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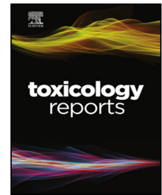

Histomorphometric studies of the effects of *Telfairia occidentalis* on alcohol-induced gonado-toxicity in male rats

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ABSTRACT

Background: Available evidence suggests that 50% of couples with infertility are male related. Over 40% of these males consume alcohol which has been reported to be a reproductive toxicant causing depletions in the epithelium of seminiferous tubules hence reducing sperm counts and sperm morphology.
Objective: To determine the effects of aqueous leaf extract of *Telfairia occidentalis* on alcohol-induced cyto-architectural changes in the testis.
Methods: Aqueous leaf extract of *Telfairia occidentalis* (*T. occidentalis*) was administered by gastric gavage at a dose of 250 mg/kg and 500 mg/kg body weight daily, while 2 g/kg body weight of ethanol at 30% v/v was administered daily to mature male Sprague–Dawley rats. The experiment was in 2 phases. Phase 1 had groups A_1–F_1 and lasted for 4 weeks while phase 2 had groups A_2–F_2 and lasted 8 weeks. Parameters tested include: testicular histology, relative volume density, sperm parameters, malondialdehyde (MDA), superoxide dismutase (SOD) and reduced glutathione.
Results: In both phases, there were depletions in the seminiferous epithelium, decreased sperm quality and increased MDA and SOD in animals that received alcohol only compared to control. Likewise, a significant increase of seminiferous epithelium of animals that received respective doses of 250 mg/kg and 500 mg/kg of *T. occidentalis* only compared to control. Animals that received *T. occidentalis* and alcohol simultaneously had a significant increase in seminiferous epithelium and sperm quality with decreased MDA level.
Conclusion: *T. occidentalis* attenuated the deleterious effects of alcohol to the cyto-architecture of the testis, protected the seminiferous epithelium, reduced oxidative stress and promoted spermatogenesis.
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1. Introduction

To most couples the desire to have one's own biological children is strong and compelling. The effects of infertility on these couples can be devastating. Infertility leads to psychological stress, anxiety and depression Centers for Disease Control and Prevention [CDC], 2012 [11]. There are over 186 million couples in developing countries alone (excluding China) who are affected by infertility [51]. Rates of infertility vary considerably from country to country; in the worst-affected areas, over 25% of couples may be unable to have children [39].

On a practical level, many families in developing countries depend on children for economic survival. So, while many people would not consider infertility a disease in itself, it is certainly a social and public health issue as well as an individual problem [51].

In Nigeria, the data on infertility indicates that disorders in males and females account for an equal proportion of infertility with the male factor being associated with a greater percentage of primary infertility [33]. Available evidence reveal that male factor infertility has not been given due prominence in issues of reproductive health [21,38,41].

[38], had attributed aspects of male infertility to alcohol consumption. Records have it that, over 42% of males with infertility are alcohol consumers [12,38]. Several studies have shown that alcohol impairs sexual performance and desire. It also decreases testosterone levels, sperm count and sperm motility contributing to fertility problems [50,6]. Studies on humans have even shown

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that heavy consumption of alcohol adversely affects sperm count [20,25]. In addition, there is evidence suggesting that long term use of alcohol will lead to permanent damage of the male gonad, including a permanent reduction in the growth and maturation of male gametes [50,52]. This may be due to the fact that alcohol atrophies the cytoplasmic mass of Leydig cells responsible for the secretion of the male sex hormone – testosterone [50]. Any decrease in testosterone will adversely affect spermatogenesis [47].

In spite of these deleterious effects, alcoholic beverages are still widely consumed domestically and socially. In our previous study, animals administered alcohol showed a correlation between decreased testosterone levels and increased oxidative stress [17]. This suggests that alcohol may be acting through oxidative stress-inducing mechanisms. Therefore, our hypothesis is that reduction in alcohol-induced oxidative stress will enhance fertility.

Antioxidants such as flavonoids, ascorbic acid and tocopherol have been reported to significantly reduce oxidative stress and improve sperm parameters [15,34]. These antioxidants and other phytochemicals have been reported present in *Telfairia occidentalis* (*T. occidentalis*) [23].

T. occidentalis is an important staple vegetable consumed in Nigeria and several other countries. The leaves are rich in minerals (such as calcium, potassium, sodium, phosphorus and magnesium), iron, antioxidants, vitamins (such as thiamine, riboflavin, nicotinamide, ascorbic acid, retinol and tocopherol). and phytochemicals such as phenols, Tannins, and Flavonoids [23,28,30]. It has a high profile of aminoacids which include; alanine, aspartate, glycine, glutamine, histidine, lysine, methionine, tryptophan, cystine, leucine, arginine, serine, threonine, phenylalanine, valine, tyrosine and isoleucine [49,23]. In view of these profertility components of *T. occidentalis*, this study is aimed at determining the effects of *T. occidentalis* on alcohol-induced cyto-architectural changes in the testis.

