

Profertility effects of aqueous leaf extract of *Telfairia occidentalis* in adult male Wistar rats

Sakpa Lucky Christopher, Onovughakpo-Sakpa Oriseseyigbemi Ejuoghanran¹, Okhimamhe Akhalumhe Festus

Departments of Anatomy and ¹Chemical Pathology, University of Benin, Benin City, Nigeria

Abstract

Telfairia occidentalis is a leafy vegetable consumed commonly for its nutritional and medicinal effects. However, few studies have been carried out on its fertility effects in males with most of such studies offering conflicting results. The present study evaluated the effects of aqueous extract of the leaves of *T. occidentalis* on male fertility in adult wistar rats. The thirty rats used for this study were randomized into groups A, B and C. Rats in groups B & C were treated with 500 and 1000mg/kg.bwt of the leaf extract respectively by gavage for 56 days while group A served as control. The sperm characteristics, male hypothalamo-pituitary gonadal axis hormones and histological studies were carried out according to existing laboratory methods. Results from all the parameters studied showed that the aqueous extract of leaves of *T. occidentalis* exhibited profertility properties in adult male wistar rats. Further research to determine the components of the leaf extract implicated in the outcomes observed and the mechanism of action would be necessary.

Key words: Profertility, *Telfairia occidentalis*, Wistar rats

INTRODUCTION

A considerable amount of research has been directed toward the study of plants with the goal of providing new and improved treatment for ailments. Most of the plants widely studied are consumed by man. One of such plants is a nutritious vegetable,

Telfairia occidentalis. *T. occidentalis* is a member of the *Cucurbitaceae* family which consists of two other species (Saani, 1982). It is a perennial, drought tolerant plant (Saalu *et al.*, 2010) widely grown and consumed as vegetable in the West Africa. It is widely known with common names such as fluted pumpkin, fluted gourd and “Ugu” as it is called in the Nigerian Igbo language (Badifu *et al.*, 1995).

Address for correspondence:

Dr. Sakpa Lucky Christopher,
Department of Anatomy, University of Benin, Benin City, Nigeria.
E-mail: sakpachristopher@yahoo.com

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The plant consists of leaves and an edible seed. It is a rich source of protein containing as much as 37% protein per 100 g of *T. occidentalis* leaf meal. It contains fats, vitamins (e.g., riboflavin, Vitamin C, and nicotinamide) and minerals such as calcium and magnesium, polyphenols, and flavonoids (Tindall, 1968; Fasuyi, 2006; Halliwell and Guttridge, 1999). In addition, it contains marked amounts of tannins, saponins, and alkaloids (Ekpenyong *et al.*, 2012).

A significant amount of literature exists on the medicinal effects of *T. occidentalis* in various tissues and organs of treated animal models. For instance, *T. occidentalis* is known to possess antidiabetic, antibacterial and anti-inflammatory properties (Oluwole *et al.*, 2003; edema and Essien, 1995; Eseyin *et al.*, 2000). Also attributed to *T. occidentalis* is its effectiveness in the treatment of hypercholesterolemia (Eseyin *et al.*, 2007) and amelioration of the effects of quinine induced testicular damage (Nwangwa *et al.*, 2006). It has also been noted that *T. occidentalis* causes significant increases in total protein and total bilirubin levels, as well as albumin, globulin and conjugated bilirubin although it has a hepatoprotective effect on the liver even at high doses of administration in Wistar rats (Ekpenyong *et al.*, 2012). Hematological studies conducted have shown that *T. occidentalis* increases packed cell volume, hemoglobin concentration (Obeagu *et al.*, 2014), and total white blood cell count (Salman *et al.*, 2008).

Although most of the chemical constituents and active ingredients in *T. occidentalis* have well documented spermatogenic properties, there has been a paucity of literature on fertility studies with *T. occidentalis* in experimental animals. The few existing literature give conflicting reports on the effects of extracts of various components of *T. occidentalis* on male fertility using different rat species. Some of the researchers reported profertility effects at low doses with antifertility effects at high doses (Saalu *et al.*, 2010, Akang *et al.*, 2010). Other researchers have reported a dose dependent testicular damage with antifertility effects (Adisa *et al.*, 2014). It was based on this premise that we decided to carry out a study on the effects of aqueous leaf extract of *T. occidentalis* on fertility indices and histological changes in the testes and epididymis of treated male Wistar rats.

