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Seed Extract and Fractions of *Telfairia Occidentalis* Attenuated Doxorubicin-Induced Testiculotoxicity in Rats

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ABSTRACT

Telfairia occidentalis Hook (cucurbitaceae) seeds, which is used in the preparation of soups and as medicine traditionally to treat various diseases in Nigeria were investigated for effect against doxorubicin-induced testicular toxicity. The seed extract (138-553 mg/kg) and fractions, dichloromethane (DCM) and aqueous, 276 mg/kg) were evaluated for testiculoprotective activity against doxorubicin-induced testicular toxicity in male rats. Testosterone level, lipid profile indices, testicular oxidative stress markers, and testis histology were used to assess the testicular protective effect of the extract. The seed extract (138-553 mg/kg) and fractions, dichloromethane (DCM) and aqueous, 276 mg/kg reduced the serum levels of total cholesterol, triglycerides, HDL, LDL and VLDL that were elevated by doxorubincin. Testosterone level, which was significantly ($p<0.05$) reduced by doxorubicin was significantly ($p<0.05$-0.01) elevated by the seed extract and fractions co-administration. The seed extract and fractions also improved significantly ($p<0.01$) GSH, GST, SOD, GPx, and CAT levels that were decreased by doxorubicin and also lowered the raised MDA level. Histology of the testes sections of extract/fractions-treated animals showed absent/or reduced the pathological features compared to the organotoxic-treated animals. The chemical pathological changes were consistent with histopathological observations, suggesting marked testicular protective potential. The anti-toxic effect of this plant may in part be mediated through the chemical constituents of the plant. The seed extract of T. occidentalis possesses anti-toxicant properties which can be exploited in the treatment of doxorubicin-related toxicities.

Keywords: *Telfairia occidentalis, anti-oxidant, oxidative tress, testicular-protective, antioxidant.*

1. Introduction

Doxorubicin, an anthracycline glycoside antibiotic with broad-spectrum of activity against various human solid tumors and hematological malignancies [7], but limited clinical usefulness due to its diverse toxicities, including cardiac, hepatic, hematological, and testicular toxicity [58]. The toxic, short-lived metabolite, semiquinone form of doxorubicin, triggers the generation of reactive oxygen species (ROS) through cascades of events. Doxorubicin-induced cardio, hepatic, testicular, and nephrotoxicities have been attributed to ROS generation, inflammatory processes, and lipid peroxidation [22,23]. Doxorubicin has also been proposed to promote free radical generation by enhancing the activities of extramitochondrial oxidative enzymes such as NADPH and xanthine oxidases and also interferes with mitochondrial iron export [5]. These free radicals cause distortion of the cell's membranes and cause organ dysfunction. Research on plants that can counteract the toxic effects of doxorubicin have been ongoing, especially on *T. occidentalis.*

Telfairia occidentalis Hook is a fluted pumpkin of the *Cucurbitaceae* family widely consumed as food in Nigeria [35]. 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 [53]. The seeds are very nutritious and are eaten roasted or boiled. The seed extract has been reported to exert antidiabetic [16], cellular antioxidant, immunodulatory, anticancer, antiinflammatory [36], antiplasmodial [35], antioxidant [40], analgesic [37,40], genotoxic and cytotoxic [30], in vivo inhibitory effect on alpha amylase and alpha glucosidase [15], genotoxic and cytotoxic [30], antiulcer [53] and antiprostatic [18] activities. Phytochemical studies of the extract have shown the presence of alkaloid, flavonoid, tannins, terpenes, saponin, and cardiac glycosides [12]. Phytochemicals such as pentadecanoic acid, hexadecanoic acid; 16-octadecenoic acid methyl ester; 9, 12-octadecadienoyl chloride (Z,Z); 9-octadecadienoic acid (Z)-, 2, 3-dihydroxypropyl ester; octadecanoic acid; hexadecanoic acid,2,3-is[(trimethylsilyl) oxy] propyl ester, 2,4-heptadien-6-ynal,(E,E); benzoic acid ; dodecanoic acid ; linoleic acid ethyl ester ; hexadecanoic acid, methyl ester ; α-phellandrene ; α-campholene aldehyde; terpinen-4-ol ; trans-β-ocimene ; borneol and stigmastan-3- ol, have been reported in the seed extract from GCMS analysis [36]

and HPLC characterisation of the seed extract and fractions revealed the presence of eleven flavonoids; kaempferol, catechin, epicatechin, anthocyanidin, narigenin, flavonones, flavones, narigenin,rutin, naringin, and resveratol in the seed extract and fractions with butanol fraction having the highest concentration. Also, alkaloids such as ribalinidine, ammodendrine, spartein and lunamarin were also found in the seed extract and fractions [53].

The present study was designed to evaluate the activities of seed extract and fractions of *T.occidentalis* against doxorubicin-induced testicular toxicity in rats.

2. Materials and Methods

Plant collection

Fresh seeds of *Telfairia occidentalis* were purchased from Itam market in Itu L. G. A, Akwa Ibom State, Nigeria, in June, 2023. The seeds were previously identified and authenticated by a taxonomist in the Department of Botany, University of Uyo, Uyo, Nigeria. Herbarium specimens (UUPH 1(b)) were deposited at the Department of Pharmacognosy and Natural Medicine Herbarium, University of Uyo.

