

SUBACUTE TOXICITY STUDY OF STEM EXTRACT OF *Telfairia occidentalis* IN RODENTS

Comment [DS21]: Rats

ABSTRACT

Telfairia occidentalis Hook (*Cucurbitaceae*) is used in Ibibio ethnomedicine for the treatment of diverse diseases such as malaria, diabetes and gastrointestinal disorders. Evaluation of subacute administration of ethanol stem extract of *Telfairia occidentalis* for effect on hematological indices, liver and kidney functions as well as lipid profile of rats was carried out. The stem extract (200, 400, 600 mg/kg body weight) was orally administered to Wistar rats daily for 30 days and the rats were sacrificed under light diethyl ether anesthesia at the completion of the administration. Subacute administration of *T. occidentalis* stem extract resulted in insignificant increase in body weights of rats especially at high doses without any significant ($p > 0.05$) effect on the weights of heart, brain, liver, kidney, spleen and testes when compared to control, but the weights of ovary was decreased significantly ($p < 0.01$) at higher doses (400 and 600 mg/kg) relative to control. The stem extract treatment did not affect the WBC, RBC and platelets counts, hemoglobin concentration, lymphocytes, monocytes, eosinophil and basophil percentages as well as bleeding and clotting times significantly ($p > 0.05$) relative to control. However, significant ($p < 0.05$) increase of neutrophils percentage at low dose (200 mg/kg) and decreased PCV percentage at higher doses (400 and 600 mg/kg) when compared to control were recorded. The stem extract administration did not cause any significant ($p > 0.05$) effect on total protein, albumin, ALP, total and conjugated bilirubin levels of rats except significantly ($p < 0.05-0.01$) elevated ALT and AST levels at low dose (200 mg/kg) of the extract when compared to control. The stem extract did not cause any significant effect ($p > 0.05$) on creatinine, potassium, sodium, chloride and bicarbonate levels of the treated rats except significantly ($p < 0.001$) elevated urea level at low dose of the extract (200 mg/kg), but caused dose-dependent and insignificant ($p > 0.05$) decreases in total cholesterol, triglyceride, HDL, LDL and VLDL levels when compared to control. The stem extract exerted

mild to moderate effect on the histologies of brain, heart, spleen, livers, kidneys, testes and a severe effect on ovaries of rats. Chronic study is advocated to investigate the effect of prolonged administration of the stem extract organs and systems of rats.

Keywords: *Telfairia occidentalis*, haematological parameters, kidney function, liver function

1. INTRODUCTION

Comment [DS22]: The introduction of the manuscript is very brief and needs additional details regarding the topic of the study

Telfairia occidentalis Hook is a fluted pumpkin of the *Cucurbitaceae* family widely consumed as food in Nigeria (Magnus *et al.*, 2024). It is a popular vegetable all over Nigeria, especially in the Niger-Delta region and the Eastern part of the country; varieties of meals are prepared from the leaves, stem and seeds of the plant (Usunomena and Okpiabhele, 2023). The various parts of the plant (seeds, leaves and stem) are used traditionally in the treatment of various ailments and diseases. Antiplasmodial activities of the seed, leaves, stem and roots of the plant have been previously reported (Okokon *et al.*, 2007; Okokon *et al.*, 2009; Okokon *et al.*, 2025a) as well as the analgesic activity of the stem (Okokon *et al.*, 2025b). Enin *et al.*, (2024) had reported on antioxidant activity and in vivo inhibitory effect on alpha amylase and alpha glucosidase of the stem extract. The anti-inflammatory effects of the leaf extract (Oluwole *et al.*, 2003) and seed extract (Okokon *et al.*, 2012) have been reported. Polyunsaturated fatty acids such as hexadecanoic acid, methyl ester, 9,12-octadecadienoic acid methyl ester (linoleic acid), 9,12,15-octadecatrienoic acid, methyl ester (linoleic acid) and 9-octadecenoic acid have been found in the various fractions of the stem extract as well as alkaloid, terpenes, saponin, flavonoid and tannin in the crude extract (Enin *et al.*, 2024). The present study was designed to evaluate the effect of stem extract of *T. occidentalis* on experimentally-induced pain in rodents. The present study was designed to evaluate the toxic effect of subacute administration of the stem extract of *T. occidentalis* on rats.

2. MATERIALS AND METHODS

2.1 Plant materials

Fresh stems of *Telfairia occidentalis* were collected from farms in Uyo metropolis in Uyo LGA, Akwa Ibom State, Nigeria. The leaves were identified and authenticated as *Telfairia occidentalis* by a taxonomist in the Department of Botany and Ecological studies, University of Uyo, Uyo, Nigeria and a voucher specimen was prepared and deposited at the herbarium of the Department of Pharmacognosy and Natural Medicine, Faculty of Pharmacy, University of Uyo.

2.2 Extraction

Fresh stems of *Telfairia occidentalis* were washed, cut into smaller pieces and dried under shade for two weeks. The stems were further pulverized to powder using electric grinder. The powdered leaf material (2 kg) was soaked in 50% ethanol (7.5 L) at room temperature (28 ± 2 °C) for 72 hours. It was thereafter filtered and the liquid filtrate was concentrated and evaporated to dryness in *vacuo* 40 °C using a rotary evaporator (BuchiLab Switzerland). The extract was weighed and stored in a refrigerator at -4 °C, until used for the proposed experiments.

3. 3 Experimental animals

Swiss albino mice and wistar rats (male and female) that were used in the study were obtained from the University of Uyo's Animal house. They were kept in standard plastic cages in a well ventilated room and left to acclimatized for a period of 10 days before the experiments. They were fed on standard pelleted diet and water *ad libitum*. The care and use of animals was conducted in accordance with the National Institute of Health Guide for the Care and Use of laboratory Animals (NIH Publication, 1996). Approval for the study was obtained from the University of Uyo's Animal Ethics Committee.

