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Testicular Damage in *Telfairia Occidentalis* Extract Treated Wista Rats

Adisa W.A.[1,*], Okhiai O.[2], Bankole J.K.[3], Iyamu O.A.[4], Aigbe O.[4]

[1]Department of Physiology, Ambrose Alli University, Ekpoma, Edo State, Nigeria
[2]Department of Nursing, Ambrose Alli University, Ekpoma, Edo State, Nigeria
[3]Department of Medical Laboratory Sciences, Ambrose Alli University, Ekpoma, Edo State, Nigeria
[4]Department of Biochemistry, Ambrose Alli University, Ekpoma, Edo State, Nigeria
*Corresponding author: williamsadewumi@yahoo.com

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Abstract Telfairia occidentalis commonly called Pumpkin or Ugwu in Nigeria is one of such plant with claimed medicinal value. No doubt this acclaimed beneficial value may be popularizing its ingestion amongst male populations on whom paucity of literature is present. Hence this study is aimed at determining the effect of Telfairia occidentalis leave extract on testicular histology archipuncture. The study involves 18 adult male rats of comparable weight. They were grouped into three (A, B, and C) where a served as control and B and C served as the test groups. The test group B and C were administered varying doses of the leave extract of Telfairia occidentallis according to body weight. This experiment lasted for four weeks after acclimatization. At the end of the four weeks of study, blood samples were obtained from the rats for analysis of testosterone value. In addition their testes were obtained and fixed in 10% formalin for histological analysis. The result showed a reducing potential effect on plasma testosterone level in a dose dependent fashion. Statistically the plasma testosterone levels of test group B (1.40±0.45ng/ml) were significant at $P < 0.05$ compared to that of control (2.70±0.96ng/ml). Furthermore the histological presentation was a dose dependent damage on testicular cells. This damages ranges from basement membrane distortions, cellular degeneration, hemorrhage, interstitial space exudations and cellular necrosis. Conclusively, judging by the result of the study. Telfairia occidentallis leave extract have potential for reducing male sexual function considering the observed effect on plasma testosterone level and may also be testiculotoxic indicated by the histological presentation of the testis.

Keywords: *wista rats, telfairia occidentalis, testosterone, semen, testis, histology, testiculotoxic, sexual function*

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1. Introduction

The use of herbs to treat disease is almost universal among non-industrialized societies, and is often more affordable than purchasing expensive modern pharmaceutical and is fast becoming revolutionalized. In some countries, it has been integrated into the health scheme despite advance in orthodox medicine. It is believed that the natural products if utilized in the correct form and dosage are less harmful than synthetic products, which most often elicit some anaphylactic response or reaction (Olatunji, 2005). *Telfairia Occidentalis* commonly called fluted pumpkin, or Ugwu plant in Nigeria is one of such plants.

Telfairia occidentalis is a tropical vine grown in West Africa as a leaf vegetable and for its edible seeds. The plant is dioecious, perennial, and drought-tolerant. It is usually grown trellised. The young shoots and leaves of the female plant are the main ingredient of Nigerian Edikang Ikong soup (Badifu *et al.*, 1995). It is a creeping vegetable shrub that spreads low across the ground with large lobed leaves, and long twisting tendrils (Horsfall and Spiff, 2005). It thrives in humid climate and well drained soils and is usually cultivated in garden and family farms around homes. Telfairia occidentalis belongs to the botanical family of cucurbitaceae. The curcubitaceae are reported to have been associated with man since 12,000BC (Esquinas-Alcazar et al., 1983).Common example of plants in this family are cucumber, watermelon, squash and melon (Oboh, 2005).

Telfairia occidentalis contain nutrients such as proteins, carbohydrate, vitamins, minerals and fiber (Fasuyi, 2006). It also contain oxalates, saponins, glycosides, flavonoids, alkaloids and resins (Tindall, 1968; Akubue *et al*., 1980). Aqueous extract of Telfairia occidentalis is reported to increase heamotogical parameter (Alada, 2000).

Interestingly, the aqueous extract of Telfairia occidentalis has been shown to be hepatoprotective against garlic induced oxidative stress (Olorunfemi *et al*., 2005) while its ethanolic extract have demonstrated hypoglycaemic properties both in normoglycaemic and alloxan-induced diabetic rats (Nwozo et al., 2004). The seeds of the plant can also be fermented for several days

and eaten as slurry (Badifu and Ogunsua, 1991. Freshly prepared Ugwu (pumpkin-Telfaira occidentalis) mixture containing Ugwu (pumpkin) fluid, raw content of egg and peak evaporated unsweetened milk administered orally is a popular heamatinic regimen used to combat anemia in pregnant women in mission hospitals in Nigeria (Olaniyam and Adeleke, 2005).

The root and leaves has been shown to contain highly toxic alkaloids and saponins (Akube, 1980). In folkloric medicine, the fresh leaves are used in the treatment of anemia, sudden attack of convulsion and malaria (Gbile, 1986; Alada, 2000; Ukwuoma and Muanya, 2005). Despite the fact that Telfairia occidentalis leaves (TOL) are widely consumed by man in the Southern Nigeria and other part of West Africa in addition to their versatile ethno medical usage, little is known about the effect of TOL on the male reproductive system except for some unverified claims by herbal practitioners that it increases sperm count and activity.

This present study is therefore aimed at investigating the role of various doses of the aqueous extract of fresh Telfairia occidentalis leaves on some reproductive parameters in male wista rats.

2. Materials and Methods

Aqueous extraction: The leaves of the plant *Telfairia occidentalis* were bought from Ekpoma main market in Edo state and then taken to the herbarium unit of the Department of botany of Ambrose Alli University, Ekpoma for identification and authentification. The leaves were washed and blended, the grinded paste was filtered and the extracted sediment was air dried for 7 days. This way, a powdery product was obtained from which aqueous extract of 100mg/kg and 200mg/kg body weights were prepared.