2. Materials and methods

2.1. Extract preparation

Healthy *T. occidentalis* plant was obtained from a local market in Lagos, Nigeria. It was authenticated in the Department of Botany of the University of Lagos and a specimen was deposited in the herbarium with a voucher number – LUH 2763. One kilogram (1 kg) of freshly cut *T. occidentalis* leaves with the stalk was separated from the stem, washed with clean water to remove dirt and sand. The water was drained and the leaves chopped. This was then squeezed and filtered with sieve to obtain an aqueous extract of the *T. occidentalis* leaves. The aqueous leaf extract was prepared at intervals of 3 days, refrigerated and served to the animals fresh [40].

2.2. Administration of extract and Alcohol

Aqueous leaf extract was administered orally by gastric gavage at a dose of 250 mg/kg and 500 mg/kg body weight daily Saalu et al. [44], while 2 g/kg body weight of ethanol was administered orally daily at a concentration of 30% v/v [17].

2.3. Experimental animals

A total of 60 adult male Sprague–Dawley rats weighing between 170 and 200 g were randomly selected from the Nigerian Institute of Medical Research (NIMR) located in Yaba, Lagos. The animals were housed in the Laboratory Animal Center, College of Medicine, University of Lagos. The animal house was well ventilated with a temperature range of 28–32 °C under day/night 12–12 h photoperiodicity. The rats were fed with standard rat chow (Pfizer Nig Ltd). They had access to water *ad libitum*. The experiment was in 2 phases. Each phase had 6 groups (A- distilled water, B- 30%v/v alcohol, C- 250 mg/kg of *T. occidentalis* D-500 mg/kg of *T. occidentalis*, E-250 mg/kg of *T. occidentalis* + Alcohol, F-500 mg/kg of *T. occidentalis* + Alcohol) with 5 rats per group. The first phase was for 4 weeks and had groups ranging from A_1 to F_1 while the second phase was for 8 weeks and had groups ranging from A_2 to F_2. All experimental protocols followed the guidelines approved by the Ethics Committee of the College of Medicine, University of Lagos, Nigeria.

2.4. Sperm count

This was done using the new improved Neubauer‘s counting chamber (Hematocytometer). The epididymal fluid was diluted with normal saline solution by adding 0.9 ml to 0.1 ml of the crushed epididymis. This chamber was then filled with sperm fluid and placed under a binocular light microscope using an adjustable light source. The ruled part was then focused and the number of spermatozoa counted in five 16-celled squares Keel and Webster, [32].

2.5. Sperm motility

The sperm motility analysis was carried out at room temperature using one epididymis of each rat. The percentage of sperm motility was calculated using the number of live sperm cells divided by the total number of sperm cells (motile and non-motile), from two samples per epididymis of each rat. All sperm cells that were not moving at all were considered to be non - motile, while the rest, which displayed some movement, were considered to be motile [53].

2.6. Testicular malondialdehyde (MDA) and testicular antioxidant enzymes

The lipid peroxidation products were estimated by measuring TBARS and were determined by modifying the method of Niehaus and Samuelson [36]. Antioxidants such as reduced glutathione (GSH) and Superoxide dismutase (SOD) were estimated by employing modified methods of [18] and [43], respectively.

2.7. Testicular histology preparation

The histology of the testes was done by modification of method reported by Kayode et al. [31]. The organs were harvested and fixed in Bouin‘s fluid for 24 h after which it was transferred to 70% alcohol for dehydration. The tissues were passed through 90% and absolute alcohol and xylene for different durations before they were transferred into two changes of molten paraffin wax for 1 hour each in an oven at 65 °C for infiltration. They were subsequently embedded and serial sections cut using rotary microtome at 5 microns. The tissues were picked up with albumenized slides and allowed to dry on hot plate for 2 min. The slides were dewaxed with xylene and passed through absolute alcohol (2 changes); 70% alcohol, 50% alcohol and then to water for 5 min. The slides were then stained with haematoxylin and eosin. The slides were mounted in DPX. Photomicrographs were taken at a magnification of x400.

2.8. Phytochemical screening

Qualitative and Quantitative phytochemical analysis of the aqueous leaf extract of *T. occidentalis* was done in accordance with Soni and Sosa [48]. While modifications on the report by Grindberg and Williams [27] on high performance liquid chromatography was adopted to quantify the vitamins.