The fresh seeds of the plant were dried on a laboratory table for 2 weeks and reduced to powder. The seed powder (1 kg) were separately macerated in 50% ethanol (5000 mL) for 72 hours. The liquid filtrates obtained were concentrated at 40˚C and all the ethanol was completely removed. The crude extract (20 g) was dissolved in 500 mL of distilled water and partitioned with equal volume of dichloromethane (DCM, 5 x 500 mL) till no colour change was observed, to obtain DCM and aqueous fractions. The extract and fractions were stored at 4˚C in a refrigerator until used for the experiment.

Animals

In this study, male albino Wistar rats (150-200 g) were used. The animals were sourced from the University of Uyo Animal House and sheltered in plastic cages. The rats were fed with pelleted standard Feed (Guinea feed) and given unlimited access to water. The study was approved by College of Health Sciences Animal Ethics Committee, University of Uyo.

Experimental design

In this study, the repeated dose model earlier described [38,43] was used, which lasted for 14 days was used. Groups I rats, which served as the untreated control were orally pretreated with 10 mL/kg/day of distilled water. Group 2 rats were given normal saline (10 mL/kg/day) but equally treated on alternate days with 1.66 mg/kg of doxorubicin hydrochloride dissolved in 0.9% normal saline for 14 days. Groups 3 - 5 rats were orally pretreated, respectively with 138 mg/kg/day, 276 mg/kg/day, and 553 mg/kg/day of *Telfairia occidentalis* dissolved in 10% Tween 80, one hour before treatment with 1.66 mg/kg of doxorubicin in 0.9% normal saline administered intraperitoneally on alternate days for 14 days. Groups 6 and 7 were pretreated with 276 mg/kg of DCM and aqueous fractions respectively, and also treated with 1.66 mg/kg of doxorubicin in 0.9% normal saline intraperitoneally on alternate days for 14 days. Group 8 rats which served as the positive control group were equally pretreated with 100 mg/kg/day of silymarin one hour before treatment with 1.66 mg/kg of doxorubicin in 0.9% normal saline administered intraperitoneally on alternate days for 14 days.

Collection of blood samples and organs

After 14 days of treatment (24 hours after the last administration) the rats were weighed again and sacrificed under light diethyl ether vapour. Blood samples were collected by cardiac puncture into plain centrifuge tubes and left for one hour. The blood samples were then centrifuged at 1500 rpm for 15 mins to separate of serum at room temperature and used for biochemical assays. The testes of the rats were surgically removed and weighed. One testis was fixed in 10 % formaldehyde for histological processes, while the other testis was briskly rinsed in ice cold 1.15% KCl solution and stored in ice cold 0.9% NaCl in a clean sample bottle.

Evaluation of progressive motility,viability,count,and the structural abnormality of sperm

The caudal piece of epididymis was isolated to retrieve the sperm samples. Initially, the epididymal part was finely minced in 5 mL of physiological-saline and was incubated for 30 min at 37 ◦ C for spermatozoa release of the epididymal ducts. Sperm progressive motility percentage was noted through the phase-contrast microscope at 400 X [25]. Sperm viability was assessed, by eosin or nigrosin staining, accompanied by microscopic evaluation. Moreover, a hemocytometer was employed to count epididymal sperm in the suspension [59]. Furthermore, morphological anomalies of the head, tail, and mid piece of sperm were determined in percentage using the method of Filler [19]. The apparent abnormal characteristics included (i) the size and shape of spermatozoa heads (bigor small heads) with lighter and emphasized curvature;(ii) intermediary pieces' defects that result in untied heads; and (iii) defects of tails (short, multiple, folded, and broken tails).

Biochemical Assays

Effect of the seed extract and fractions on testis oxidative stress markers

The oxidative markers assays were performed on testes homogenates of rats that were used in this study in order to assess antioxidative stress potentials of the extract and fractions. Homogenates of the stored testes samples were made in a ratio of 1 g of wet tissue to 9 mL of 1.25% KCl by using motor driven Teflon-pestle. The homogenates were centrifuged at 7000 rpm for 10 min at 4˚C and the supernatants were used for the assays of superoxide dismutase (SOD) [31], catalase (CAT) [46], glutathione peroxidase (GPx) [29], reduced gluthathione (GSH) [13], and malondialdehyde (MDA) content [17].

Histopathological studies

The excised testes fixed in 10 % buffered formalin were used for histological processes. They were processed and stained with haematotoxylin and eosin (H&E) [11], according to standard procedures at the Department of Chemical Pathology, University of Uyo Teaching Hospital, Uyo, Nigeria. Morphological changes observed and recorded in the excised organs of the sacrificed animals. Histologic pictures were taken as micrographs.

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 ± SEM and significance relative to control was considered at $p<0.001$ and $p<0.05$.

3. Results

Effect of seed extract and fractions of *T. occidentalis* on body and testes weights of rats with doxorubicin-induced toxicity

Administration of *T.occidentalis* seed extract and fractions to rats with doxorubicin-induced organs toxicities caused considerable improvement of body weights compared to the organotoxic group. The crude extract caused a significant ($p<0.01$) dose-dependent effect when compared to the organotoxic group, with the dichloromethane fraction-treated group exerting the highest effect. The testis weight of the group treated with doxorubicin only was found to be reduced when compared to that of the normal control group though not statistically significant ($p>0.05$). However, treatment of rats with doxorubicin-induced toxicities with the seed extract and fractions of *T.occidentalis* improved the testis weights though insignificantly ($p>0.05$), except in the group treated with aqueous fraction (Table 1).