Experimental animals: A total number of 18 male Wistar Rats weighing between 195 -264gm were randomly selected from the animal house of College of Medicine, A.A.U Ekpoma. They were housed in well ventilated labelled wooden cages at the site of the experiment. The cages were designed to secure the animals properly especially from insect and wide animals and the cages were cleaned daily. The animals were allowed to acclimatize for two weeks, during which they were fed with rat feed (pellated diet) and water and were carefully observed until the administration of Telfairia Occidentalis commenced.

The animals were separated into 3 groups of 6 rats each and labeled A, B and C group; i.e control, test group B and test group C respectively. Group A rats were fed with rat feed and water, group B and C were fed with rat feed, water and administered 100mg/kg and 200mg/kg body weight of aqueous extract of telfairia occidentalis respectively orally using a metal canula for a period of 4 weeks.

Materials: Heparinised tubes, syringe (5ml), canular**,** cotton wool**,** chloroform**,** formalin-normal saline**,** dissecting kit/surgical apparatus, hormonal assay kit/testosterone, microscope, microscope slides, sterilized containers.

3. Sample Collection and Analysis

Blood collection: Blood samples were collected via assessing the jugular vein into tubes containing heparin as anticoagulant using syringes(5ml). These were taken to the laboratory for the analysis of the plasma testosterone using hormonal assay kit.

Semen and testes collection: The testes were assessed following incision of the lower abdomen and semen collected from the epididymis for semen analysis. The testes collected were fixed into10% formalin for histological evaluation.

Semen Analysis: Semen to evaluation was done using methods described by Zemjanis (). In these methods, motility was evaluated with one drop each of semen sample and 2.9% sodium citrate buffered under a cover-slip and viewed under x 40 of microscope. Semen smears prepared were stained with wells and Awastain for morphology and Eosin-nigrosin for live-dead ratio. The spermatozoa concentration was also evaluated using the improved Neubar Chamber method of counting.

Tissue processing: The tissues were processed using automatic tissue processor according to the processing schedule used in Obafemi Awolowo University Teaching Hospital, Ile-Ife Osun state Nigeria. The fixed plastic tissue cassettes in 10% formalin were automatically processed by passing them through different grades of alcohol. After the last grade, the tissues were removed from the plastic cassettes and placed at the center of the metallic tissue mould and then filled with molten paraffin wax. They were also left to become solid after which they were placed in the refrigerator at 5 degree Celsius for 15minutes. The blocks were then removed from the metallic case using a knife and trimmed.

The blocks were then sectioned using 3nm on a rotary microtome. The sections were floated in the water bath at 55 degree Celsius and picked up by the use of a clean frosted end slides. The floated end slides were now placed on a hot plate for 40 minutes for adequate attachment of the sections on the slide, after which the sections were dewaxed, hydrated, air dried and stored in a slide box ready for staining with haematoxylin and eosin.

Statistical analysis: The data obtained were analysed using the student T-test, and then expressed as mean ± standard deviation at a confidential interval of $p < 0.05$ considered significant. These were then presented with suitable tables and chart. In addition, histological evaluation were presented with tissue micrograph (H and E × 100)

4. Results

Hormonal assay: Table 1, shows the distribution of plasma testosterone levels in rats treated with Telfairia occidentalis for four weeks compared to control rats not fed on the plant substance. The results showed a reducing potential effect on plasma testosterone level in a dose dependent fashion. Specifically, testosterone level for the control (2.70±0.96ng/ml) was higher compared to those of test group B (1.40±0.69ng/ml) and that of test group C (1.30±0.45ng/ml). Statistically, the plasma testosterone levels of test group B and C were significant at $P < 0.05$ compared to that of control. However, the plasma testosterone levels for test group B and C was not significantly different. Figure 1 is a bar chart

representation of the distribution of plasma testoterone level amongst the different groups.

Table 1. Plasma testosterone level (X±SD) in rats treated with Telfairia Occidentalis for four weeks compared to control

Parameter	Group		
	Control	Test group B	Test group C
Testosterone (ug/ml)	2.70±0.96	1.40±0.69*	1.30±0.45*

Values are mean±SD; * indicate significant difference at p<0.05 when compared with control.

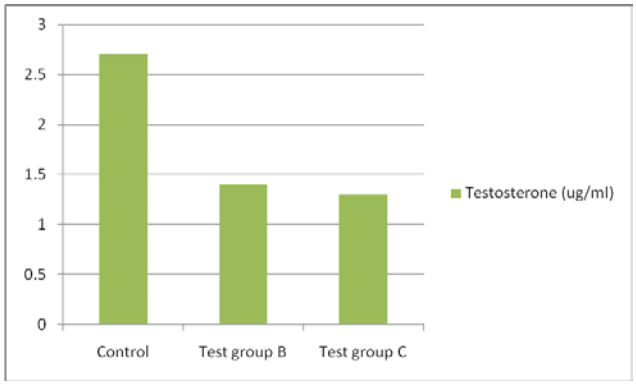

Figure 1. Bar chart representation of plasma testosterone level in rats treated with Telfairia Occidentalis for four weeks compared to control

Semen analysis: The result of the various parameters evaluated on the semen is as shown in Table 2 below. The result shows a significant decrease (P<0.05) in sperm count and morphology in group B and C compared with control. In like, manner the sperm motility result shows that there were significant decrease (P<0.05) of fully active sperms in group B and C compared with control. However there were significant increase (P<0.05) of slightly active sperms in group C compared with the control group and no statistical significant increase in group B compared with the control, while the number of dead increased significantly (P<0.05) in group B and C compared with control.