2.4 Determination of median lethal dose (LD₅₀)

The median lethal dose (LD₅₀) of the extract was estimated using albino mice by oral route using a modified method of Lorke (1983) as earlier described by Okokon *et al.* (2019). This was carried in two phases. The first phases involved oral administration of 3 different doses of the stem extract (10,100 and 1000 mg/kg) to groups of three mice each. The second phase was done using four doses

Comment [DS23]: Authors should mention the active ingredients of ethanolic extract of stems of *Telfairia occidentalis* (in table)

(2000, 3000, 4000 and 5000 mg/kg) and 3 mice per group were orally administered with the stem extract. The animals were observed for manifestation of physical signs of toxicity such as writhing, decreased motor activity, decreased body/limb tone, decreased respiration and death. The number of deaths in each group within 24 hours was recorded. The LD₅₀ was calculated as geometrical means of the maximum dose producing 0% (a) and the minimum dose producing 100% mortality (b).

$$LD_{50} = \sqrt{ab}.$$

2.5 Subacute toxicological study

Adult wistar rats of both sexes were used in this study. They were weighed and randomly divided into four groups of 6 animals each and treated as follows; groups I, II, and III were administered 200, 400 and 600 mg/kg of the stem extract respectively on alternate days for 30 days. Group IV were administered with distilled water (10 mL/kg) for the same period of time. The weights of the animals in all the treatment groups were weighed periodically to monitor the weight increases. At the end of the treatment period (100 days), the animals were again weighed and sacrificed under light ethyl ether vapour. Blood samples were collected by cardiac puncture into EDTA-bottles and plain bottles. Blood samples in EDTA bottles were used for haematological study such as bleeding time, clotting time, full blood counts etc. Sera were separated from the blood samples in plain bottles and stored at -20°C until used for biochemical determinations such as liver function test, kidney function test, and lipid profile

The effects of the extract on some organs were studied. The organs; liver, kidney, spleen, brain, ovary, testis, and heart of rats in both males and females groups were respectively removed surgically and fixed in 10% buffered formalin. The organs were weighed, processed, sectioned and stained using hematoxylin and eosin (H&E) according to standard procedures.

2.6 Haematological Analysis

Blood samples collected from each diethyl ether euthanized rat into different Ethylene Diamine Tetra-acetic Acid (EDTA)-coated sample bottles were analysed for all the haematological

indices (RBC, HGB, PCV, platelets, WBC and differentials – neutrophils, eosinophils, basophils, lymphocytes and monocytes). These parameters were analysed using automated Haematology analyser according to manufacturer's protocols (Sysmex Haematology-Coagulation Systems®, Model KX-21N, Sysmex Incorporation, Kobe, Japan) at the University of Uyo Teaching Hospital. Also, the bleeding and clotting times of each rat were also determined.

2.7 Biochemical Analysis

Using a centrifuge (Nikon optical Co., Japan), whole blood of each sacrificed rat collected through cardiac puncture into different plain sample bottles were centrifuged at 2500 rpm for 15 min at 10 °C to obtain the serum. The obtained sera were analysed for the concentrations and activities of certain biomarkers of liver and kidney functions. The determinations were done spectrophotometrically using Randox Analytical Kits® according to standard procedures of manufacturer's protocols (Tietz, 1976) at the Chemical Pathology Department of University of Uyo Teaching Hospital.

2.8 Liver Function Test

The following parameters were determined; Aspartate aminotransferase (AST), Alanine aminotransferase (ALT), Total Cholesterol, Alkaline phosphatase (ALP), Total plasma protein, Total and combined bilirubin. The determinations were done spectrophotometrically using Randox analytical kits according to standard procedures of manufacturer's protocols (Tietz, 1976) at the Chemical Pathology Department of University of Uyo Teaching Hospital.

2.9 Kidney Function Test

The following biochemical parameters were determined as markers of kidney function using diagnostic kits at the Chemical Pathology Department of University of Uyo Teaching Hospital; Levels of electrolytes (Na, K, Cl, and HCO₃), creatinine, and urea.

2.10 Determination of the effect of the crude extract on the lipid profile (Serum TG, TC, HDL, LDL, VLDL levels) of the treated rats

Serum cholesterol, triglyceride and high density lipoprotein (HDL) levels of the treated rats were measured by enzymatic colorimetric methods using Randox diagnostic kits. The low and very low-density lipoprotein (LDL and VLDL) levels were estimated from the formula of Friedwald *et al.*, 1972).

2.11 Histopathological Examination

The kidney, liver, pancrea, heart, brain and spleen of each animal that was used in the study was surgically harvested and fixed in buffered formalin. They were then processed and stained with haematotoxylin and eosin (H&E) according to standard procedures at Department of Chemical Pathology, University of Uyo Teaching Hospital, Uyo. Morphological changes were observed and recorded in the excised organs of the sacrificed animals. Histologic pictures were taken as micrographs.

2.12 Statistical analysis

Data collected were analyzed using one way analysis of variance (ANOVA) followed by Tukey's multiple comparison post-test (Graph pad prism software Inc. La Jolla, CA, USA). Values were expressed as mean \pm SEM and significance relative to control were considered at $p < 0.05$.

3.RESULTS

3.1 Determination of Median lethal dose (LD₅₀)

Administration of stem extract of *T. occidentalis* (10 - 5000 mg/kg) orally did not cause any mortality to the animals groups administered. Moreover, no physical toxic signs of the extract was observed. The median lethal dose (LD₅₀) of stem extract of *T. occidentalis* was therefore estimated to be =5000 mg/kg.

3.2 Effect of subacute administration of stem extract on Body weight of rats

The effect of stem extract on body weight of rats treated with stem extract of *T.occidentalis* for 30 days is shown in Table 1. There was considerably increase in body weights of rats treated the extract

in all groups similar to that of the control group though non dose-dependently. The body weight gain in the low dose (200 mg/kg) treatment group was significantly ($p < 0.001$) lower than that of the control, while the body weight increases of rats treated with middle dose (400 mg/kg) of the extract was higher than that of control but not statistically significant ($p > 0.05$) (Table 1).

3.3 Effect of subacute administration of leaf extract on organs weights of rats

Treatment of rats with the stem extract (200-600 mg/kg) for 30 days did not cause any effect on the weights of heart, brain, liver, kidney, spleen and testes. However, decreases in the ovary weights of rats treated with higher doses of the extract (400 and 600 mg/kg) were observed which were significant ($p < 0.01$) when compared statistically to the control group (Table 2).

3.4 Effect of subacute administration of stem extract on haematological parameters of rats

The results of the effect of subacute administration of ethanol stem extract of *T. occidentalis* (200-600 mg/kg) on haematological parameters of rats is shown in Table 3. Subacute administration of stem extract of *T. occidentalis* to rats for 30 days did not affect the WBC, RBC and platelets counts, hemoglobin concentration, lymphocytes, monocytes, eosinophil and basophil percentages significantly ($p > 0.05$) when compared to control. However, neutrophils percentage of the group treated with 200 mg/kg of the stem extract was significantly ($p < 0.05$) increased and significant ($p < 0.05-0.01$) decreases in PCV percentage was observed in the group treated with the middle and high doses (400 and 600 mg/kg) of the extract when compared to control (Table 3). Moreover, treatment of rats with stem extract of *T. occidentalis* (200 - 600 mg/kg) for 30 days did not affect significantly ($p > 0.05$) the bleeding and clotting time of rats in all doses used when compared to control (Figures 1).

3.5 Effect of subacute administration of stem extract on liver function indices of rats

Administration of stem extract of *T. occidentalis* (200 - 600 mg/kg) to rats for 30 days did not cause any significant ($p > 0.05$) effect on total protein, albumin, ALP, total and conjugated bilirubin levels

of rats when compared to control (Table 4). However, ALT and AST levels of the rats were significantly ($p < 0.05-0.01$) elevated in the group treated with the low dose (200 mg/kg) of the extract when compared to control (Table 4).