Effect of *T. occidentalis* seed extract and fraction on testes oxidative stress markers of doxorubicin-induced testes toxicity:

Table 2 shows the effect of *T.occidentalis* seed extract/fractions on testes oxidative stress markers of the rats. Administration of doxorubicin (1.66 mg/kg i.p) on alternate days for 14 days caused significant ($p<0.05$-0.001) decreases of CAT activity and GSH levels when compared to control, while SOD and GPx were insignificantly decreased. The MDA level was also significantly ($p<0.01$) elevated by doxorubicin treatment when compared to the normal control. However, concomitant administration of seed extract/fractions of *T. occidentalis* (138 - 553 mg/kg) with doxorubicin for 14 days caused significant ($p<0.05$-0.001) and non-dose-dependent elevations of GPx and GSH activities in the treated rats groups when compared to the organotoxic groups, with the DCM fraction exerting the highest effect. Dose-dependent and significant ($p<0.05$-0.001) increase in SOD levels of the extract/fractions treated groups were recorded with DCM having the highest effect. Similarly, CAT activity was elevated in extract/fractions treated groups non dose-dependently with the middle dose (126 mg/kg) and silymarin treated groups having the highest effect. MDA levels in various extract/fractions treated groups were non-dose-dependently decreased with the most significant effect ($p<0.001$) recorded in DCM fraction treated group when compared to organotoxic control. (Table 2).

Effect of the seed extract and fractions of *T. occidentalis* on serum testosterone level of rats with doxorubicin-induced testicular toxicity

Administration of doxorubicin (1.66 mg/kg i.p) on alternate days for 14 days was found to significantly ($p<0.001$) cause decreases of serum testosterone levels of rats when compared to controls. However, concomitant administration of seed extract and fractions of *T.occidentalis* (138-553 mg/kg) and silymarin with doxorubicin for 14 days caused significant ($p<005$-0.01) dose-dependent elevation of the testosterone levels of the treated rats when compared to the organotoxic groups with the DCM fraction having the highest effect (Figure 1).

Effect of seed extract and fraction of on Seminal analysis of rats with doxorubicin –induced organs toxicities

Table 3 shows the seminal analysis of semen from rats with doxorubicin–induced organs toxicities. The semen in all treatment groups was found to be milky in appearance, while the semen volume in the group administered with doxorubicin alone was found to be small (0.01 mL). There was a marked improvement in the semen volume of rats treated with the seed extract and fractions (0.02 -0.04 mL) with the DCM fraction-treated group having the highest volume (0.043 mL). The PH of the semen samples from all the groups was 8.0. The extract/fraction-treated groups were found to have a higher percentage of viable sperm cells (78-90%) compared to the doxorubicin only-treated group (55%). silymarin treated group had 90% viable cells while control had 80%. Viscosity of semen in all the groups was normal. The average number of cells was 33.25×10^6 in the doxorubicin only treated group, but non-dose dependent improvements were observed in extract / fractions treated groups with high dose (553 mg/kg) having 130.75×10^6 cells, while DCM fraction treated group recorded average cell of 126.75×10^6 cells and silymarin group 91.0×10^6 cells. The percentage of active sperm cells in the extract-treated groups ranged from 55-80% non non-dose-dependent compared to 50 % recorded in the testosterone only group. The percentage of dead sperm cells in the doxorubicin only group was found to be about 10% compared to 5% in the middle dose (276 mg/kg) and high dose (553 mg/kg) treated groups as well as the groups treated with the various fractions. The standard drug (silymarin) group had 5 % dead sperm cell (Table 3).

Table 1: Effect of T.occidentalis seed extract on body and testes weights of rats with doxorubicin-induced toxicity

PARAMETERS/ TREATMENT	Dose mg/kg	Testis	Body weight		
			Before	After	% increase in body weight
Normal control	-	2.74±0.16	176.28±17.97	198.25± 6.61	12.46
Doxorubicin	1.66	2.44±0.10	169.66 ± 6.80	181.66±13.24	5.72
Silymarin+DOX	100	2.59±0.23	180.33± 10.86	190.66± 13.24	5.72
Extract+DOX	138	2.99±0.17	176.0± 11.13	191.0± 6.08	8.52
	276	2.62±0.10	167.0± 7.57	185.0 ± 8.73	10.77
	553	2.74±0.25	177.66± 7.42	193.33 ± 5.48	8.82
Aqueous fraction	276	2.65±0.05	187.66± 17.89	195.33± 17.70	4.08
DCM fraction	276	2.72±0.21	162.66± 12.66	180.33± 9.56	10.86

Data are expressed as mean ±SEM. significant at dp<0.001 when compared to normal control; ap< 0.05, bp< 0.01, cp< 0.001 when compared to organotoxic control. n = 5

Table 2: Effect of T.occidentalis seed extract and fractions on testes oxidative stress markers of rats with doxorubicin-induced toxicity