Table 2. Effects of Telfairia Occidentalis on Semen Quality

	Group A	Group B	Group C
Sperm count (10^6/ml)	61.33 ± 1.90	43.67 ± 3.02 *	33.17 ± 1.01 *
Morphology (%)	99.17 ± 0.83	91.17 ± 1.54 *	78.83 ± 0.31 *
Motility:			
Fully Active	89.17 ± 0.83	72 ± 1.59 *	59.83 ± 0.65 *
Slightly Active	10 ± 0.00	10.83 ± 098	22.67 ± 0.92 *
Dead	0.83 ± 0.83	17.17 ± 1.01*	17.50 ± 0.62 *

The data is given in mean ± SEM; the value of P is taken to be significant for P <0.05

5. Histological Observation

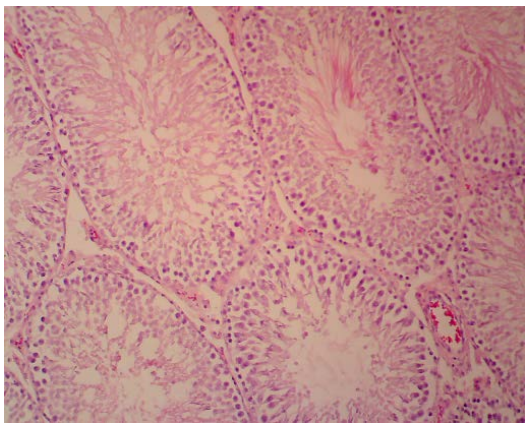

Plate 1. Testis (control Section; H&E x100) showing normal histological features with intact seminiferous tubules. (As presented for the rats in the control group; rat 1 to 6)

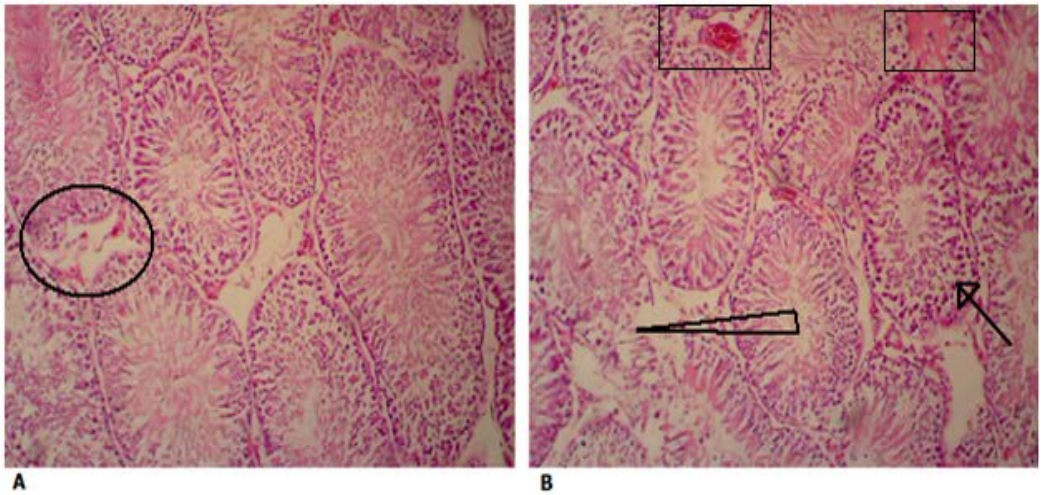

Plate 7A &B. Testis (Test Group B1; H&E x100) showing basement membrane distortions as encircled in Plate A and as indicated by the 'triangular arrow' in Plate B. Note also the necrotic cells in Plate B ('line arrow') and interstitial space exudation ('squared')

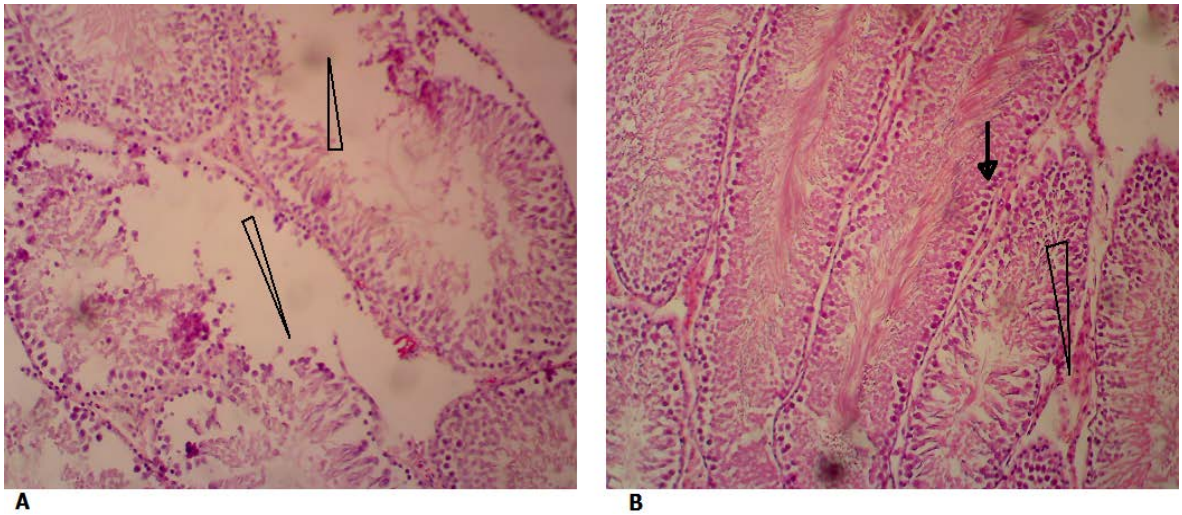

Plate 8A&B. Testis (Test Group B2; H&E x100) showing severe basement membrane distortions and cellular degeneration (in Plate A). Note also the interstitial space exudates ('triangular arrow') and the deeply staining cells in Plate B (line arrow)