3.6 Effect of subacute administration of leaf extract on kidney function parameters of rats

Treatment of rats for 30 days with stem extract of *T. occidentalis* (200-600 mg/kg) did not cause any significant effect ($p > 0.05$) on the levels of creatinine, potassium, sodium, chloride and bicarbonate of the treated rats when compared to control. However, the level of urea was significantly ($p < 0.001$) increased at the low dose of the extract (200 mg/kg) when compared to control (Table 26).

3.7 Effect of subacute administration of stem extract on lipid profile indices of rats

Treatment of rats with stem extract of *T. occidentalis* (200-600 mg/kg) caused dose-dependent but insignificant ($p > 0.05$) decreases in the levels of total cholesterol, triglyceride, HDL, LDL and VLDL of rats treated for 30 days when compared to control. (Table 27).

Table 1: Effect of subacute administration of *Telfairia occidentalis* stem extract on body weights of rats

Treatment	Dose	Initial body weight (Kg)	Final body weight (Kg)	Weight gain (Kg)
R&G /Extract	(mg/kg)			
Control	0.2ml	153.3 ± 9.40	207.6 ± 5.69	54.3 ± 2.81
<i>T. occidentalis</i>	200	170.3 ± 6.88	201.0 ± 17.00	30.7 ± 3.86 ^a
	400	171.6 ± 1.66	232.0 ± 8.50	60.4 ± 2.74
	600	164.0 ± 9.53	209.6 ± 2.40	45.6 ± 3.41

Data are expressed as mean ± SEM. Significant at ^ap>0.001 when compared to control . n = 6.

Table 2: Effect of subacute administration of *Telfairia occidentalis* stem extract on organs weights of rats

TREATMENT	DOSE(mg/kg)	Heart (mg)	Brain (mg)	Liver (mg)	Kidney(mg)	Spleen (mg)	Testes (mg)	Ovary (mg)
Control	10 mg/ml	0.66± 0.07	1.65±0.06	6.00± 0.35	1.19±0.03	0.62± 0.04	2.41±0.21	0.07±0.01
Crude extract	200	0.70±0.04	1.65±0.15	6.11±0.45	1.31±0.12	0.77± 0.11	3.07± 0.36	0.06±0.01
	400	0.66±0.02	1.71±0.06	6.29±0.25	1.14±0.03	0.74±0.02	3.31± 0.47	0.04±0.01 ^b
	600	0.71±0.02	1.73±0.01	6.10±0.38	1.21±0.06	0.65± 0.05	2.77± 0.18	0.04±0.01 ^b

Data are expressed as MEAN ± SEM, Significant at ^bp< 0.01, when compared to control. (n=6).

Table 3 : Effect of subacute administration of *Telfairia occidentalis* stem extract on heamatological parameters of rats

Treatment	Dose	WBC (L)	NEUT. (%)	LYM (%)	MONO (%)	ESINO (%)	BASO (%)	RBC (L)	HGB (g/dL)	PCV (%)	PLATELETS. (L)
Control normal saline	10 mg/ml	6.27±0.05	17.26±1.46	77.96±2.43	2.93±0.52	0.33± 0.03	0.50± 0.05	6.12± 0.46	13..53±0.14	43.76±2.27	754.0± 35.90
Crude extract	200	7.81±0.98	23.63±1.46	73.73±1.93	1.43±0.68	0.66±0.17	0.66± 0.17	8.40 ± 0.15	14.63± 0.36	46.96±1.17	775.0± 82.21
	400	4.48±0.85	13.65±1.72	82.26±1.74	2.60±1.42	0.46± 0.32	0.46± 0.03	4.85± 1.63	9.13± 2.73	28.23±1.34 ^c	542.3± 30.52
	600	6.99±0.99	21.33±1.74	75.56±3.01	3.68±0.69	0.30± 0.17	0.36± 0.23	7.77± 0.27	12.44± 2.06	34.93±1.96 ^b	769.0± 95.04

Data are expressed as MEAN ± SEM, Significant at ^ap<0.05, ^bp<0.01, when compared to control. (n=6).

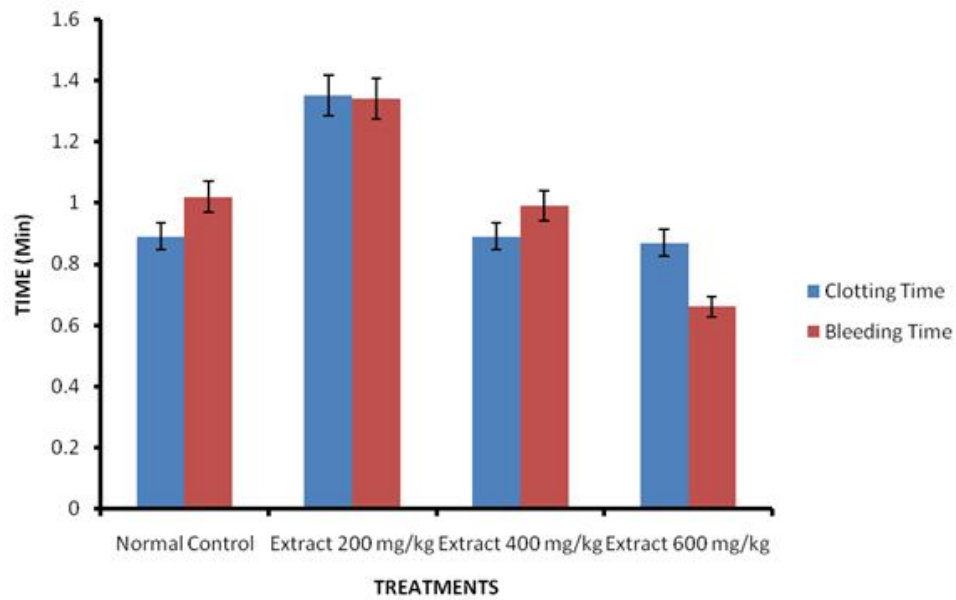


Figure 1: Effect of subacute administration of *Telfairia occidentalis* stem extract on clotting and bleeding times of rats

Data are expressed as MEAN \pm SEM, (n=6).