Treatment	Dose mg/kg	SOD (U/mL)	CAT (U/g of protein)	GPx (µg/mL)	GSH (µg/mL)	MDA (µMol/mL)
Control	10	0.17± 0.01	3.51±0.40	0.043±0.0008	1.92±0.03	0.43±0.02
Doxorubicin	1.66	0.11±0.01	1.39± 0.26^{a}	0.036±0.002	1.64±0.06^{b}	0.61±0.01^{c}
Crude extract	138	0.20±0.01	3.63± 0.43^{e}	0.031±0.0008^{b}	1.42±0.04^{f}	0.53±0.01
	276	0.22±0.03^{d}	4.30±0.35^{f}	0.044±0.0005^{d}	2.06± 0.03^{f}	0.54±0.01
	553	0.15±0.08^{e}	3.54± 0.02^{d}	0.042±0.0001	1.92± 0.06^{e}	0.61±0.01^{c}
Aqueous Fraction	276	0.24±0.04^{e}	2.66±0.06	0.041±0.0008	1.89±0.008^{d}	0.49± 0.03^{d}
DCM fraction	276	0.40±0.02^{c,f}	3.63±0.43^{e}	0.052±0.002^{f}	2.30± 0.09^{c,f}	0.30± 0.06^{a,f}
Silymarin	100	0.21±0.02^{d}	4.31±0.88^{f}	0.044±0.001^{d}	1.96±0.02^{f}	0.52±0.01

Data are expressed as MEAN ± SEM, Significant at ap<0.05, bp<0.01, cp<0.001, when compared to control;Significant at dp<0.05, ep<0.01, fp<0.001 compared to organotoxic group. (n=5)

Table 3: Effect of seed extract and fractions of Telfairia occidentalis on seminal analysis of rats with doxorubicin-induced testicular toxicity

Parameters	Normal Control	Doxorubicinonly	Silymarin	Extract 138 mg/kg	Extract 276 mg/kg	Extract 553 mg/kg	Aqueous fraction	DCM fraction
App	Milky	Milky	milky	milky	milky	milky	milky	milky
Volume	0.03ml	0.01ml	0.02ml	0.02ml	0.03ml	0.03ml	0.03ml	0.043ml
PH	8.0	8.0	8.0	8.0	8.0	8.0	8.0	8.0
Viability	80%	55%	90%	78%	80%	90%	90%	90%
Viscosity	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
Average cells (cell x10^{6})	108.50	60.24	125.75	114.50	78.75	130.75	97.00	116.75
Normal	70%	65%	75%	70%	75%	65%	70%	70%
Abnormal	30%	35%	25%	30%	25%	35%	30%	30%
Active	90%	50%	70%	60%	70%	55%	60%	80%
Sluggish	5%	40%	25%	30%	25%	40%	35%	15%
Dead	5%	10%	5%	10%	5%	5%	5%	5%
Sperm	500	200	600	400	450	600	600	600

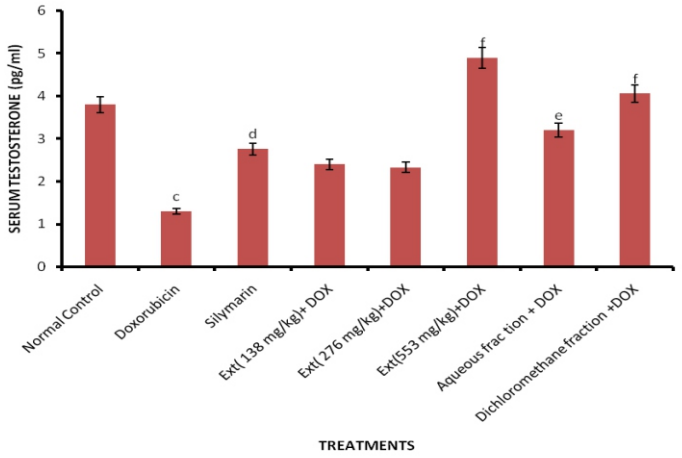


Figure 1: Effect of T. occidentalis seed extract and fractions on serum testosterone levels of rats with doxorubicin-induced testicular toxicity.

Data are expressed as MEAN ± SEM, Significant at ^{c}p<0.001 when compared to normal control; ^{d}p<0.05; ^{e}p<0.01; ^{f}p< 0.001, when compared to organotoxic control. (n=5).

Effect of seed extract and fractions of *T. occidentalis* on histology of rat testis in doxorubicin-induced testicular toxicity

Histological sections of testes of rats receiving various treatments at magnification (x100) stained with H&E method revealed that Group 1 (normal control, CONT) rat treated with distilled water (10 mL/kg) showed A normal histo-architecture with well-presented seminiferous tubules having well-defined basement layer, well-lined spermatogenic cells and arrays of spermatozoa within the tubular lumen, and well-presented Leydig cells and blood vessels within the interstitial connective tissue and no evidence of pathological changes were seen. The organotoxic group (Group 2, T+CONT) treated with doxorubicin (1.66 mg/kg) showed an artrophying histo-architectural, with areas of spermatogenic cells degeneration and altered spermatogenic processes, with widened tubular lumen, vacuolated and degenerating leydig cells within the interstitial space and degenerating spermatogenic cells within the seminiferous tubules (Figure 2). Rats in group 3 (T+STD) treated with 100 mg/kg of silymarin and doxorubicin (1.66 mg/kg) showed mildly affected testicular tissue, demonstrating a mild histo-architectural alteration, with area of vacuolated and degenerating leydig cells, within the interstitial connective tubule. Group 4 (T+LDE) treated with 138 mg/kg of *T.occidentalis* seed extract and doxorubicin (1.66 mg/kg) showed a moderately affected testicular tissue demonstrating moderate histo-architectural alteration, with area of altered spermatogenic processes, with widened tubular lumen, within the seminiferous tubules. Group 5 (T+MDE) treated with 276 mg/kg of *T.occidentalis* seed extract and doxorubicin (1.66 mg/kg) showed a moderately affected testicular tissue, demonstrating moderate histo-architectural alteration, with an area of altered spermatogenic processes having a widened tubular lumen, within the seminiferous tubules. Group 6 (T+HDE) treated with 553 mg/kg of *T.occidentalis* seed extract and doxorubicin (1.66 mg/kg) showed testicular tissue demonstrating moderate histo-architectural alteration, with area of altered spermatogenic processes, with widened tubular lumen, within the seminiferous tubules. Group 7 (T+AQE) treated with 276 mg/kg of aqueous fraction of *T.occidentalis* seed and doxorubicin (1.66 mg/kg) showed a normal histo-architecture with well-presented seminiferous tubules having well-defined basement layer, well-lined spermatogenic cells and arrays of spermatozoa within the tubular lumen and well-presented Leydig cells within the interstitial connective tissue. Group 8 (T+DCME) treated with 276 mg/kg of dichloromethane fraction of *T.occidentalis* seed and doxorubicin (1.66 mg/kg) showed a moderate histo-architectural alteration, with an area of altered spermatogenic processes having widened tubular lumen within the seminiferous tubules (Figure 2).

