.53 \pm 0.014
250 mg/kg	0.65 \pm 0.031
500 mg/kg	0.70 \pm 0.027
750 mg/kg	0.61 \pm 0.023

**Figure 2.** A photomicrograph of the transverse section of the liver of male albino rat after a 28-day oral administration of the crude root extract of *Boscia senegalensis* showing normal architecture as obtained in the control (H and E. $\times 100$).

the testes revealed a normal testicular architecture. However, there was a significant increase ($P < 0.001$) in the mean diameter of the testicular seminiferous tubule of the rats in the treated group when compared to the control group (Table 5 and Figures 4-7).

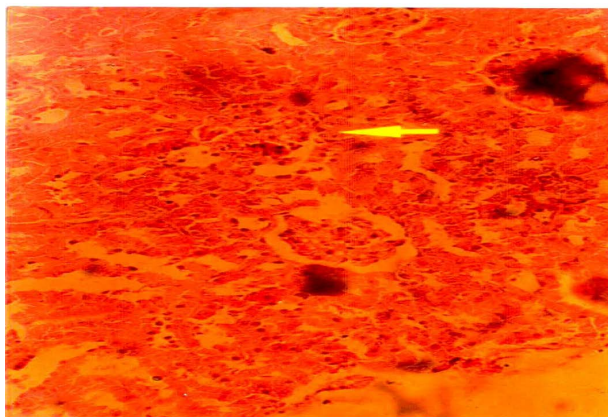


Figure 3. A photomicrograph of the transverse section of the kidney of male albino rat after a 28-day oral administration of the crude root extract of *Boscia senegalensis* showing a normal architecture as obtained in the control (H and E. $\times 100$).

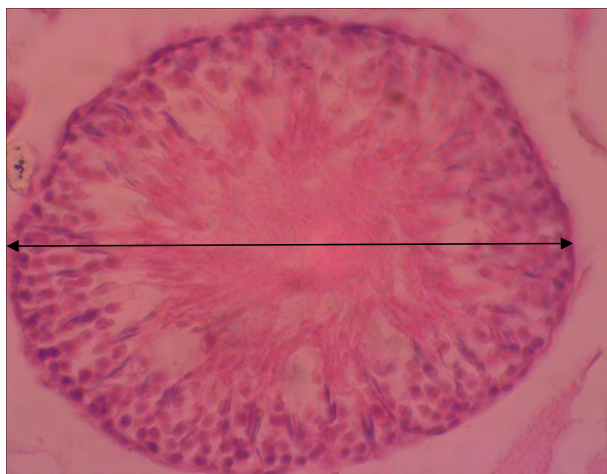


Figure 4. A photomicrograph of the transverse section of the testis of male albino rat after a 28-day oral administration of normal saline (control) showing a relatively short seminiferous tubular diameter (H and E $\times 400$).

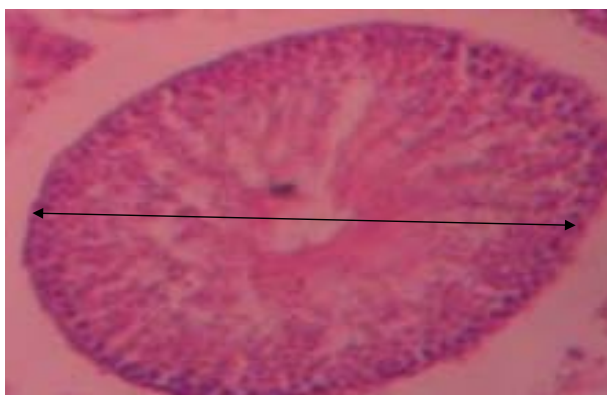


Figure 5. A photomicrograph of the transverse section of the testis of male albino rat after a 28-day oral administration of 250 mg/kg of the crude root extract of *Boscia senegalensis* showing a significantly longer seminiferous tubule diameter compared to the control group (H and E. $\times 400$).

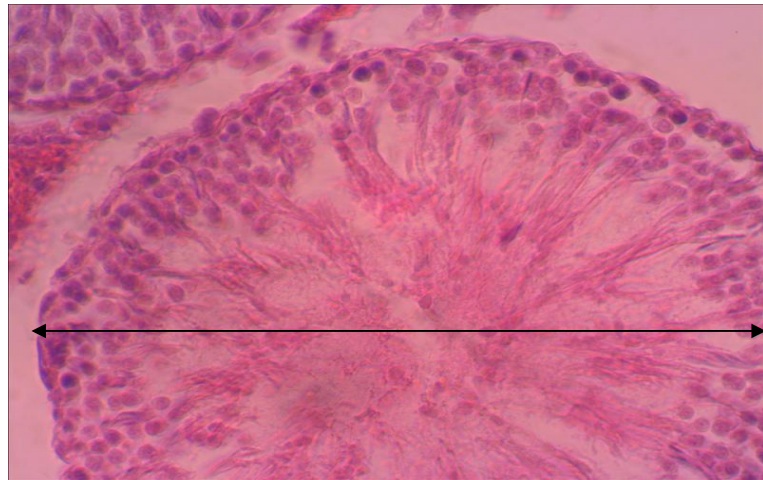


Figure 6. A photomicrograph of the transverse section of the testis of male albino rat after a 28-day oral administration of 500 mg/kg of the crude root extract of *Boscia senegalensis* showing a significant longest seminiferous tubule diameter when compared to the other treated group (H and E. $\times 400$).