Table 4: Effect of subacute administration of *Telfairia occidentalis* stem extract on liver function parameters of rats

TREATMENT	DOSE (mg/ kg)	Total Protein (mg/dL)	Albumin (mg/dL)	ALT (IU/L)	ALP (IU/L)	AST (IU/L)	Total Bilirubin (μ mol/l)	Combined Bilirubin (μ mol/l)
Control	10 mg/mL	67.80 \pm 1.39	40.66 \pm 1.50	23.0 \pm 2.09	30.60 \pm 1.69	37.20 \pm 1.49	7.62 \pm 0.34	5.52 \pm 0.39
Crude extract	200	69.80 \pm 1.77	43.60 \pm 1.60	37.0 \pm 1.70 ^c	26.40 \pm 1.07	46.40 \pm 2.06 ^b	9.36 \pm 0.37	6.58 \pm 0.32
	400	71.20 \pm 2.45	44.80 \pm 2.08	22.40 \pm 0.92	26.0 \pm 0.70	37.0 \pm 1.51	7.70 \pm 0.31	5.06 \pm 0.38
	600	76.80 \pm 1.46	45.0 \pm 0.70	17.40 \pm 0.92	27.0 \pm 0.70	32.20 \pm 1.49	6.74 \pm 0.34	4.48 \pm 0.12

Data are expressed as MEAN \pm SEM, Significant at ^ap<0.05, ^bp< 0.01, ^cp< 0.001, when compared to control. (n=6).

Table 5: Effect of subacute administration of *Telfairia occidentalis* stem extract on kidney function parameters of rats

TREATMENT	DOSE (mg/kg)	CREATININE (mg/kg)	UREA (mg/dl)	BICARBONATE (mMol/L)	SODIUM (mMol/L)	POTASSIUM (mMol/L)	CHLORIDE (mMol/L)
Control normal saline	10 mg/mL	68.0± 2.09	3.16± 0.15	27.40± 1.88	151.0±3.96	4.92± 0.20	53.0± 1.41
Crude extract	200	64.40±2.50	13.06± 0.15 ^c	28.00± 0.86	149.40±2.58	4.90± 0.20	55.40± 1.20
	400	74.40± 7.05	3.64± 0.40	28.00±0.70	128.80± 2.28	3.96± 0.09	49.20± 0.86
	600	64.40± 4.22	2.96± 0.25	25.20±0.86	144.20±2.53	4.60± 0.23	49.40± 1.88

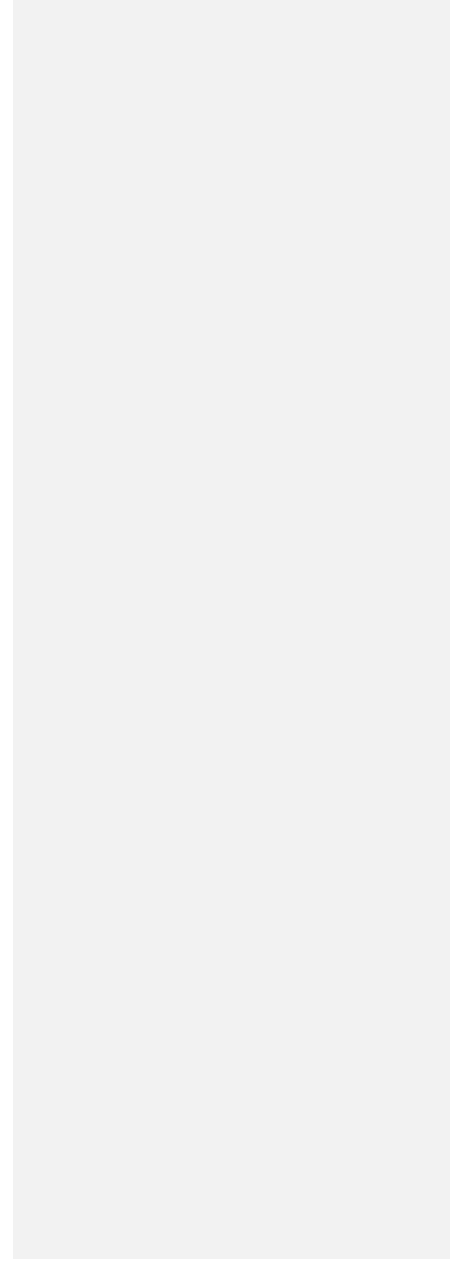
Data are expressed as MEAN ± SEM, Significant at ^cp< 0.001, when compared to control. (n=6).

Table 6: Effect of subacute administration of *Telfairia occidentalis* stem extract on lipid profile of rats

TREATMENT	DOSE mg/kg	TOTAL CHOLESTEROL (mMol/L)	TRIGLYCERIDE (mMol/L)	HDL-C (mMol/L)	LDL-C (mMol/L)	VLDL (mMol/L)
Control	10 mL/kg	2.42± 0.12	1.16± 0.11	1.25± 0.12	1.69± 0.05	0.52± 0.05
Crude extract	200	2.40± 0.14	0.98± 0.04	1.10± 0.05	1.65± 0.10	0.44± 0.02
	400	2.30± 0.11	0.93± 0.10	1.09± 0.09	1.62± 0.08	0.42± 0.04
	600	2.10± 0.17	0.90± 0.09	0.99± 0.08	1.50± 0.13	0.41± 0.04

Data are expressed as MEAN ± SEM. (n=6).

UNDER PEER REVIEW



3.8 Effect of subacute administration of stem extract on histology of organs

Figures 2 - 8 show the effects of subacute administration of ethanol stem extract of *T. occidentalis* to rats for 30 days on histology of some organs. The stem extract (200 - 600 mg/kg) caused varying defects on the histology of the organs. The stem extract administration affected the brain tissue of the treated rats moderately, with the lateral prefrontal cortex of the cerebral hemisphere having moderately altered brain tissue with karyolysis of the neural cells, presence of vacuolated neural cells and presence of blood vessels within the cerebral matrix (Figure 2). Moderate effects were also observed on the histo-structure of cardiac tissues of the treated rats showing presence of inter-muscular vascular hemorrhage and fibrosis within the cardiac myometrium (Figure 3). Moderate effect of the stem extract on the cyto-structure of the spleen were observed following subacute administration of the extract with treated rats' spleen tissues showing moderately altered splenic histo-structure with the red pulp having areas of proliferating and degenerating nodular cells, central artery with areas of degenerated lymphocytic cells within the splenic matrix (Figure 4). Moderately altered hepato-architecture with increased degenerating and vacuolated hepatocytes, widespread micro-vesicular steatosis and organic deposits within the blood vessel and hepatic lobules were observed at lower doses (200 and 400 mg/kg) of the extract, while high dose (600 mg/kg) had severely altered hepato-architecture with areas of degenerated hepatic cells, increased degenerating and vacuolated hepatocytes and widespread micro-vesicular steatosis (figure 5). The histo-architecture of renal tissues of treated rats had a slight abnormal renal histo-architecture with slight widened bowman's space and was slightly affected at low dose (200 mg/kg). Moderate effects were seen at higher doses (400 and 600 mg/kg) of the extract exhibiting atrophying renal micro-architecture, having areas of hemorrhagic blood

vessels within the renal cortical matrix and degenerating tubules with vacuolated ductal cells. There was no pathological effect on the control kidney (figure 6). The stem extract (200 -600 mg/kg) administration caused moderate histo-architectural alteration on testicular tissues with areas of spermatogenic cells degeneration and altered spermatogenic processes, with widened tubular lumen within the seminiferous tubules (figure 7). The low dose of the stem extract (200 mg/kg) affected the ovaries of the treated rats moderately demonstrating abnormal histo-structure with an area of altered follicular cells division and degenerating follicular cells, while higher doses (400 and 600 mg/kg) caused severe effects on the ovaries with sections showing abnormal histo-structure with degenerating follicles having area of vacuolated and degenerated follicular cells, degenerating secondary follicle, and atrophying follicle within the ovarian stroma (Figure 8).