The present study is geared toward enhancing the availability of applicable knowledge on the effects of *T. occidentalis* on reproduction by evaluating the effects of the aqueous leaf extract on rat testicular histology, sperm characteristics, and male hormone profile.

METHODS



Plate 1: *Telfairia occidentalis* leaves and fruit at Sakpa Research Gardens

Telfairia occidentalis leaves and fruit at Sakpa Research Gardens

Methodology

Fresh leaves of *T. occidentalis* were obtained from Sakpa, Benin City, Edo State Nigeria and authenticated by Mr. Sunny Nweke at the Department of Plant Biology and Biotechnology, University of Benin, Benin City, Nigeria [Plate 1]. The leaves were washed and weighed (6.83 kg). The aqueous leaf extract was prepared according to the method by Sakpa and Uche-Nwachi (2014). The extract was kept in a sterile labeled container and refrigerated. The stock solution to be administered to the rats was prepared by dissolving 10 grams of the extract in 20 ml of distilled water to give a concentration of 500 mg/ml. The choice of aqueous leaf extract in this experiment was based on the fact that it is closest to the conventional method of consumption of *T. occidentalis*.

Thirty-six adult male Wistar rats weighing between 200 and 280 g were used for the study. The animals were housed in the Animal House of the Department of Anatomy, University of Benin, Benin City, Nigeria and cared for in accordance with the guidelines of the Research and Ethics Committee of the College of Medical Sciences, University of Benin, who gave ethical clearance for the research. The rats were acclimatized for 2 weeks prior to the experiment and were fed with Growers Marsh (Bendel Feed and Flour Mill Ltd.) with access to clean water throughout the entire study period of 8 weeks.

The animals were divided into three groups (groups A, B, and C) consisting of 12 rats each. Each group was further randomized into two subgroups (A1, A2, B1, B2, C1, and C2) consisting of six rats each. The animals in groups A1 and A2 which served as control had feed and clean water *ad libitum* for 4 and 8 weeks, respectively. The animals in subgroups B1 and

C1 received 500 mg/kgbw daily of aqueous leaf extract of *T. occidentalis* administered by gavage for 4 and 8 weeks, respectively, while the animals in treatment subgroup B2 and C2 received 1000 mg/kgbw daily of aqueous leaf extract of *T. occidentalis* administered by gavage for 4 and 8 weeks, respectively.

At the commencement of the experiment, at weekly intervals and at the end of each experimental period, the rats were weighed and the weights recorded. The rats were anesthetized using chloroform inhalation and sacrificed. The testes and epididymis were accessed through a ventral midline abdominal incision to harvest the relevant organs. Both testes and left caudal epididymis were fixed in Bouins fluid for histological studies while the right caudal epididymis was used to study sperm characteristics. The testes and left caudal epididymis were individually weighed before processing. The tissues were fixed, processed, and stained with hematoxylin and eosin. Photomicrographs were prepared from photographs taken at $\times 400$ and $\times 100$ magnifications for study of histological changes. Blood was obtained through direct cardiac puncture for hormone assay.

The right caudal epididymis was dissected free of adherent tissue and weighed and later incised longitudinally. The sperms were flushed out with 1 ml of 0.9% normal saline into a sterile 10 ml universal container and slides were prepared and examined for motility and total sperm count according to the method by Besley *et al.*, (1980). Score for morphological abnormalities and assessment of livability was performed using the method by Attehsahin *et al.*, (2006) and Mortimer (1989), respectively. The slides were examined under the microscope and photographs taken for the preparation of photomicrographs. Hormone assay was carried out using the Enzyme Immunoassay Test

kit according to the method of Uotila, 1981 for follicle stimulating hormone (FSH), leuteinizing hormone (LH), and prolactin. Progesterone was assayed by the method of Tietz, (1995); Estradiol (E2) by the methods of Hall, (1988) and Ratcliffe *et al.*, (1988) while testosterone was assayed by the method of Tietz, (1986).

Data were collected into a proforma and analyzed using the International Business Machines Statistical Product and Service Solutions (IBM-SPSS) version 20.0 Chicago IL. Means were also calculated and compared using one way analysis of variance. The level of statistical significance was set at $P < 0.05$. Charts and tables were drawn using Microsoft Office Excel, 2007.