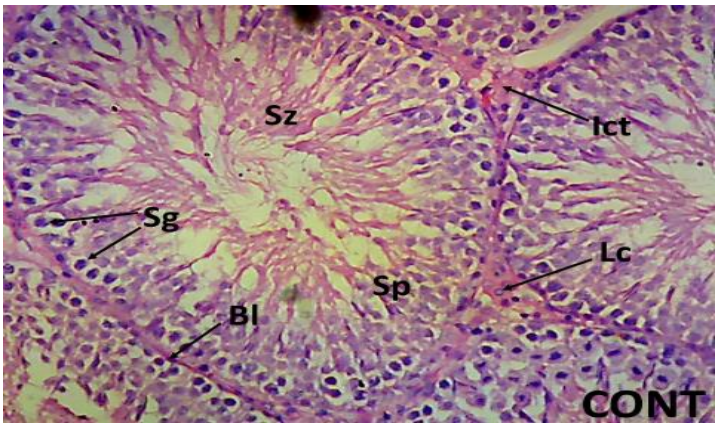
Effect of seed extract and fraction of *T.occidentalis* on lipid profile of rats with doxorubicin –induced organs toxicities

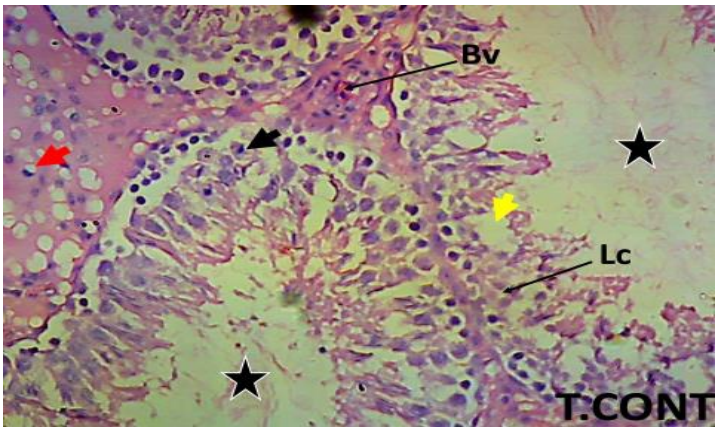
Administration of doxorubicin (1.66 mg/kg) was observed to caused significant ($p<0.05$-0.001) elevation in levels of total cholesterol, triglyceride, high density lipoprotein, low density lipoprotein, and very low density lipoprotein. These raised levels of total cholesterol, triglyceride, high-density lipoprotein, low density lipoprotein, and very low density lipoprotein were significantly ($p<0.05$-0.001) reduced when compared to organotoxic group following concomitant treatment with seed extract and fractions of *T. occidentalis* and silymarin. However, the reductions in total cholesterol and low density lipoprotein were dose-dependent, while non dose-dependent reductions were observed in triglyceride, high density lipoprotein and very low density lipoprotein levels (Table 4).

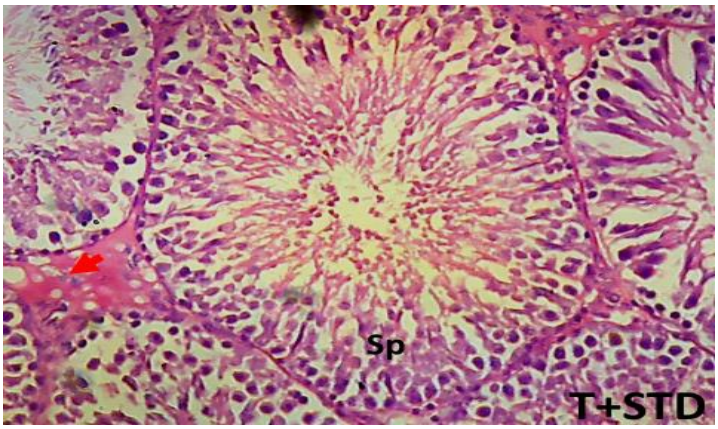
Table 4: Effect of T. occidentalis seed extract and fractions on lipid profile parameters of rats with doxorubicin-induced toxicity