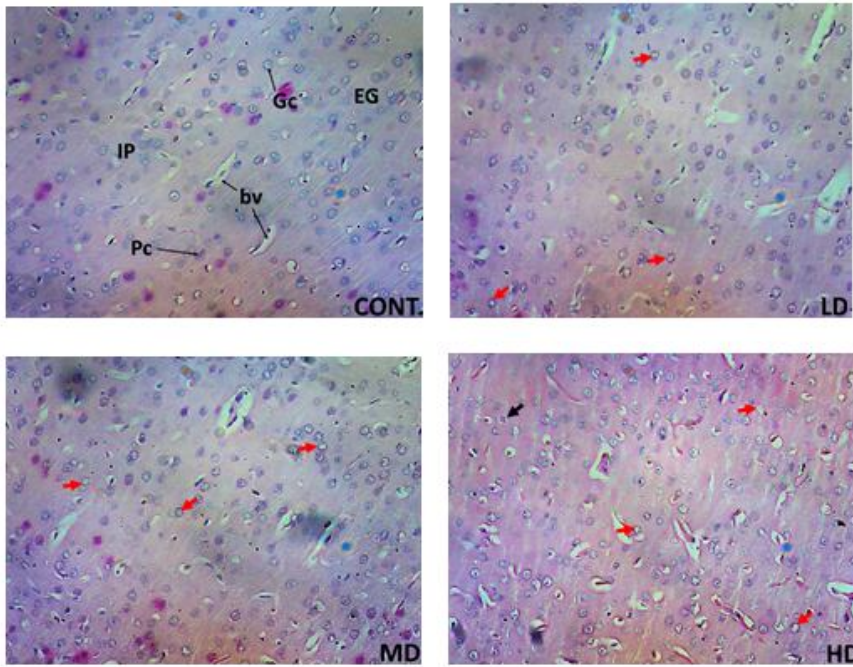


Figure 2: Photomicrograph of the transverse sections of prefrontal cortex area of brain tissues of rats treated with distilled water (**CONT**), stem extract of *T.occidentalis* at 200 mg/kg (**LD**), 400 mg/kg (**MD**) and 600 mg/kg (**HD**) showing prefrontal cortex tissue, External granular cell layer (EG), granular cells (Gc), pyramidal cells (Pc), Internal pyramidal cell layer (IP), and blood vessels (bv) within the cerebral matrix

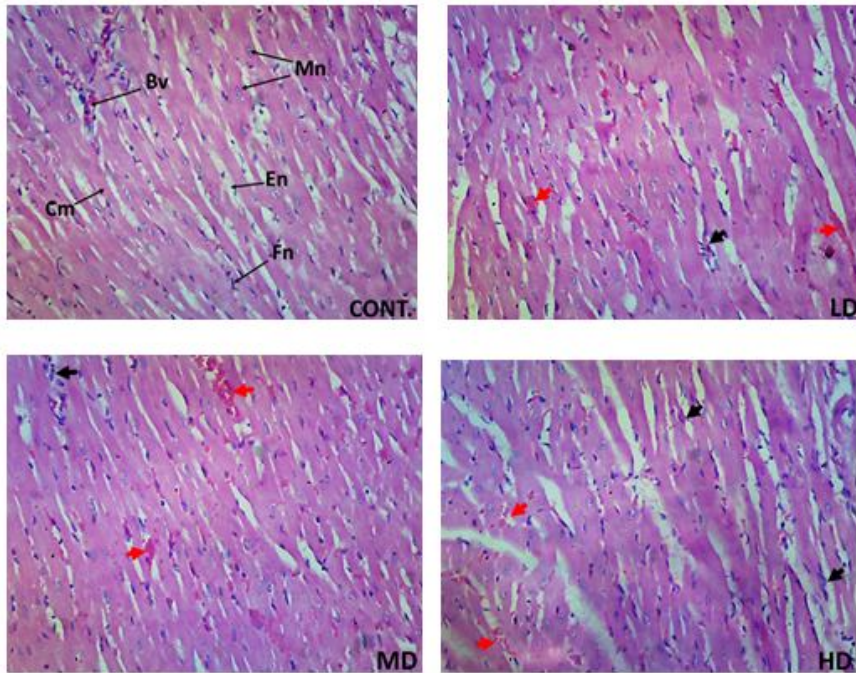


Figure 3: Photomicrograph of the transverse sections of hearts of rats treated with distilled water (**CONT**), stem extract of *T.occidentalis* at 200 mg/kg (**LD**), 400 mg/kg (**MD**) and 600 mg/kg (**HD**) heart tissues showing cardiac myocytes (Cm), myocyte nuclei (Mn), fibrocyte nuclei (Fn) and endomysium (En) and blood vessels (Bv), presence of inter-muscular vascular hemorrhage (red arrow) and fibrosis (black arrow) within the cardiac myometrium