Table 1
Sperm motility and sperm count after 4 weeks.

Groups	Description	Motility (%)	Count(10^6/ml)
A_1	Control (distilled water)	98.5 ± 0.71^	88.3 ± 9.90^
B_1	Alcohol (30%v/v)	42.0 ± 1.41*	24.7 ± 1.27*
C_1	250 mg/kg of *T. occidentalis*	93.5 ± 4.95^	95.9 ± 3.82^
D_1	500 mg/kg of *T. occidentalis*	96.5 ± 2.12^	91.1 ± 1.70^
E_1	250 mg/kg of *T. occidentalis* + Alc	61.5 ± 9.19*^	69.8 ± 13.4^
F_1	500 mg/kg of *T. occidentalis* + Alc	75.5 ± 6.36*^	86.4 ± 8.70^

v: volume; T. occidentalis: *Telfairia occidentalis*
Values are expressed as mean ± Standard deviation.
* $p < 0.05$ compared with A_1; ^ $p < 0.05$ compared with B_1. One-Way ANOVA.

2.9. Morphometry

The primary aim was to estimate the volumes of seminiferous tubule epithelium (seminiferous epithelium) and interstitium in the testis. This was done in accordance with Howard and Reed [29] and Baines et al. [8]. Four sections per testis, and six microscopical fields per section, were randomly chosen for analysis. Fields were sampled as images captured on a Leica DM750 brightfield microscope (Germany) via LAZ software. Volume densities of testicular ingredients were determined by randomly superimposing a transparent grid comprising 35 test points arranged in a quadratic array. Test points falling on a given testis and its ingredients were summed over all fields from all sections. The total number of points hitting on a given ingredient (lumen (E_L), epithelium (E_E), interstitium (E_I)), divided by the total number of points hitting on the testis sections (E_T) multiplied by 100, provided an unbiased estimate of its%volume density/volume fraction.

2.10. Statistics

The sperm parameters were log transformed to improve normality of data. Differences between groups were compared using one way ANOVA and LSD *post hoc* test. This was done using the SPSS. The data was expressed as mean ± standard deviation [4].

3. Results

Sperm motility and sperm count analysis

After the first phase of the study, there was a significant decrease in sperm motility of group B_1 when compared to groups A_1, C_1, D_1, E_1 and F_1 ($p < 0.05$). There was also significant decrease in groups E_1 and F_1 compared to group A_1. The sperm count of group B_1 decreased significantly compared to groups A_1, C_1, D_1, E_1 and F_1 ($p < 0.05$; Table 1).

After the second phase of the study, the sperm motility showed a significant decrease in group B_2 compared to group A_2. There was also a significant increase in groups C_2, D_2, E_2 and F_2 compared to group B_2. In the sperm count, there was a significant decrease in sperm count of group B_2 compared to groups A_2, C_2, D_2, E_2 and F_2. There was also a significant increase in groups C_2 and F_2 compared to group A_2 ($p < 0.05$; Table 2).

3.1. MDA and antioxidant enzymes

MDA levels increased significantly in group B_1 when compared to groups A_1, C_1, D_1, E_1 and F_1 in the first phase of the study. The SOD levels of group D_1 decreased significantly when compared to group B_1. There was no significant difference in GSH when compared to groups A_1 or B_1 (Table 3).

In the second phase of the study, there was a significant increase in MDA level of group B_2 when compared to groups A_2, C_2, D_2, E_2 and F_2. SOD levels in groups B_2 and C_2 increased significantly compared to group A_2. The GSH levels of groups E_2 and F_2 increased significantly compared to group B_2. Also, there was a significant increase in GSH of group F_2 compared to group A_2 (Table 4).

3.2. Testicular histology

Cross section of the testis of animals after the first phase of the study showed that groups A_1, C_1, D_1, E_1 and F_1 had a normal cellular composition in their germinal epithelium with sperm cells in the lumen and a normal interstitium. However, animals in group B_1 that received alcohol only, showed a marked depletion of spermatogenic cells as depicted in Fig. 1.

Cross section of the testis of animals after the second phase of study showed that groups A_2, C_2 and D_2 showed normal cellular composition with no obvious aberrations compared to group B_2 that showed marked depletion of spermatogenic cells with a widened and a hypo-cellular interstitium (Fig. 2). Groups E_2 and F_2 showed normal cellularity in the germinal epithelium but a widened and hypocellular interstitium (Fig. 2).