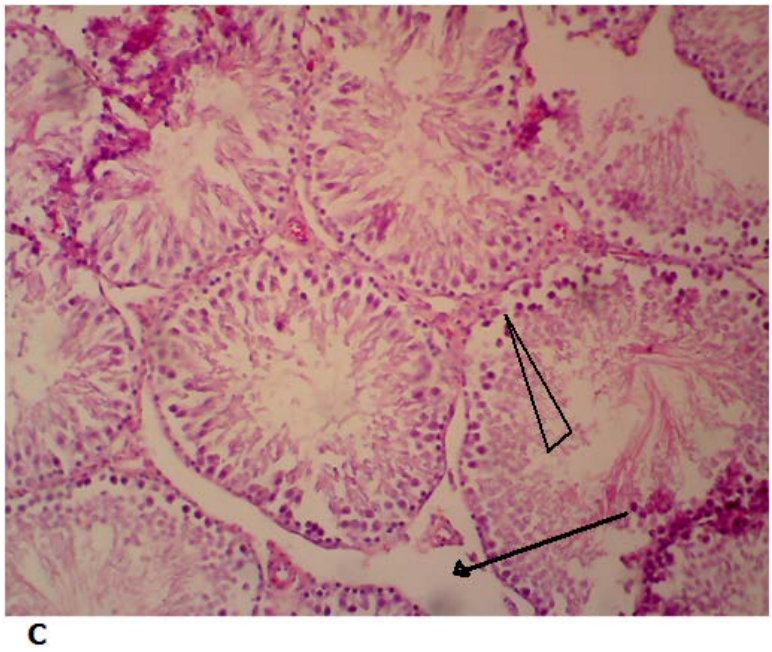

Plate 8C. Testis (Test Group B2; H&E x100) showing interstitial space enlargement ('line arrow') with cellular degeneration (in Plate A)

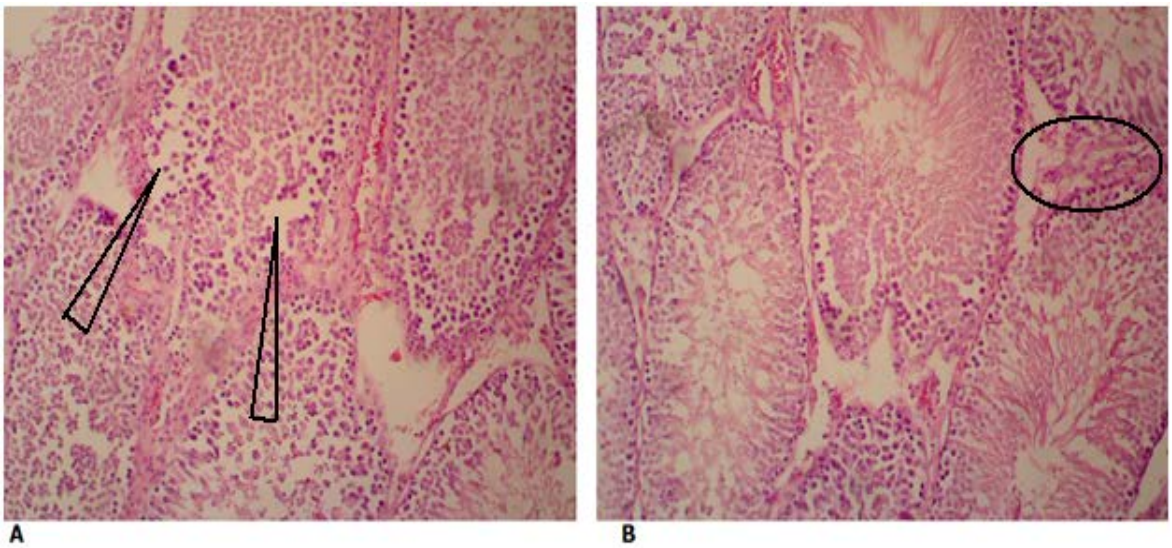

Plate 9A&B. Testis (Test Group B3; H&E x100) showing mild cellular degeneration (in Plate A) ('triangular arrows'). Note also the interstitial space exudates ('encircled')

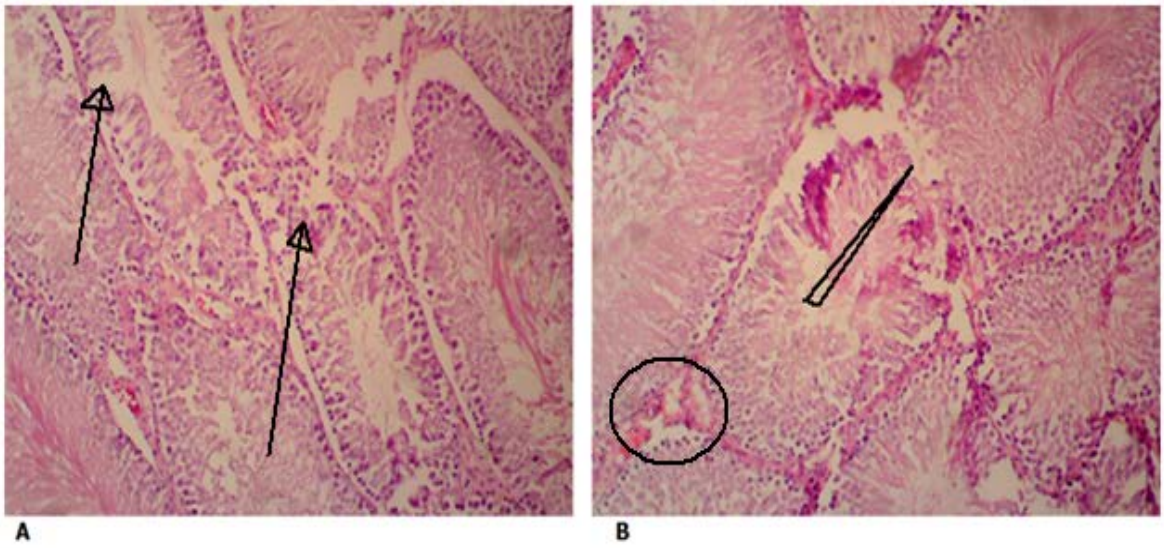

Plate 10 A&B. Testis (Test Group B4; H&E x100) showing cellular degeneration (line arrow in Plate A), basement membrane distortions in Plate B ('triangular arrow') and interstitial space exudates (encircled)