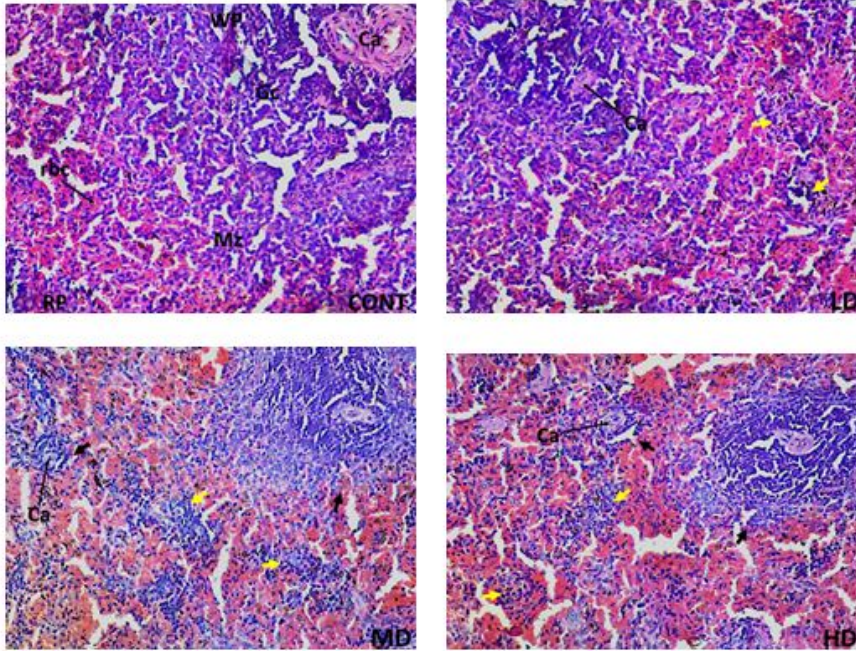


Figure 4: Photomicrograph of the transverse sections of spleens of rats treated with distilled water (**CONT**), stem extract of *T.occidentalis* at 200 mg/kg (**LD**), 400 mg/kg (**MD**) and 600 mg/kg (**HD**) spleen tissues showing white pulp (WP) germinal center (Gc), central artery (Ca),marginal zone (Mz), red pulp (RP), red blood cells (rbc), areas of proliferating and degenerating nodular cells (yellow arrow), central artery (Ca) with areas of degenerated lymphocytic cells (black arrow)

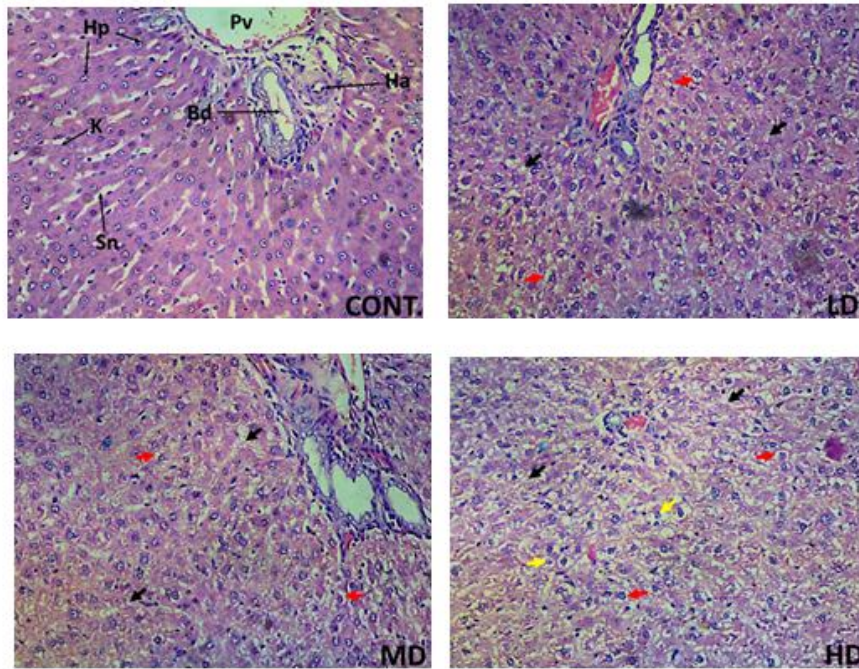


Figure 5: Photomicrograph of the transverse sections of livers of rats treated with distilled water (CONT), stem extract of *T.occidentalis* at 200 mg/kg (LD), 400 mg/kg (MD) and 600 mg/kg (HD) liver tissue showing portal vein (Pv), hepatic artery (Ha), Bile duct (Bd), hepatocytes (Hp), Kupfer cells (K), sinusoids (Sn), organic deposits (Od) degenerating and vacuolated hepatocytes (red arrow), widespread micro-vesicular steatosis (black arrow) and organic deposits (Od), degenerated hepatic cells (yellow arrow).

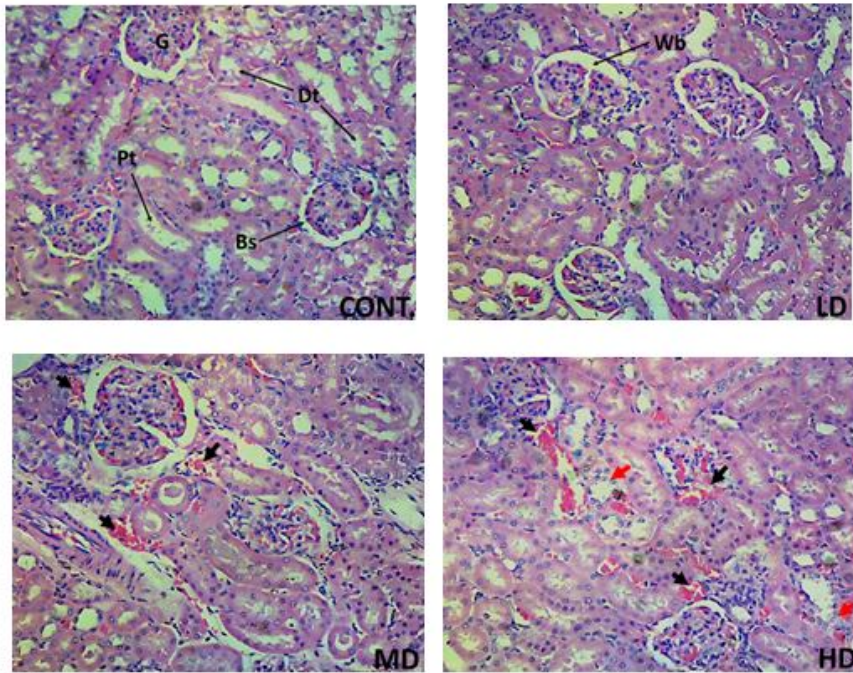


Figure 6: Photomicrograph of the transverse sections of kidneys of rats treated with distilled water (**CONT**), stem extract of *T.occidentalis* at 200 mg/kg (**LD**), 400 mg/kg (**MD**) and 600 mg/kg (**HD**) kidney tissues showing glomeruli (G) bowman's space (Bs), proximal convoluted tubules (Pt) distal convoluted tubules (Dt), widened bowman's space (Wb), areas of hemorrhagic blood vessels (black arrow), vacuolated ductal cells (red arrow)