RESULTS

Animal/Organ Weight

There was remarkable weight gain of rats in all the experimental groups. The treatment groups recorded higher weight gain when compared with the control groups. The increase in weight of treatment rats for 4 and 8 weeks was statistically significant ($P = 0.008$ and $P = 0.001$, respectively), when compared with the control rats. There was epididymal and testicular weight gain in all treatment groups, a finding which was absent in the control group. Statistically significant differences were recorded in comparison of mean testicular weights (weights of left and right testes compared separately) of 8 weeks treatment groups B2 and C2 to 8 weeks control group A2 ($P < 0.001$) [Table 1]; and also in comparison of mean testicular weights of rats in 4 weeks treatment groups B1 and C1 to 4 weeks control group A1 ($P < 0.001$) [Table 2], although there was no predeletion for left or right testes/epididymis in the patterns for weight gain.

Table 1: Body, testicular and epididymal weights of rats at end of 4 weeks of experiment

	Four weeks control	Four weeks treatment group		P value (ANOVA)
	group (group A1)	Group B1 (500 mg/kgbw)	Group B2 (1000 mg/kgbw)	
Body weight gain (g)	21.67 \pm 1.67	25.00 \pm 0.00	25.00 \pm 0.00	0.008*
Weight of right testis (g)	1.02 \pm 0.06	1.18 \pm 0.01	1.22 \pm 0.01	0.001*
Weight of left testis (g)	1.04 \pm 0.06	1.19 \pm 0.01	1.21 \pm 0.01	0.002*
Weight of right epididymis (g)	0.17 \pm 0.01	0.19 \pm 0.01	0.20 \pm 0.03	0.228
Weight of left epididymis (g)	0.18 \pm 0.01	0.19 \pm 0.01	0.20 \pm 0.003	0.485

*Statistically significant at $P < 0.05$. ANOVA - Analysis of variance

Table 2: Body, testicular and epididymal weights of rats at end of 8 weeks of experiment

	Eight weeks control group	Eight weeks treatment group		P value (ANOVA)
	(group A2)	Group C1 (500 mg/kgbw)	Group C2 (1000 mg/kgbw)	
Body weight gain (g)	40.00 \pm 0.00	56.67 \pm 4.41	51.67 \pm 1.67	0.001*
Weight of right testis (g)	1.39 \pm 0.05	1.70 \pm 0.01	1.70 \pm 0.00	<0.001*
Weight of left testis (g)	1.40 \pm 0.05	1.71 \pm 0.02	1.70 \pm 0.03	<0.001*
Weight of right epididymis (g)	0.20 \pm 0.01	0.21 \pm 0.03	0.21 \pm 0.01	0.770
Weight of left epididymis (g)	0.20 \pm 0.01	0.22 \pm 0.00	0.21 \pm 0.01	0.064

*Statistically significant at $P < 0.05$. ANOVA - Analysis of variance

Testicular/Epididymal Histological Photomicrographs

Figures 1a and 4a showed normal architecture of the seminiferous tubules of control animals at 4 weeks and 8 weeks respectively with normal architecture and sequential arrangement of cells of the spermatogenic series. The lumina of the seminiferous tubules contained tufted tails of spermatozoa. The tufted tails were densely packed in rats that received 500 mg/kgbw of aqueous leaf extract of *T. occidentalis* for 4 weeks [Figure 2a] and more densely packed in rats receiving 500 mg/kgbw of extract for 8 weeks [Figure 5a].

Rats receiving 1000 mg/kgbw of the extract had remarkably more densely packed spermatozoa in their seminiferous tubules [Figure 3a and 6a] when compared with rats receiving lower doses of extract for same periods. The finding was in keeping with increased spermatogenesis.

Figures 1b and 4b showed normal epididymal architecture for 4 and 8 weeks control rats, respectively. The lumina contain spermatozoa and in addition, there was presence of the normal luminal gap between the epithelia lining of the epididymis and the contained spermatozoa. This gap became progressively narrowed on administration of 500 mg/kgbw of aqueous leaf extract of *T. occidentalis* for 4 and 8 weeks, respectively [Figure 2b and 5b]. The luminal gap present in the epididymis was found to be narrowed at 4 weeks (in rats receiving 1000mg/Kg bw of *T. occidentalis* extract)