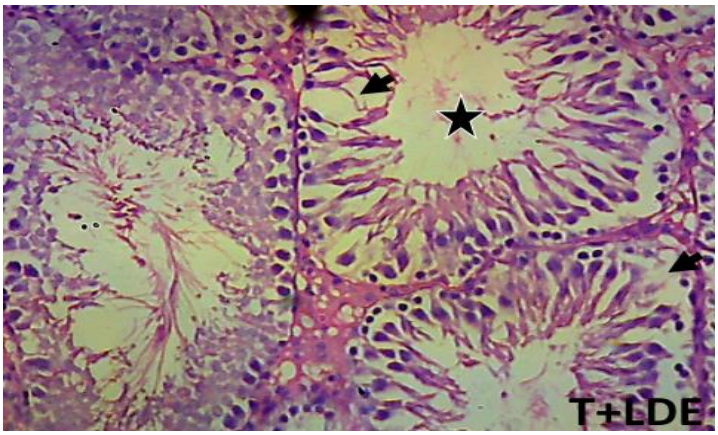
Treatment	Dose mg/kg	Total Cholesterol (mMol/L)	Triglyceride (mMol/L)	HDL-C (mMol/L)	LDL-C (mMol/L)	VLDL (mMol/L)
Control	10	3.33± 0.20	1.21±0.03	1.30±0.02	2.70± 0.22	0.56± 0.02
Doxorubicin	1.66	4.60± 0.15^{b}	1.55±0.05^{c}	1.60±0.06^{a}	3.70± 0.09^{c}	0.70± 0.02
Crude extract	138	3.80±0.11	1.31± 0.03^{e}	1.44±0.01	2.95± 0.11^{e}	0.60±0.01
	276	3.33±0.23^{f}	1.35±0.03^{d}	1.45±0.04	2.49± 0.20f	0.61±0.01
	553	2.63±0.17^{f}	1.14±0.02^{f}	1.23±0.04^{e}	1.92± 0.13^{f}	0.51±0.01^{e}
Aqueous Fraction	276	2.53±0.17^{f}	1.08±0.07^{f}	1.23±0.06^{e}	1.78± 0.15^{a,f}	0.49± 0.03^{e}
DCM fraction	276	2.40±0.17^{f}	1.08±0.02^{f}	1.19±0.07^{e}	1.70± 0.14^{a,f}	0.49± 0.04^{e}
Silymarin	100	3.00±0.28^{f}	1.09±0.06^{f}	1.20±0.07^{e}	2.29± 0.24^{f}	0.48± 0.04^{e}

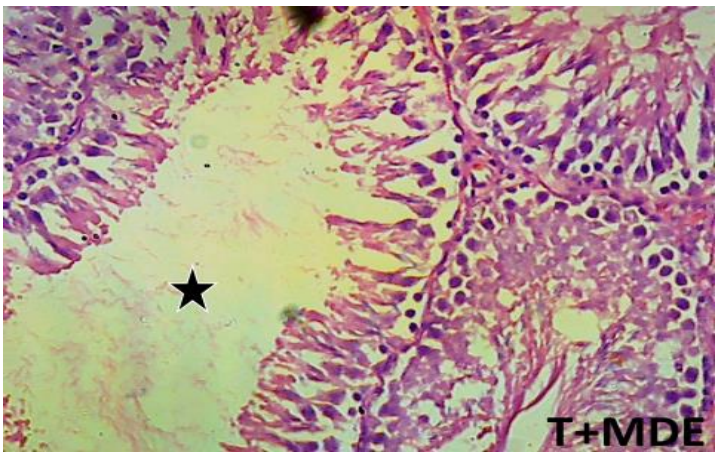
Data are expressed as MEAN ± SEM, Significant at $^{a}p<0.05$, $^{b}p<0.01$, $^{c}p<0.001$, when compared to control; Significant at $^{d}p<0.05$, $^{e}p<0.01$, $^{f}p<0.001$ compared to organotoxic group. (n=5)