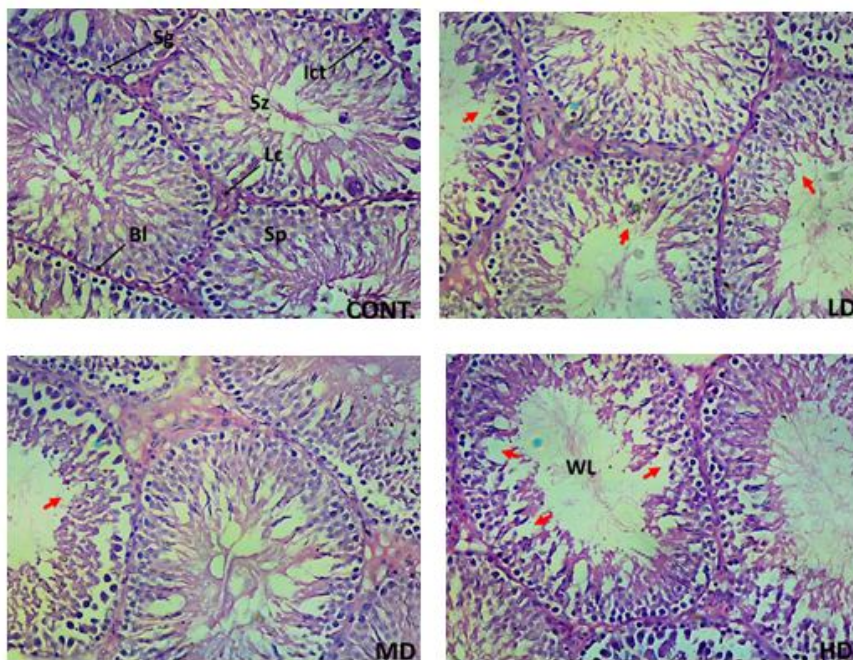


Figure 7: Photomicrograph of the transverse sections of testes of rats treated with distilled water (**CONT**), stem extract of *T.occidentalis* at 200 mg/kg (**LD**), 400 mg/kg (**MD**) and 600 mg/kg (**HD**) showing basement layer (Bl), spermatogenic cells (Sp), arrays of spermatozoa (Sz), Leydig cells (Lc) and blood vessels (Bv), areas of spermatogenic cells degeneration and altered spermatogenic processes (red arrow), with widened tubular lumen (WL).

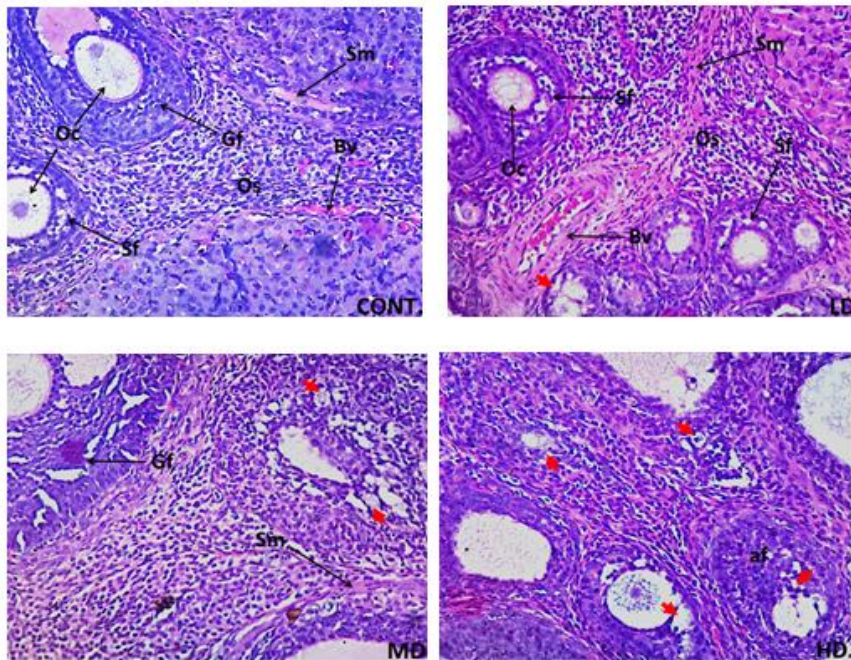


Figure 8: Photomicrograph of the transverse sections of ovary of rats treated with distilled water (**CONT**), stem extract of *T.occidentalis* at 200 mg/kg (**LD**), 400 mg/kg (**MD**) and 600 mg/kg (**HD**) showing Graffian follicle (Gf), secondary follicles (Sf), Oocytes (Oc), blood vessels (Bv), smooth muscles (Sm) within the ovarian stroma (Os). (H&E x 100).

DISCUSSION

In this study, subacute administration of the stem extract caused various degrees of weight gains in all the treatment groups which were insignificantly higher than control at higher doses (200 and 600 mg/kg) but significantly lower at the low dose (200 mg/kg) when compared to control. Changes in body weights are markers of adverse effects of drugs and it is considered statistically significant if a body weight loss is more than 10% (Tepongning *et al.*, 2018). In this study, there were moderate increases in body weights of rats in all the extract treated groups but these increases were not significantly ($p>0.05$) different from that of the control group except at the low dose indicating that feeding habit of the rats was not adversely affected by the administration of the extract and there were no adverse effects of the extract on the body growth processes of rats.

Treatment of rats with the stem extract (200-600 mg/kg) for 30 days did not cause any effect on the weights of heart, brain, liver, kidney, spleen, pancreas and testes, although slight increases in the weights of heart, brain, liver, kidney, spleen,

pancreas and testes of treated rats were observed but these were not significant when compared statistically to the control group. However, significant ($p < 0.01$) weight decreases were observed in the ovary of rats treated with higher doses (400 and 600 mg/kg) of the extract. Weights of internal organs are used to assess injury and toxicities and damaged organs due to toxicity are often identified by hypertrophy of the organs, which presents as oedema in such organ due to inflammation of the organ (Farah *et al.*, 2013; Ping *et al.*, 2013). The decrease in weights of ovary in high doses (400 and 600 mg/kg) treated groups indicates serious toxicity potential as reflected in the histological results

Plant extracts and other chemical compounds are evaluated for possible effect on hematological parameters to assess their toxic potentials on blood (Bashir *et al.*, 2015). Subacute administration of leaf extract of *T.occidentalis* to rats for 30 days did not affect the WBC, RBC and platelets counts, hemoglobin concentration, lymphocytes, monocytes, eosinophil and basophil percentages significantly ($p > 0.05$) when compared to control. However, neutrophils percentage of the group treated with 200 mg/kg of the stem extract was significantly ($p < 0.05$) increased and significant ($p < 0.05-0.01$) decreases in PCV percentage was observed in the group treated with the middle and high doses (400 and 600 mg/kg) of the extract when compared to control. These suggest hemolytic effect of the extract and/or suppression of erythropoiesis (the rate of production of erythrocytes)(Berinyuy *et al.*, 2015). These imply that the oxygen-carrying capacity of the blood can be affected and indicative of the extract's anaemia inducing potentials. The lack of significant effect on the platelets counts observed explains the insignificant effect on the bleeding and clotting times of the treated rats when compared to control, indicating no effect on the blood clotting mechanism.