3.3. Morphometric (stereological) analysis

After the first phase of the study, the volume density of the germinal epithelium of group A_1 had no significant difference with group B_1. However, there was a significant increase in groups C_1, D_1, E_1 and F_1 when compared to groups A_1 and B_1. The lumen density significantly decreased in group B_1 compared to group A_1. The interstitium had a significant increase in group B_1 compared to A_1. There was also a significant decrease in the interstitium of groups C_1, D_1, E_1 and F_1 compared to group B_1 as reported in Table 5.

After the second phase of the study, there was no significant difference in the volume density of the germinal epithelium of groups A_2 and B_2. However, the volume density of the germinal epithelium of group B_2 decreased compared to group A_2 ($p > 0.05$). There was a significant increase in groups C_2, D_2 and E_2 when compared with group B_2. The volume density of the lumen also showed no significant difference between groups A_2 and B_2 but there was a significant decrease in groups D_2 and E_2 compared with group B_2. The interstitium of group B_2 increased compared to A_2. There was a significant decrease in groups C_2, D_2 and E_2 when compared to group B_2 (Table 6).

3.4. Phytochemical screening

Qualitative analysis of *T. occidentalis* leaves shows the presence of flavonoids, tannins, phlobatannins, terpenoids, cardiac glycoside, saponins, alkaloids and steroids (Table 7). After the quantitative analysis as depicted in Table 8, total saponins, total phenol and total flavonoids had higher values compared to total taninins and the total alkaloids present. There were also high values of vitamins A, C and E (Table 8).