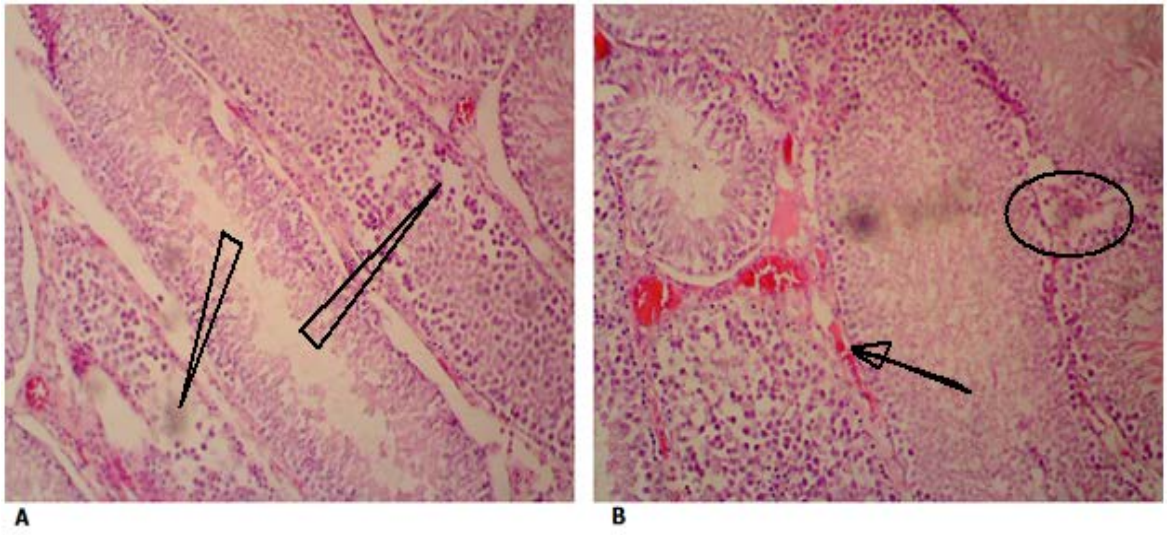

Plate 11 A&B. Testis (Test Group B5; H&E x100) showing mild cellular degeneration (line arrows in Plate A) with mild heamorrhage and interstitial space exudations ('encircled')

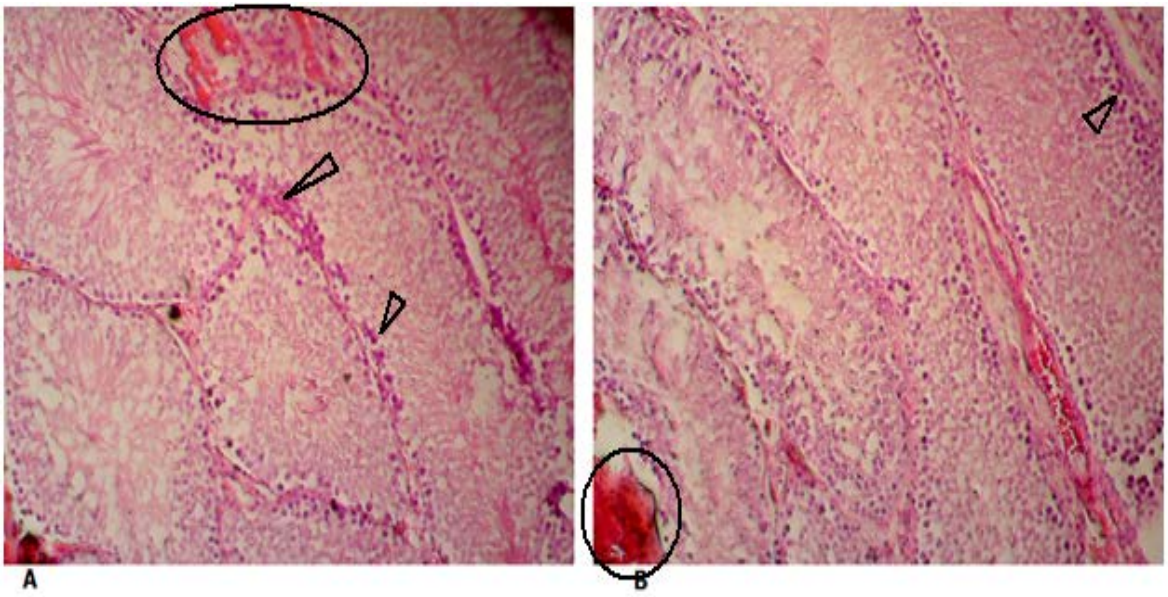

Plate 12A&B. Testis (Test Group B6; H&E x100) showing cellular necrosis (arrows A) with heamorrhage ('encircled')