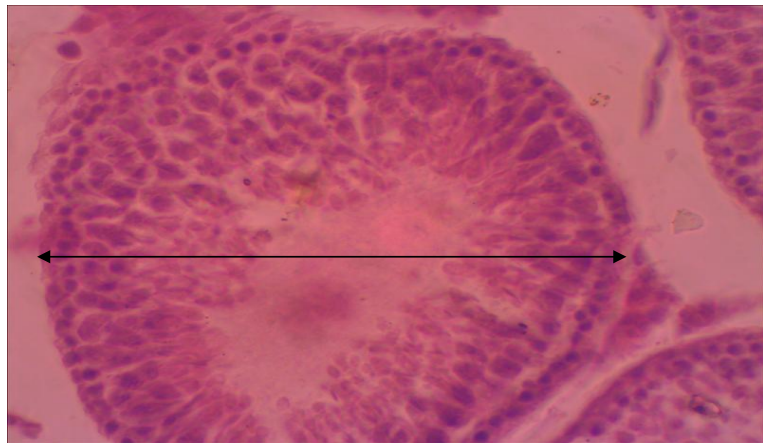


Figure 7. A photomicrograph of the transverse section of the testis of male albino rat after a 28-day oral administration of 750 mg/kg of the crude root extract of *Boscia senegalensis* showing a significant long seminiferous tubule diameter than the control group (H and E. $\times 400$).

Table 5. Effect of daily administration of the extract for 28 days on the mean diameter of testicular seminiferous tubules.

Treatment group (mg/kg)	Normal/saline	250	500	750
Mean diameter	20.71 \pm 0.67	23.95 \pm 1.07*	31.40 \pm 0.91*	26.65 \pm 0.63*

Values are expressed as mean \pm SD g = gram, n = 10, p < 0.001, * = significant values.

The study on the area of the testicular interstitial cells of the Leydig only revealed a more significant increase (p < 0.01) in the mean area at 250, 500 mg/kg body weight while a lesser significant increase (p < 0.05) was observed at the 750 mg per kg when compared to the control group (Table 6 and Figure 8).

Table 6. The effect of crude extract of *Boscia senegalensis* on the mean area of interstitial space of the Leydig cells of the testis of male albino rat. After a 28 day oral administration.

Treatment group (mg/kg)	Normal/saline	250	500	750
Mean area (n ² m ²)	1426.2 ± 507.6	2305.5 ± 720.8*	4423.1 ± 2596.9*	4008.1 ± 12,439.9*

Values expressed as mean ± SD gram, n = 10, p < 0.001, * = significant values.



Figure 8. A photomicrograph of the transverse section of the male rat testis showing the effect of a 28-day oral administration of 250 mg/kg of crude root extract of *Boscia senegalensis* on the interstitial spaces of the Leydig showing significantly larger average area of interstices than the control (H and E. ×100).

4. Discussion

The phytochemical screening generally revealed the presence of phytosteroid and triterpenoids and more specifically the presence of saponnins which may be contributory to toxicological and fertility effect of the testes [12]. Beyond the efficacy of herbal remedies, there is always a serious concern for safety more so; previous studies have shown that there is no safe drug but rather safe dose of a drug [13]. The results showed that a single oral dose administration of up to 6000 mg/kg body weight of the extract did not produce any death or visible adverse effects in rats indicating that the extract is relatively safe. And 5000 mg/kg for acute oral toxicity is generally considered a safe point, at which a test substance is practically non-toxic or non-lethal after an acute exposure [11]. In Nigeria and Africa, researchers have quoted 2000 mg/kg body weight in rats and mice to be safe [14] [15]. Supportive of the safety profile of the plant, a 28-day oral administration of the extract produced no significant ($P > 0.05$) effect on the creatinine, albumin, total protein concentration at all doses and there was a significant reduction in liver enzymes suggesting that the extract may even serve as a hepatoprotective agent. Though there was a significant increase ($P < 0.05$) in urea concentration at 250 mg/kg body weight dose. The hematological evaluation showed that the extract on a prolonged administration has no significant effect ($P > 0.05$) on the white blood cell count, red blood cell, lymphocytes,

haemoglobin and mean corpuscular haemoglobin concentration but a significant reduction ($P < 0.05$) in platelet concentration suggesting that the plant extract may cause thrombocytopenia upon prolonged oral administration at 750 mg/kg body weight dose.

There was a significant increase in the relative weight of the testis, area of the interstitial space and the diameter of the seminiferous tubules when compared to the control group. These increasing effects were observed to be highest at 500 mg/kg body weight dose for both the mean seminiferous tubules diameter and mean area of the interstitial space suggesting the highest androgenic and spermatogenic effect of the extract at this dose.

5. Conclusion

The plant root extract may be relatively safe (6000 mg/kg body weight) following oral administration in white albino rat and possesses testicular histopathological effect probably due to the presence of alkaloids saponins, triterpenoids and anthraquinones.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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