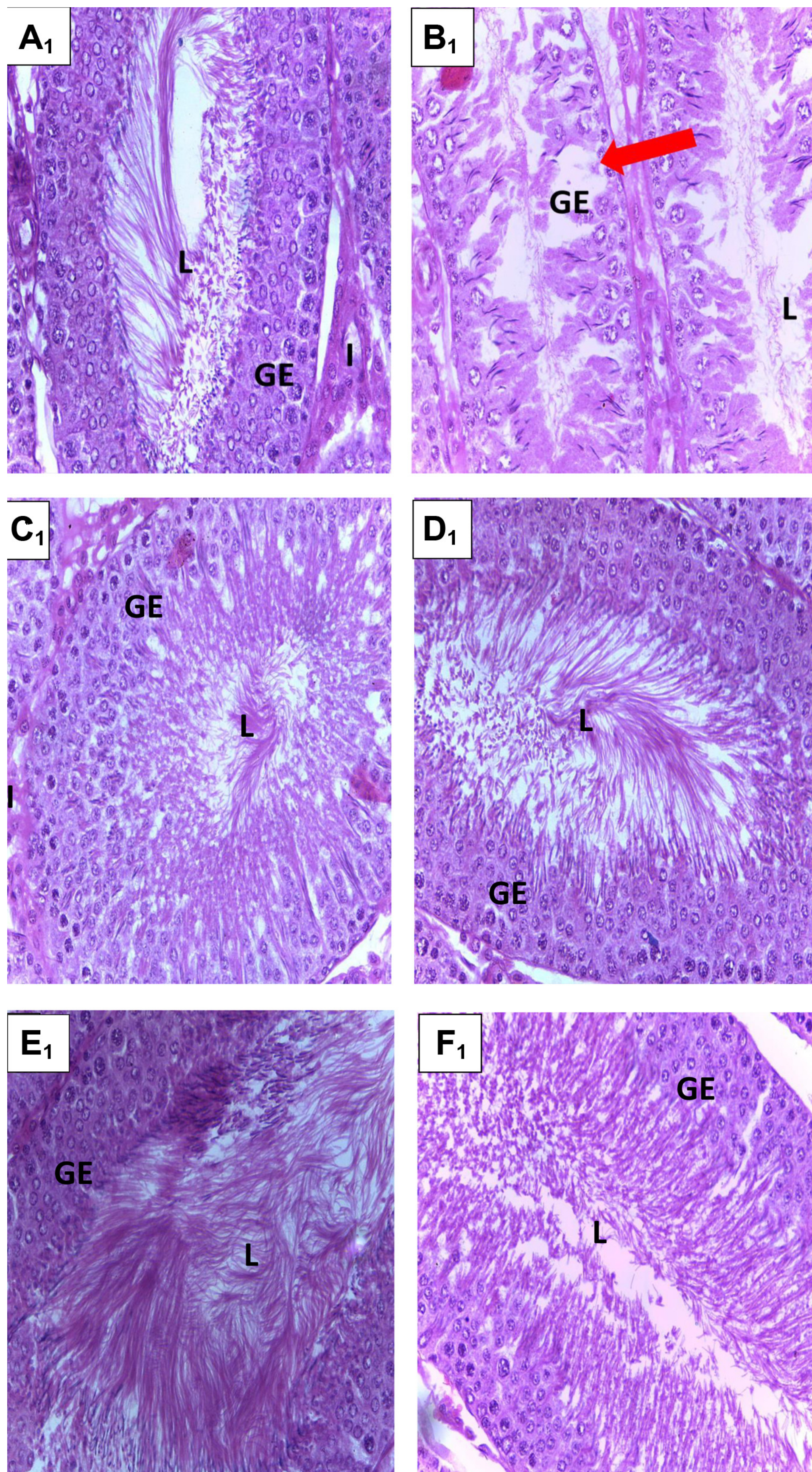

Fig. 1. Photomicrographs of testis (×400) after 4 weeks of administration, showing normal cellularity in germinal epithelium (GE), lumen (L) filled with sperm cells and interstitial cells of Leydig in the interstitium (I) in groups A_1 (control), C_1 (250 mg/kg body weight of *T. occidentalis*), D_1 (500 mg/kg body weight of *T. occidentalis*) and E_1 (250 mg/kg body weight of *T. occidentalis* + Alc) and F_1 (500 mg/kg body weight of *T. occidentalis* + Alc). Group B_1 (alcohol 30%v/v) showing depleted spermatogenic cells of the GE.