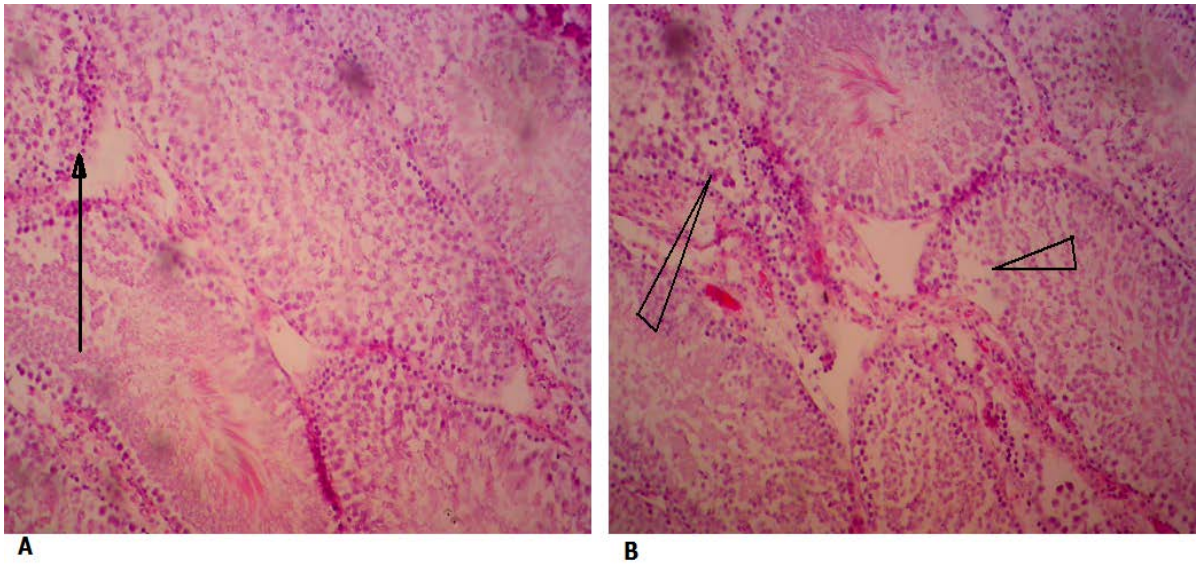

Plate 13A &B. Testis (Test Group C1; H&E x100) showing cellular degeneration and deeply staining cells (see arrows)

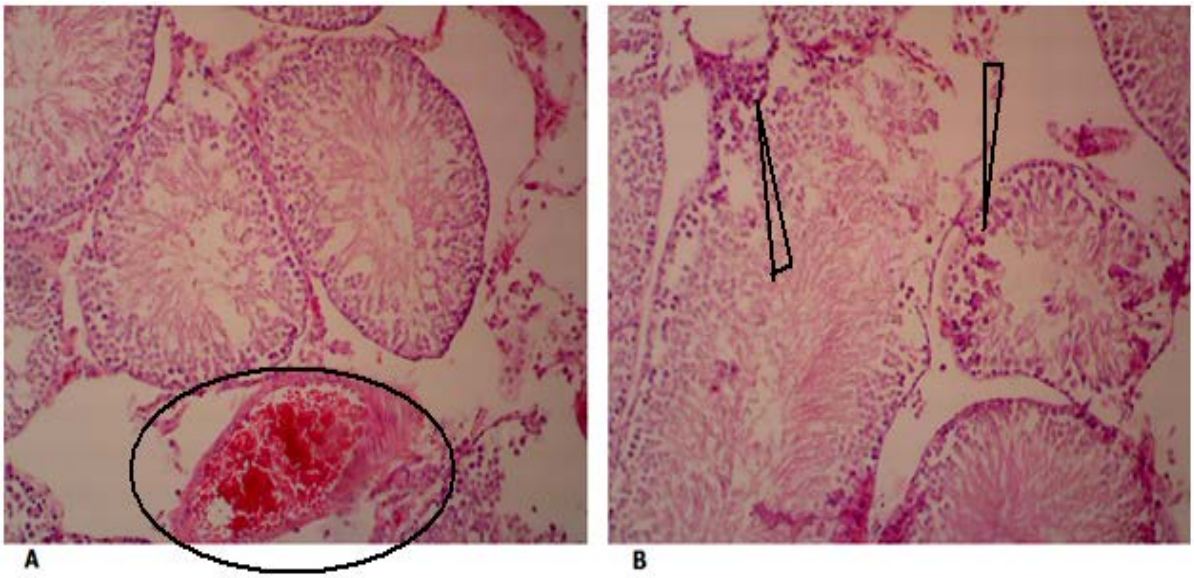

Plate 14A &B. Testis (Test Group C2; H&E x100) showing cellular degeneration and vessel congestion (encircled)

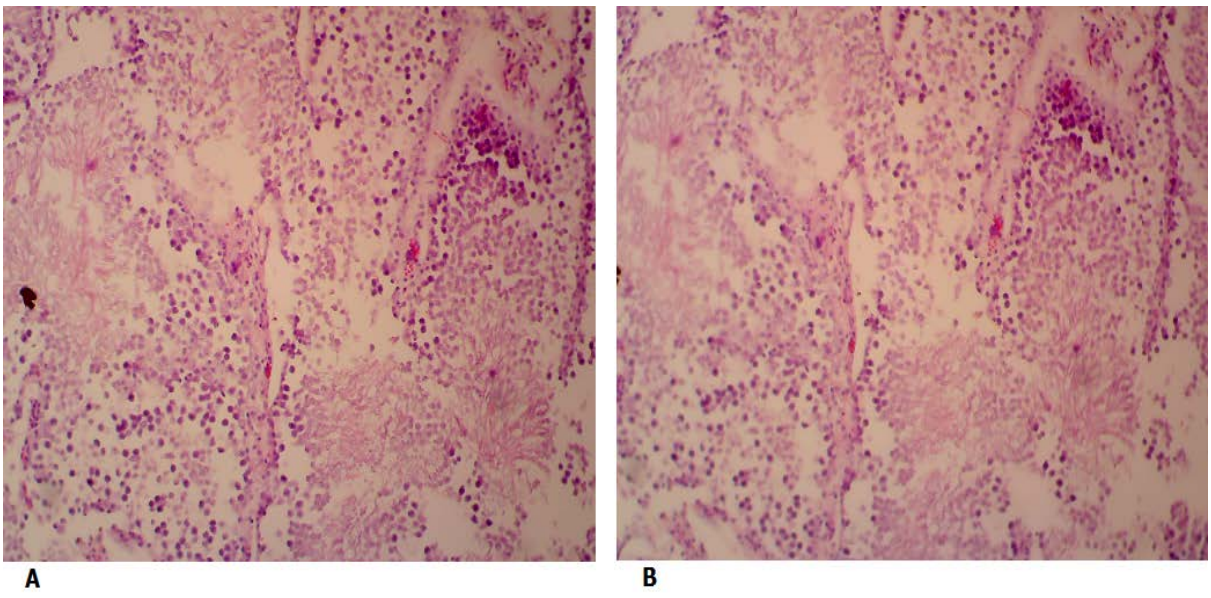

Plate 15A &B. Testis (Test Group C3; H&E x100) showing cytoarchitectural distortions with indistinct seminiferous tubules and numerous disoriented sperm cells