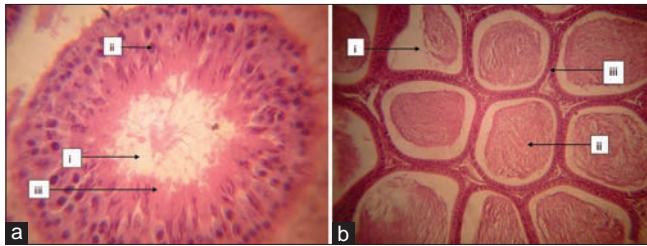


Figure 1: (a) Photomicrograph of seminiferous tubule of control rat at 4 weeks (H and E, $\times 400$), showing normal architecture with: (i) Lumen containing tufted tails of spermatozoa, (ii) spermatocyte and (iii) spermatozoon. (b) Photomicrograph of epididymis of control rat at 4 weeks (H and E, $\times 100$), showing: (i) Normal luminal gap, (ii) spermatozoa and (iii) epithelium

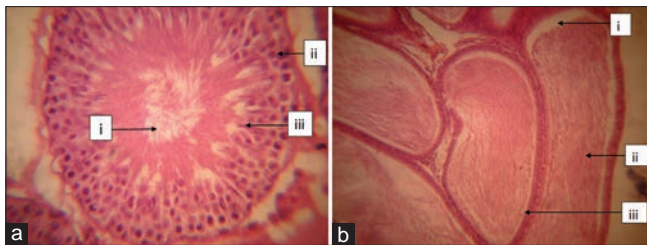


Figure 3: (a) Photomicrograph of seminiferous tubule of experimental rat at 4 weeks treatment with 1000 mg/kgbw of aqueous leaf extract of *Telfairia occidentalis* (H and E, $\times 400$), showing: (i) Lumen filled with tufted tails of spermatozoa, (ii) spermatocyte and (iii) spermatozoon. (b) Photomicrograph of epididymis of experimental rat at 4 weeks treatment with 1000 mg/kgbw of aqueous leaf extract of *Telfairia occidentalis* (H and E, $\times 100$), showing: (i) Narrowed luminal gap, (ii) spermatozoa and (iii) epithelium

and almost obliterated in treatment rats of subgroup C2 that received 1000 mg/kgbw of the plant extract for 8 weeks [Figure 6b].

Hormone Assay

Table 3 showed the gonadotrophin and sex hormone levels for both control and study groups. There was relative increase in gonadotrophin (FSH and LH) levels from control through groups B1, B2, C1, C2 but these increases were not statistically significant ($P > 0.05$). Prolactin levels fluctuated in the treatment groups but were less than the controls ($P > 0.05$).

There was progressive increase in testosterone levels between the control rats in the 4 and 8 week groups and the treatment groups for the same period. The increase was more marked in the high dose sub groups (1000 mg/kgbw) and these increases were statistically significant ($P = 0.025$ and $P = 0.016$, respectively), for 4 and 8 weeks study groups. Estrogen levels showed relative decrease in the study groups when compared to the controls and this was not statistically significant for the 4 weeks study period ($P = 0.828$) but significant for the 8 weeks group ($P = 0.048$). Testosterone/estrogen ratio increased from the control (0.24) to subgroups B2 (0.40) for the 4 weeks study group and from 0.21 for control to 0.41 in the subgroup C2 for 8 weeks study group. There was no specific pattern of change in progesterone levels during the study.

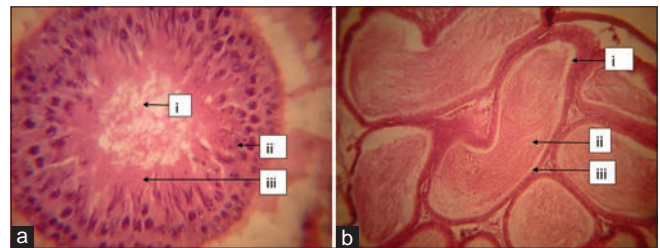


Figure 2: (a) Photomicrograph of seminiferous tubule of experimental rat at 4 weeks treatment with 500 mg/kgbw of aqueous leaf extract of *Telfairia occidentalis* (H and E, $\times 400$), showing: (i) Lumen containing tufted tails of spermatozoa, (ii) spermatocyte and (iii) spermatozoon. (b) Photomicrograph of epididymis of experimental rat at 4 weeks treatment with 500 mg/kgbw of aqueous leaf extract of *Telfairia occidentalis* (H and E, $\times 100$), showing: (i) luminal gap, (ii) Spermatozoa and (iii) epithelium