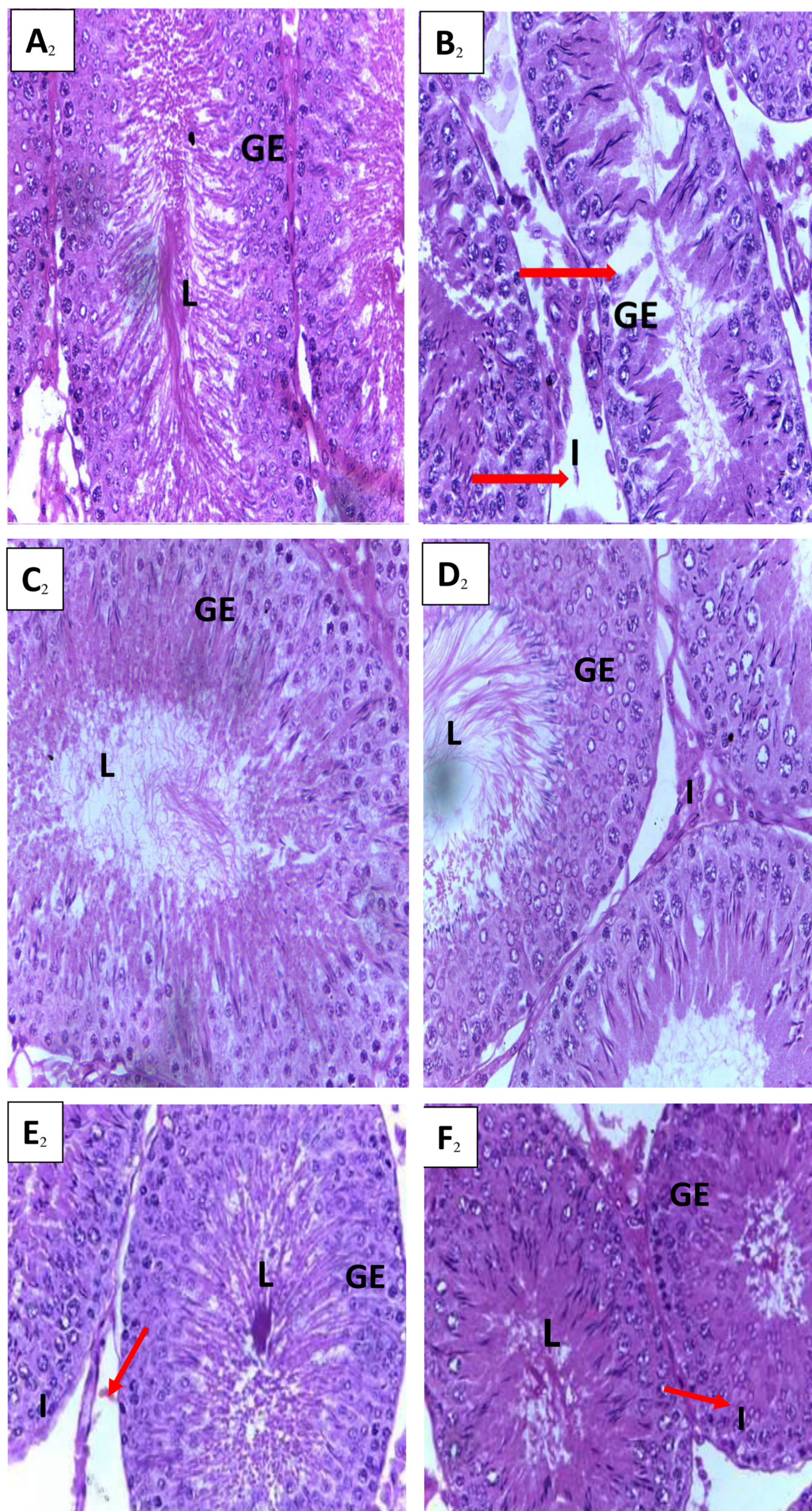

Fig. 2. Photomicrographs of testis of second phase (×400). A_2 (control), C_2 (250 mg/kg body weight of *T. occidentalis*), D_2 (500 mg/kg body weight of *T. occidentalis*) showing normal GE. Groups E_2 (250 mg/kg body weight of *T. occidentalis* + Alcohol), F_2 (500 mg/kg body weight of *T. occidentalis* + Alcohol). Group B_2 (alcohol 30%v/v) showing depleted spermatogenic cells in the germinal epithelium (GE) after 8 weeks of administration.

Table 2
Sperm motility and sperm count after 8 weeks.

Groups	Description	Motility (%)	Count (10^6/ml)
A_2	Control (Distilled water)	67.5 ± 3.54	48.95 ± 1.06
B_2	Alcohol (30%v/v)	32.5 ± 3.54*	31.2 ± 1.27*
C_2	250 mg/kg of *T. occidentalis*	71.0 ± 1.41^	79.25 ± 1.48*^
D_2	500 mg/kg of *T. occidentalis*	57.5 ± 3.54^	56.05 ± 2.62^
E_2	250 mg/kg of *T. occidentalis* + Alc	62.5 ± 3.54^	57.55 ± 3.61^
F_2	500 mg/kg of *T. occidentalis* + Alc	67.5 ± 2.12^	77.4 ± 3.39*^

v: volume; T. occidentalis: Telfairia occidentalis.
Values are expressed as mean ± Standard deviation.
* $p < 0.05$ compared with A_2; $\hat{p} < 0.05$ compared with B_2. One-Way ANOVA.

4. Discussion

At the end of both phases of this study, alcohol significantly reduced sperm count and sperm motility by subjecting the spermatozoa to increased oxidative stress-induced damage because their plasma membranes contain large quantities of polyunsaturated fatty acids (PUFAs) [5,9] and their cytoplasm contains low concentrations of scavenging enzymes [45,46]. Increased formation of ROS has been correlated with a reduction of sperm motility [2,7]. The link between ROS and reduced motility may be due to a cascade of events that result in a rapid lose of intracellular ATP leading to axonemal damage and sperm immobilization [9,16].

It was also observed that malondialdehyde (MDA) a by-product of lipid peroxidation [17,45] was increased in the animals that received alcohol only. This depicts an increase in lipid peroxidation. This study further showed that an increased lipid peroxidation elicited an increased production of superoxide dismutase to neutralize the effects of the free radicals. This is in consonant with the findings by [17] but contradicts that of [37] and [19] who reported that acetaldehyde a product of the biotransformation of alcohol *in situ* might harm testicular function indirectly by decreasing

Table 3
MDA and antioxidant enzymes analysis after 4 weeks.

Groups	Description	MDA (nmol/ml)	SOD (min/m/protein)	GSH (μmol/ml)
A_1	Control (distilled water)	6.87 ± 0.19	107.71 ± 5.20	0.063 ± 0.03
B_1	Alcohol (30%v/v)	12.10 ± 0.52*	120.66 ± 3.92	0.042 ± 0.03
C_1	250 mg/kg of *T. occidentalis*	6.34 ± 0.29^	113.01 ± 15.80	0.101 ± 0.09
D_1	500 mg/kg of *T. occidentalis*	5.92 ± 0.76^	82.23 ± 23.84^	0.021 ± 0.01
E_1	250 mg/kg of *T. occidentalis* + Alc	7.54 ± 0.38^	125.31 ± 10.49	0.031 ± 0.03
F_1	500 mg/kg of *T. occidentalis* + Alc	6.21 ± 0.47^	114.64 ± 4.74	0.018 ± 0.01

v: volume; T. occidentalis: Telfairia occidentalis; MDA: Malondialdehyde; SOD: Superoxide dismutase; GSH: Glutathione reductase
Values are expressed as mean ± Standard deviation.
* $p < 0.05$ compared with A_1; $\hat{p} < 0.05$ compared with B_1. One-Way ANOVA.