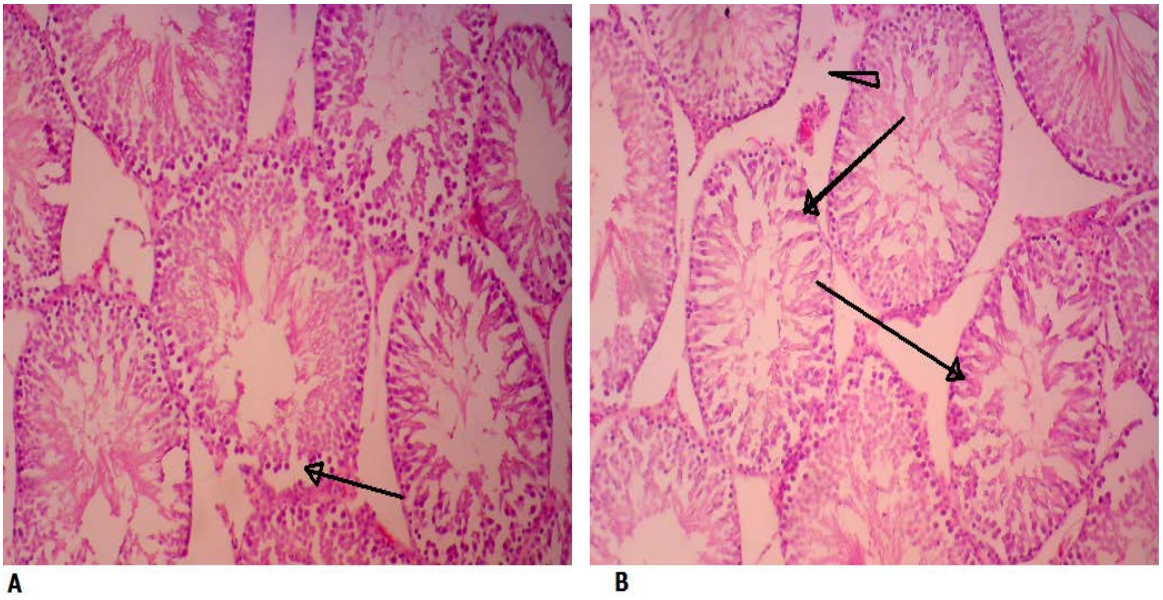

Plate 16A &B. Testis (Test Group C4; H&E x100) showing reduction in cell population with interstitial space exudations

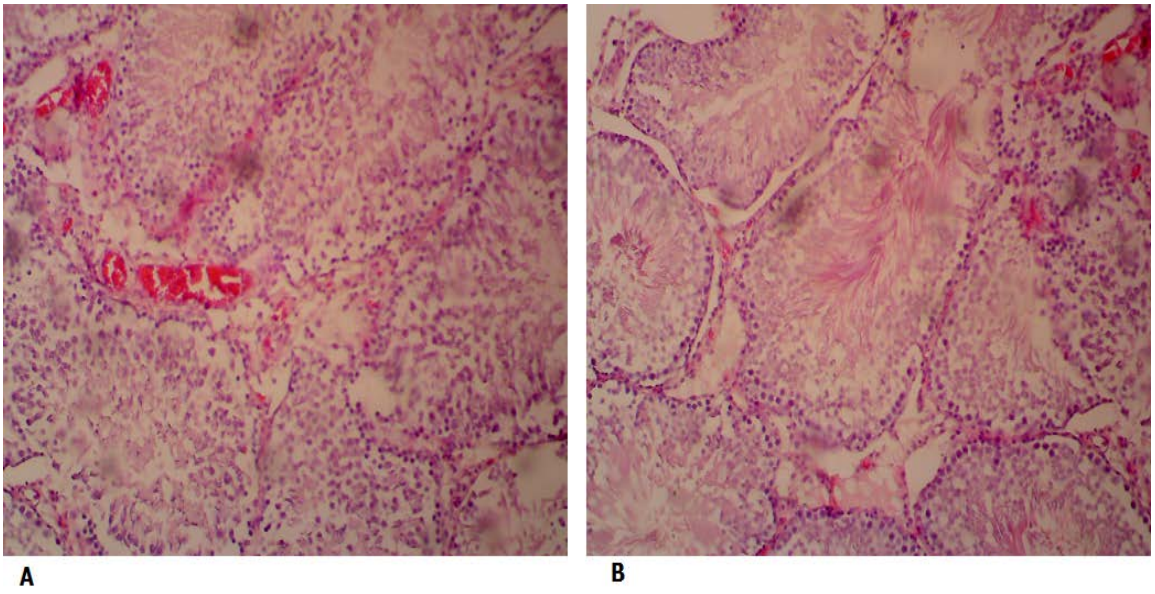

Plate 17A &B. Testis (Test Group C5; H&E x100) showing intact seminiferous tubules with cellular proliferation and intact blood vessels