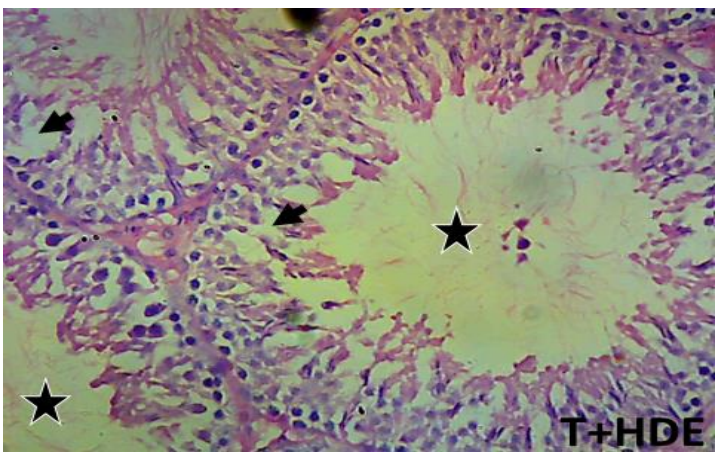












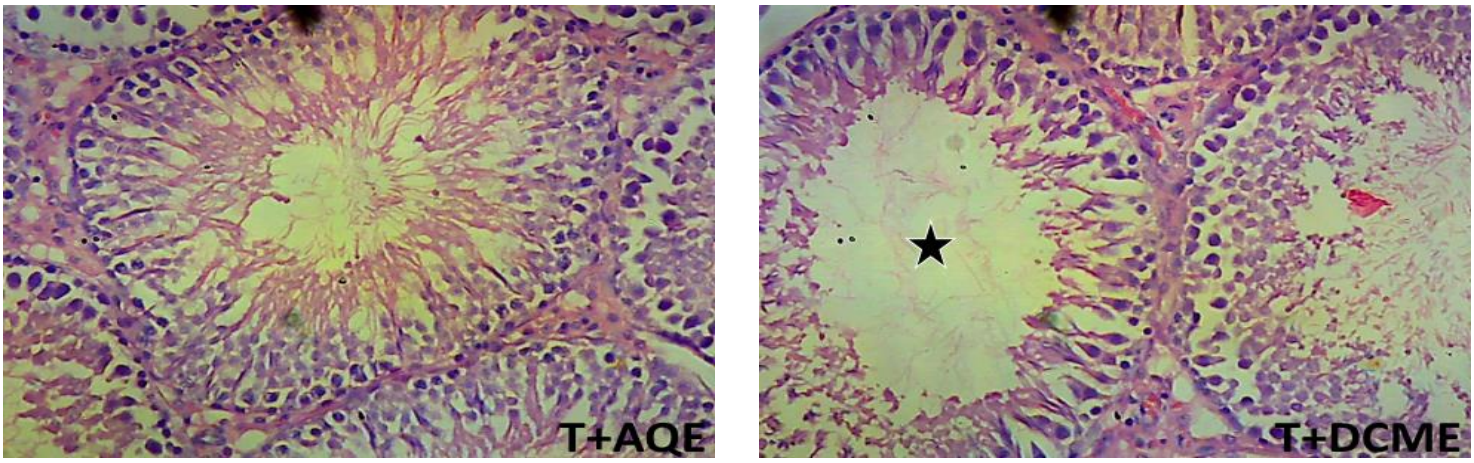


Figure 2: Photomicrographs of sections of testes of rats treated with distilled water (**CONT**), doxorubicin only,1.66 mg/kg (**T.CONT**), Sylimarin,100 mg/kg and DOX(**T+STD**), *T occidentalis*, 138 mg/kg and DOX (**T+LDE**),*T.occidentalis*, 276 mg/kg and DOX (**T+MDE**), *T.occidentalis,* 553 mg/kg and DOX (**T+HDE**), Aqueous fraction,276 mg/kg and DOX (**T+AQE**) and DCM fraction, 276 mg/kg and DOX (**T+DCME**) showing well-defined basement layer (Bl), well-lined spermatogenic cells (Sp) and arrays of spermatozoa (Sz) within the tubular lumen, and well-presented Leydig cells (Lc) and blood vessels (Bv), spermatogenic cells degeneration and altered spermatogenic processes (red arrow), with widened tubular lumen (black star), vacuolated and degenerating leydig cells (red arrow) within the interstitial space and degenerating spermatogenic cells (black arrow) within the interstitial connective tissue (H&E x100)

4. Discussion

Doxorubicin, an anthracycline use in the treatment of tumors [27,51], exhibits serious undesirable effects on the male reproductive system [48], such as disruption of normal reproductive functions via the induction of oxidative stress. Investigations of agents with antagonistic potentials to counteract the toxic effects of doxorubicin are ongoing. This study was carried out to assess effects of seed extract of *T. occidentalis* on doxorubicin-induced male reproductive system toxicity.

The exact mechanisms through which doxorubicin induces toxicity in male reproductive system, though reported widely, have not been properly elucidated. The drug reportedly retards testicular growth, suppresses spermatogenesis, leading to male infertility through imposing oxidative stress and cellular apoptosis [57]. It also causes double-strand DNA breaks and cell death by intercalating into DNA strands [20,27] in meiotically dividing spermatocytes and spermatogonia [6]. Spermatogenesis suppression, sperm motility impairment, increase percentage of abnormal spermatozoa, decreased body and testicular weights, reduced testosterone level and testicular failure through oxidative stress and cell apoptosis in testicular tissue have also been reported [21]. In this study, doxorubicin was found to cause significant increase in the percentages of dead and abnormal cells. Also decreased seminal volume and sperm count were observed in the doxorubicin alone treated group. Similarly, testes from rats treated with doxorubicin alone were found to have marked degenerated germ cells and spermatozoa, supporting previous reports [21]. However, all these toxic effects of doxorubicin were alleviated by concomitant administration of seed extract and fractions of *T.occidentalis,* as animals treated with the seed extract/fractions had high sperm cell count, greater seminal volume, low level of dead cells as well as normal testicular architecture, portraying the extract's potentials in preventing doxorubicin-induced testicular toxicity probably through the antioxidative stress activity of its phytochemical components. The antioxidative burst and antioxidant activities of the seed extract and fractions of *T.occidentalis* previously reported [34,36,40] may be responsible for these activities.

Moreso, the findings observed in this study further support the antioxidant potentials of the plant. These activities may have contributed to the observed protective effects in this study.