In this study, administration of leaf extract of *T.occidentalis* (200-600 mg/kg) to rats for 30 days exerted insignificant ($p>0.05$) effects on total protein, albumin, ALP, total and conjugated bilirubin levels of rats when compared to control. However, ALT and AST levels of the rats were significantly ($p<0.05-0.01$) elevated in the group treated with the low dose (200 mg/kg) of the extract when compared to control. Decreases in serum proteins and albumin levels are indicators of hepatic damage due to reduced capability of the hepatocytes to synthesize enough serum proteins (Shin *et al.*, 2010; Yousef *et al.*, 2010), whereas increased bilirubin (total and conjugated) could be used to assess the excretory function of the liver (Kaplan *et al.*, 1979; Yakuba *et al.*, 2003), these were not observed in this study. The results suggest that the extract had no effect on the secretory/excretory as well as synthetic functions of the liver at all doses used.

In this study, significantly elevated activities of AST and ALT were observed following subacute administration of the leaf extract of *T.occidentalis* especially at low dose (200 mg/kg) of the extract. Cellular leakage of enzymes occurs often when the cell architecture and integrity is damaged. Accordingly, the presence of enzymes in the serum above their normal level is a pointer to clinical diagnosis of variety of pathological conditions. Serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) have been reported by numerous authors as markers for acute and chronic hepatocellular damage (Dufour *et al.*, 2000). The extract at low dose may have affected the liver integrity leading to leakage of these enzymes. Alkaline phosphatase (ALP) is a 'marker' enzyme for the plasma membrane and endoplasmic reticulum; it is therefore an ectoenzyme of the plasma membrane and it is often used to assess the integrity of the plasma membrane (Shittu *et al.*, 2015). However, it was

not affected by the extract treatment. The results further suggest mild toxic potential of the extract at low dose.

Blood urea nitrogen (BUN) produced in the liver is derived from the diet or tissue sources and is excreted in the urine via the kidney. Serum urea accumulates in the serum in renal disease when the rate of production exceeds that of excretion (Mayne, 1994). Serum creatinine is basically derived from endogenous sources by tissue creatinine breakdown (Mayne, 1994). Therefore, elevation of urea and creatinine levels in the serum had been taken as the index of nephrotoxicity (Ali *et al.*, 2001; Flaoyen *et al.*, 2001). In this study, treatment of rats for 30 days with stem extract of *T.occidentalis* (200-600 mg/kg) resulted in significant ($p<0.001$) increased of urea level at the low dose of the extract (200 mg/kg) when compared to control. However, the extract did not cause any significant effect ($p>0.05$) on the levels of creatinine, potassium, sodium, chloride and bicarbonate of the treated rats when compared to control. This suggests that the extract is nephrotoxic at the low doses. The electrolytes concentrations were not affected by the extract treatment, suggesting that the glomerular filtration rate was not affected by the extract treatment at doses studied.

Alterations in the concentration of major lipids like cholesterol, high-density lipoprotein cholesterol, low density lipoprotein cholesterol and triglycerides can give useful information on the lipid metabolism as well as predisposition of the heart to atherosclerosis and its associated coronary heart diseases (Yakubu *et al.*, 2008). High blood cholesterol concentration is an important risk factor for cardiovascular disease (Abolaji *et al.*, 2007). Therefore, the slightly reduced levels of serum total cholesterol, triglyceride, LDL, and VLDL especially at higher doses by the extract though

insignificant may be clinically beneficial to the animals as the extract is unlikely to be associated with cardiovascular risk at these doses. Similarly, the decreased levels of serum triacylglycerol by the extract may be explained by a reduced lipolysis (Yakubu *et al.*, 2008). The reduction in the levels of VLDL, LDL and HDL in this study reveals a strong hypolipidemic activity of the stem extract perhaps due to inhibitory activity on lipolysis which is due to the activities of its phytoconstituents and may be an indication that the extract may not predispose the animals to atherosclerosis and coronary heart diseases (Philip, 1995; Jackson, 1996; Mayes, 1996; Panagiotakos *et al.*, 2003).

On the histology, subacute administration of ethanol stem extract of *T.occidentalis* to rats for 30 days produced varying degrees of abnormalities ranging from mild to moderate defects on histology of the heart, ovary, liver, kidney, brain, spleen and testis. Moderately altered hepato-architecture with increased degenerating and vacuolated hepatocytes, widespread micro-vesicular steatosis and organic deposits within the blood vessel and hepatic lobules were observed at lower doses (200 and 400 mg/kg) of the extract, while high dose (600 mg/kg) had severely altered hepato-architecture with areas of degenerated hepatic cells, increased degenerating and vacuolated hepatocytes and widespread micro-vesicular steatosis. These results corroborated the significantly raised ALT and AST levels observed in the study at the low dose. The histo-architecture of renal tissues of treated rats had a slight abnormal renal histo-architecture with slightly widened bowman's space and was slightly affected at low dose (200 mg/kg). This result supports the raised urea level observed at the low dose. Moderate effects were seen at higher doses (400 and 600 mg/kg) of the extract exhibiting atrophying renal micro-architecture, having areas of hemorrhagic blood vessels within the renal cortical matrix and

degenerating tubules with vacuolated ductal cells. There was no pathological effect on the control kidney. Moderate effects were also observed on the histo-structure of cardiac tissues of the treated rats showing presence of inter-muscular vascular hemorrhage and fibrosis within the cardiac myometrium, suggesting a mild effect on the heart. The stem extract (200 -600 mg/kg) administration further caused moderate histo-architectural alteration on testicular tissues with areas of spermatogenic cells degeneration and altered spermatogenic processes, with widened tubular lumen within the seminiferous tubules, indicating a possible effect on the male reproductive system. The low dose of the stem extract (200 mg/kg) affected the ovaries of the treated rats moderately demonstrating abnormal histo-structure with an area of altered follicular cells division and degenerating follicular cells, while higher doses (400 and 600 mg/kg) caused severe effects on the ovaries with sections showing abnormal histo-structure with degenerating follicles having area of vacuolated and degenerated follicular cells, degenerating secondary follicle, and atrophying follicle within the ovarian stroma. Thus, suggesting an effect on the female reproductive system. Moderate effects of the stem extract on the cyto-structure of the spleen were observed following subacute administration of the extract with treated rats' spleen tissues showing moderately altered splenic histo-structure with the red pulp having areas of proliferating and degenerating nodular cells, central artery with areas of degenerated lymphocytic cells within the splenic matrix, indicating a possible toxic effect on the spleen. The stem extract administration affected the brain tissue of the treated rats moderately, with the lateral prefrontal cortex of the cerebral hemisphere having moderately altered brain tissue with karyolysis of the neural cells, presence of vacuolated neural cells and presence of blood vessels within the cerebral matrix. These results indicate mild

adverse effects on the brain cells which might also affect some functions of the CNS.

5. CONCLUSION

The results of this investigation suggest that subacute treatment of rats with stem extract of *Telfaira occidentalis* could have mild to moderate effects on the brain, heart, liver, kidney, spleen and testes with serious effect to the ovary. However, low dose was found to be more toxic than high doses in some cases. It recommended that high doses should be avoided.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript

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