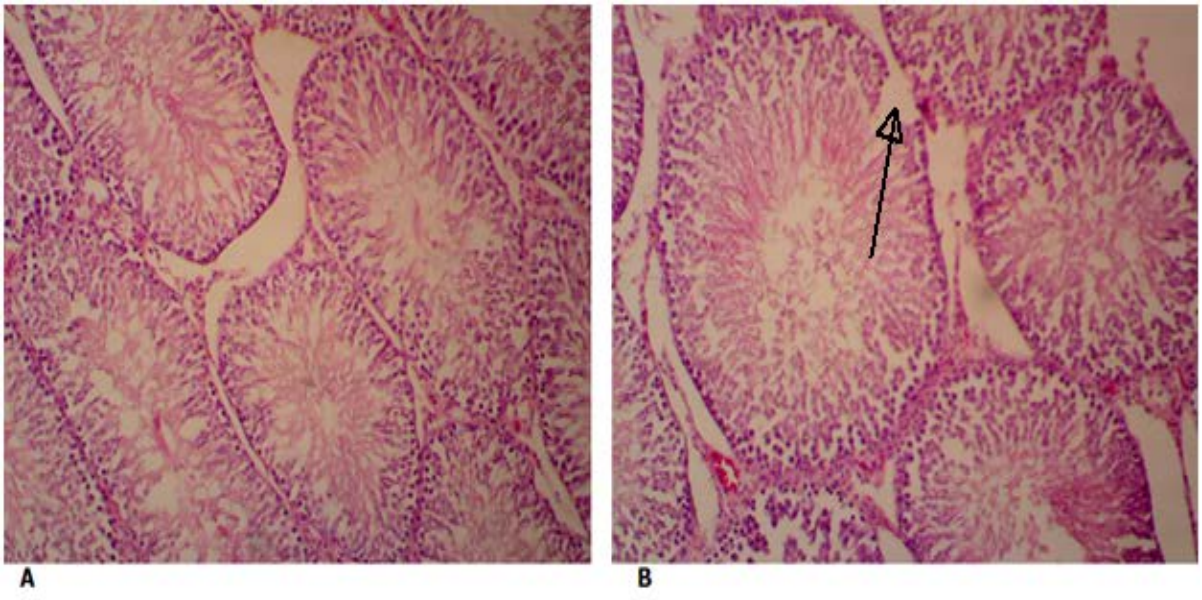

Plate 18A &B. Testis (Test Group C6; H&E x100) showing intact seminiferous tubules with mild basement membrane distortions (arrow in Plate B)

6. Discussion

It is widely accepted that consumption of plant foods in adequate amounts is associated with numerous health benefits rooted in their various physiological effects as a result of their phytochemical and nutritional constituents (Hunter and Fletcher, 2002). However, the finding of this study disagrees with this fact that plant in adequate amounts is associated with numerous health benefits considering the impact of *Telfairia occidentalis* on semen parameters, plasma testosterone value and the histological presentations on the testes reported in this study. The semen parameters investigated in this study occupy a position of relevance in male fertility studies. Hence any factor(s) which damages the testicles such as infections, toxic agents, malnutrition or heat will result in the production of subfertile spermatozoa. Specifically, the finding of this study shows that *Telfairia occidentalis* has a reducing effect on semen quality, plasma testosterone values and has potentials for testicular cell damage in a dose dependent fashion. This finding is in accordance with the finding of Saalu et al. (2010) who demonstrated that *Telfairia occidentalis* leaves extract possesses testiculotoxic potentials in Sprague-Dawley rats.

On the other hand, the present findings disagree with those of Saalu et al. (2010) and Muanya et al (2011) with regard the testiculo-protective properties reported with the ingestion of *Telfairia occidentalis* leaves extract in term of sperm motility, sperm progressivity and percentage normal spermatozoa count reported by them. The variations observed in this study as compared to previous studies where its protective potentials have been reported may be owned to the dose differences used. This is suggested considering the higher doses and period of administration employed in this study.

In another line of thought, the findings of this study may be justified considering the phytochemical constituents of the plant. This is sequel to the fact that pathology have been traced to the presence of alkaloids (Burkil, 1994), which have also been observed to be a constituent of *Telfairia occidentalis* (Badifu and Ogunsu, 1991). In fact, the roots and leaves of *Telfairia occidentalis* have been shown to contain highly toxic alkaloids and saponins (Akube, 1980). In addition, Taitzoglou *et al*.,(2001) as well as Nworgu *et al*., (2007) reported that a chemical constituent of *Telfairia occidentalis*, tannins even though they are classified as antioxidants; at a high dose, they could become pro-oxidant, increasing lipid peroxidation.

The relationship of the observed findings may be explained by the fact that reduced fluid secretion and tubular contraction is seen with many testicular toxicants. In many cases this is a secondary consequence of germ cell loss, but in others it appears to be an early event and probably represents disturbed Sertoli cell function (Dianne, 2001). Since tubular fluid secretion is an androgen-dependent function, compounds that cause significant reductions in testicular testosterone levels will reduce fluid secretion and reduce tubular diameter as a secondary effect. Hence, the observed histological variations from those of the control are justified by the findings in plasma testosterone values.

However, Nwangwa et al. (2007) has reported that *Telfairia occidentalis* has regenerative effect on the histology of rat testes. The question is how true this physiological homeostasis couple with the degree of damages done? Worrisome is the fact that *Telfairia occidentalis* leaves are widely consumed by many in the Southern Nigeria and other parts of West Africa in addition to their versatile ethno medical usage, due to some unverified claims by herbal practitioners (Saalu et al., 2010).

Judging by the findings of this study, higher doses coupled with a longer period of administration of *Telfairia occidentalis* may provoke varying degrees of testicular degeneration, deranged sperm parameters and worsened testicular oxidative status. In line with this fact, *Telfairia occidentalis* have been shown to induce degenerative changes in several organs (Ajayi *et al.* 2004). Though the pathogenesis of this effect is still unclear, alkaloids that are observed in *Telfairia occidentalis* may be implicated (Badifu and Ogunsua, 1991; Burkill, 1994). In accordant, it was previously postulated that these alkaloids could be bioactivated to release metabolites, which bind to cell molecules and cross-link DNA cause cytotoxicity. Also, Tannins at a high dose could become pro-oxidant, increasing lipid peroxidation (Taitzoglou *et al*., 2001; Nworgu *et al*., 2007). This explains for the drop in sperm count, morphology and motility including hormonal level as well as testicular degeneration observed. The poor plasma testosterone observed may also be similarly explained as the histological integrity of the testis is fundamental to the production and quality of the spermatozoa (Oyeyemi *et al*., 2008).

Conclusively, the results of the present study suggest that *Telfairia occidentalis* leaf extract have potential for reducing male sexual function considering the observed effect on semen quality, plasma testosterone level and histological presentation of the testis. It is therefore on the basis of this finding that we recommend a moderate and not often consumption of T*elfairia occidentalis*.

Acknowledgement

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