The membranes of the male germ cells have a high amount of polyunsaturated fatty acids which is one of the targets of reactive oxygen species [45]. Thus, spermatozoa are vulnerable to oxidative damage because of the high amount of lipids in their membranes, and this makes them lose their integrity and become less motile [45]. This effect could have contributed to the low sperm count, high percentages of dead and abnormal cells observed in the group treated with doxorubicin only in this study. Doxorubicin has been shown to impair male fertility by causing germ cell oxidative stress and apoptosis [1,14]. It has also been demonstrated to impair spermatogenesis [24] and steroidogenesis [31,39,44]. Exposure to doxorubicin appears to affect testicular integrity at both prepubertal and post-pubertal stages of development. *In vitro* studies with prepubertal mouse testis have demonstrated significant loss in germ cell number following exposure to doxorubicin at concentrations that were equivalent to human therapeutic doses [47]. Further, studies have demonstrated early testicular developmental arrest and long-term germ cell DNA damage following prepubertal doxorubicin exposure [54]. These effects were observed in the study as marked degenerated germ cells and spermatozoa were seen in the histological sections of the testes. However, concomitant administration of the seed extract/fractions protected the testes as the toxic effects observed in the doxorubicin alone treated group were absent or mild compared to the group treated with doxorubicin alone. The high susceptibility of testes and sperm to doxorubicin -induced testicular oxidative stress may be due to a weak anti-oxidant defense system in testicular tissue and semen [33]. Besides, earlier studies demonstrated that antioxidant supplementation improved the quality of the semen profile in infertile men [50].

The levels of lipid profile indices such as total cholesterol, triglyceride, HDL, LDL and VLDL were significantly elevated in doxorubicin only treated group when compared to normal control group. These observations are in accordance with the previous reports [2,42].

However, these increases in lipid profile parameters were reduced significantly when compared to organotoxic group following concomitant treatment with seed extract and fractions of *T. occidentalis.*

Lipids are a very important part of the reproductive system. Cholesterol is considered to be the precursor of steroid hormones. Steroidogenesis plays an important role in the synthesis of spermatogenesis hormones. The biosynthesis of testosterone from pregnenolone is carried out by steroidogenesis enzymes including 17β hydroxysteroid dehydrogenase (17β-HSD) 3β and hydroxysteroid dehydrogenase (3β- HSD). It has been reported that doxorubicin result in the downregulation of these enzymes [41,49]. Adipocytes are the main sites for triacylglycerol storage. It has been found that DOX downregulates adipogenesis *in vitro* by decreasing the expression of PPARγ [3]. Doxorubicin inhibits spermatogenesis by causing defects in epididymal adipose tissue [52], which is very important for normal spermatogenesis [9]. Also, studies using adult rat models of DOX exposure have reported decreased testosterone, follicle-stimulating hormone (FSH), and luteinizing hormone (LH) levels, decreased sperm count, motility and viability, and increased abnormally formed spermatozoa [10]. The findings of this investigation show that the seed extract and fractions were able to prevent the toxic effects of doxorubicin on the male reproductive system as evident in the reduced lipid profile indices and improved testosterone levels and sperm densities in the extract/fractions-treated groups. This suggests that the seed extract and fractions may have enhanced steriodogenesis as well as spermatogenesis, which are inhibited by doxorubicin. This result further confirms the testicular protective potential of the *T.occidentalis* seed extract.

Oxidative stress plays an essential role in doxorubicin-induced toxicity through the formation of reactive oxygen species (ROS) [4]. The results of the doxorubicin alone treated group in this study revealed significant elevation of testicular MDA levels and a significant decrease in testicular SOD, CAT, GPx and GSH when compared with the DOX group. These results are in accordance with previous reported studies [26]. However, co-treatment of seed extract/fractions and DOX elevated the levels of these endogenous antioxidants revealing the free radicals scavenging potentials of the seed extract/fractions and its antioxidative stress activity, which is due to the activities of its phytochemical constituents, such as flavonoids, monoterpenes and polyunsaturated fatty acids previously reported [36,53] to be present in this seed extract.

The dichloromethane and butanol fractions of the seed extract has been found to contain some pharmacological active compounds especially flavonoids like kaempferol, catechin, epicatechin, anthocyanidin, rutin flavones, flavonones, narigenin, naringin among others which are potent antioxidant compounds [53] and are likely to contribute to the observed activities in this study. Kumar *et al.* [28] had reported on the activities of some phyto-components with compound nature of flavonoids; palmitic acid (hexadecanoic acid ester and n-hexadecanoic acid), unsaturated fatty acid and linolenic (docosatetraenoic acid and octadecatrienoic acid) as anti-inflammatory, antioxidant, hypocholesterolemic, cancer preventive, hepatoprotective, among others. These compounds could have contributed to the observed anti-inflammatory, antioxidant, hypocholesterolemic, cardioprotective, renoprotective, testiculoprotective and hepatoprotective activities of the seed extract observed in this study.

Besides, antioxidant compounds such as borneol and terpen-4-ol[8,56], present in the extract may have played a role in the antioxidant/antioxidative stress activity observed in this study. Similarly, phytosterols such as Stigmastan-3-ol, 5-chloro-, acetate, (3a, 5a)-, present in this extract have been reported to have preventive effects on the development of diseases due to reactive oxygen species [55]. Moreover, Yoshida and Niki [60] showed the antioxidant effects of the phytosterols against lipid peroxidation. This radical scavenging activity of the phytochemical components of this extract could have accounted for all the activities observed in this study and may be the mechanism of action of the seed extract.

2. Conclusion

The findings of this study showed that the root extract and fractions of *Telfairia occidentalis* possess testicular protective potentials against doxorubicin-induced testicular toxicity. These properties can be attributed to the antioxidant and antioxidative stress activities of its phytochemical constituents. Thus, the root can be use to alleviate and/or prevent doxorubicin-induced male reproductive system toxicity.

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Conflicts of interest

There is no conflict of interest

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