Table 4
MDA and testicular antioxidant enzymes analysis after 8 weeks.

Groups	Description	MDA (nmol/ml)	SOD (min/mg/protein)	GSH (μmol/ml)
A_2	Control (distilled water)	9.42 ± 0.95	32.27 ± 0.91	0.154 ± 0.01
B_2	Alcohol (30%v/v)	16.75 ± 2.90*	54.07 ± 15.3*	0.136 ± 0.06
C_2	250 mg/kg of *T. occidentalis*	8.68 ± 0.48^	58.93 ± 14.16*	0.195 ± 0.03
D_2	500 mg/kg of *T. occidentalis*	8.75 ± 0.22^	39.58 ± 5.87^	0.229 ± 0.13
E_2	250 mg/kg of*T. occidentalis* + Alc	9.70 ± 1.90^	39.56 ± 4.43^	0.275 ± 0.04^
F_2	500 mg/kg of*T. occidentalis* + Alc	9.63 ± 0.86^	35.4 ± 2.36^	0.308 ± 0.00*^

v: volume; T. occidentalis: Telfairia occidentalis; MDA: Malondialdehyde; SOD: Superoxide dismutase; GSH: Glutathione reductase
Values are expressed as mean ± Standard deviation.
* $p < 0.05$ compared with A_2; ^ $p < 0.05$ compared with B_2. One-Way ANOVA.

Table 5
Morphometric analysis of testis after 4 weeks administration.

Groups	Description	Germinal epithelium (%)	Lumen (%)	Interstitium (%)
A_1	Control (distilled water)	63.70 ± 0.99	15.00 ± 0.71	21.25 ± 0.35
B_1	Alcohol (30%v/v)	62.60 ± 0.14	10.24 ± 1.08*	27.10 ± 1.27*
C_1	250 mg/kg of *T. occidentalis*	69.20 ± 2.12*^	11.89 ± 1.54	18.90 ± 0.56^
D_1	500 mg/kg of *T. occidentalis*	69.55 ± 1.06*^	12.83 ± 2.93	17.35 ± 3.75^
E_1	250 mg/kg of *T. occidentalis* + Alcohol	71.98 ± 0.46*^	11.95 ± 1.34	16.05 ± 1.77*^
F_1	500 mg/kg of *T. occidentalis* + Alcohol	68.05 ± 0.92*^	11.40 ± 0.99	20.50 ± 1.84^

v: volume; T. occidentalis: Telfairia occidentalis
Values are expressed as mean ± Standard deviation.
* $p < 0.05$ compared with A_1; ^ $p < 0.05$ compared with B_1. One-Way ANOVA.

Table 6
Morphometric analysis of testis after 8 weeks.

Groups	Description	Germinal epithelium(%)	Lumen (%)	Interstitium (%)
A_2	Control (distilled water)	61.09 ± 3.41	14.60 ± 0.41	24.33 ± 3.78
B_2	Alcohol (30%v/v)	50.50 ± 0.71	16.50 ± 0.14	37.05 ± 6.43
C_2	250 mg/kg of *T. occidentalis*	64.25 ± 1.06^	13.95 ± 0.78	21.80 ± 0.28^
D_2	500 mg/kg of *T. occidentalis*	64.90 ± 0.14^	13.30 ± 0.14^	21.80 ± 0.28^
E_2	250 mg/kg of *T. occidentalis* + Alcohol	65.15 ± 0.21^	13.70 ± 0.42^	21.10 ± 0.71^
F_2	500 mg/kg of *T. occidentalis* + Alcohol	50.80 ± 4.53	14.20 ± 1.70	35.00 ± 2.83

v: volume; T. occidentalis: Telfairia occidentalis.
Values are expressed as mean ± Standard deviation.
*$p < 0.05$ compared with A_2; ^$p < 0.05$ compared with B_2. One-Way ANOVA.

Table 7
Qualitative Phytochemical Analysis of Aqueous Extract of *Telfairia occidentalis*.

S/No	Phytochemicals	Status
1	Flavonoids	+
2	Tannins	+
3	Phlobatannins	+
4	Terpenoids	+
5	Cardiac glycosides	+
6	Alkaloids	+
7	Steroids	+
8	Saponins	+
9	Coumerins	–
10	Quinones	–

Table 8
Quantitative phytochemical analysis of aqueous extract of Telfairia occidentalis.

S/No	Phytochemicals	Quantity
1	Total Phenols (%)	15.36
2	Total Alkaloids (%)	5.62
3	Total Saponins (%)	16.2
4	Total Flavonoids (%)	12.36
5	Total Tannins (%)	7.33
6	Vitamin C (mg/g)	3.06
7	Vitamin A (mg/g)	3.28
8	Vitamin E (mg/g)	2.46

antioxidant defenses. The observed increase in MDA with animals that consumed alcohol alone may also infer that an increase in SOD alone may not suffice for effective testicular antioxidant defense against lipid peroxidation.

This study also demonstrated that *T. occidentalis* increased testicular antioxidant enzymes and decreased MDA levels when administered alone. It also prevented the ravaging effects of alcohol on sperm count, sperm motility and testicular antioxidant enzymes when administered with alcohol. The findings from this study has shown that *T. occidentalis* is rich in antioxidant constituents such as flavonoids, saponins, vitamin E, vitamin C and vitamin A. This is in concordance with reports form Kayode and Kayode [30]. Therefore, it is plausible deducing that these rich antioxidant constituent of *T. occidentalis* boosted the testicular non-enzymatic and enzymatic antioxidants to effectively scavenge the free radicals preventing lipid peroxidation. The consequence is hereby reflected in the increased sperm count and sperm motility. This finding is in concordance with the reports by [42] and [9]. Moreso, vitamin E, a chain-breaking, non-enzymatic antioxidant also found in *T. occidentalis* inhibits lipid peroxidation in membranes by scavenging peroxyl ($RO^{\bullet}$) and alkoxyl ($ROO^{\bullet}$) radicals [45]. The ability of vitamin E to maintain a steady state rate of peroxyl radical reduction in the plasma membrane depends on the recycling of vitamin E by external reducing agents such as ascorbate (present in *T. occidentalis*) or thiols Saleh and Agarwal, 2002 [45]. The improved sperm parameters are also attributed to the amino acid content of *T. occidentalis* [23]. Amino acids such as alanine, glycine, cystine and arginine which are present in *T. occidentalis* have been reported to preserve sperm cells and improve their motility [10,3]

In this study, alcohol depleted spermatogenic cells and reduced the volume density of the germinal epithelium. This is in concert with our previous study [3] and the study by [35] who reported that ethanol significantly inhibited the proliferative activity of the spermatogonia in all stages of the seminiferous tubules cycle, it significantly decreased the seminiferous tubules diameter, degenerated germ cells and decreased the number of Leydig cells. For decades, it has been made clear that testosterone which is produced by the interstitial cells of Leydig is a necessary prerequisite for the maintenance of established spermatogenesis [54]. The reduced cellularity of the interstitium in testis of animals that treated with only alcohol would consequently lead to a decrease in testosterone resulting in the poor spermatogenesis observed.

T. occidentalis aqueous leaf extract also maintained the histoachitecture of the testis, increased the proliferative activity of spermatogonia and maintained the volume density of the interstitium compared to the control animals. *T. occidentalis* has been reported to be rich in carotene which reduces lipid peroxidation [22,24]. From our observation, when *T. occidentalis* aqueous leaf extract was administered concomitantly with alcohol; it protected the testis from the pernicious effects of alcohol. This protective nature of *T. occidentalis* is enhanced by some of its phytochemical constituents: the presence of ascorbic acid which is known for its protection on cell membranes and its scavenging effects on free radicals [22,9]. In clinical trials, vitamin E supplementation has been found to increase fertilization rates possibly by improving membrane integrity, reducing oxidative damage and lipid peroxidation potential [26,14]. It is plausible that the antioxidants and micronutrients example zinc and folates in *T. occidentalis* [24] militated against the ravaging effects of alcohol on the testis. Moreover, Aitken and Roman [1], reported that flavonoids, vitamins A, C and E promote spermatogenesis. Hence, the increase in the volume density of the germinal epithelium of animals administered only *T. occidentalis* is a consequence of the positive effects of its multivitamins and phytochemicals. Albeit, the reason why *T. occidentalis* when administered with alcohol was more effective at 250 mg/kg per body weight as compared to 500 mg/kg per body weight remains indefinable although Iron – pro-oxidant may be a plausible culprit [13].

In conclusion, alcohol depleted the germinal epithelium and caused widening and hypocellularity of the interstitium. *T. occidentalis* however, did not only promote germinal epithelial growth but protected the cyto-architecture of the testis from the damaging effects of alcohol. Hence, *T. occidentalis* leaves augments spermatogenesis and attenuates alcohol-induced oxidative stress via an antioxidant system of activities.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.toxrep.2015.06.009

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