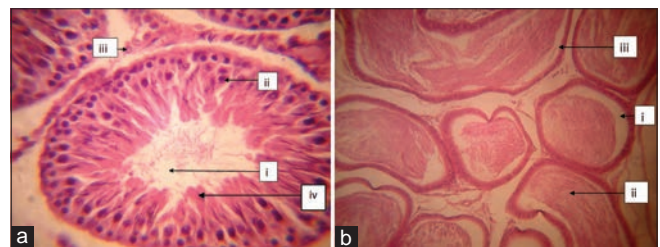


Figure 4: (a) Photomicrograph of seminiferous tubule of control rats at 8 weeks (H and E, $\times 400$), showing: (i) Lumen containing tufted tails of spermatozoa, (ii) spermatocyte and (iii) interstitial cell, and (iv) spermatozoon. (b) Photomicrograph of epididymis of control rat at 8 weeks (Hand E, $\times 100$), showing: (i) Normal luminal gap, (ii) spermatozoa and (iii) epithelium

Sperm Characteristics

The livability of the spermatozoa for all groups was consistently above 80% rising to as much as 90.2% in subgroup C2 on prolonged administration of 1000 mg/kgbw of *T. occidentalis* extract. The sperm count progressively increased from 4 weeks to 8 weeks and in animals receiving low and high doses of the extract. This increase in sperm count was statistically significant ($P = 0.025$) at 4 weeks and 8 weeks ($P = 0.036$) with a mean difference of

17.16×10^6 and 25.33×10^6 spermatozoa per ml of semen between subgroups B2 and C2 with their respective 4 and 8 weeks controls. Presence of abnormal sperm cells was very low in all experimental groups (with the highest average being <7% for any subgroup in the study). There was also an increase in number of motile sperms which in part may be from the increased number of normal viable spermatozoa due to the effect of the extract at 4 weeks which was further enhanced at 8 weeks with the 8 weeks values being significantly higher than normal control values ($P = 0.018$) [Tables 4 and 5].

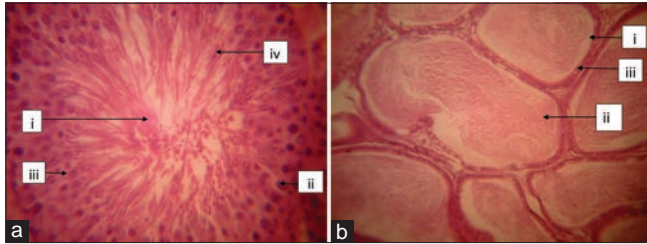


Figure 5: (a) Photomicrograph of seminiferous tubule of experimental rats after 8 weeks treatment with 500 mg/kgbw of aqueous leaf extract of *Telfairia occidentalis* (H and E, x400), showing: (i) Lumen containing tufty tails of spermatozoa, (ii) spermatocyte, (iii) spermatid, and (iv) spermatozoon. (b) Photomicrograph of epididymis of experimental rat at 8 weeks treatment with 500 mg/kgbw of aqueous leaf extract of *Telfairia occidentalis* (H and E, x100), showing: (i) narrowing of luminal gap, (ii) spermatozoa and (iii) epithelium

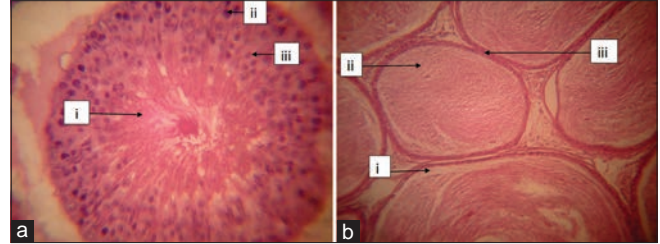


Figure 6: (a) Photomicrograph of seminiferous tubule of experimental rats after 8 weeks treatment with 1000 mg/kgbw of aqueous leaf extract of *Telfairia occidentalis* (H and E, x400), showing: (i) Lumen filled with tufty tails of spermatozoa, (ii) spermatocyte and (iii) spermatozoon. (b) Photomicrograph of seminiferous tubule of experimental rat at 8 weeks treatment with 1000 mg/kgbw of aqueous leaf extract of *Telfairia occidentalis* (H and E, x100), showing: (i) Marked narrowing of the luminal gap, (ii) spermatozoa and (iii) epithelium

Table 3: Hormone assay of rats in all experimental groups

	Four weeks			P	Eight weeks			P
	Control	Study			Control	Study		
		500 mg	1000 mg			500 mg	1000 mg	
FSH (U/L)	7.28±2.62	6.08±3.65	5.30±2.56	0.527	6.72±3.43	5.08±3.63	4.90±3.63	0.631
LH (U/L)	3.35±1.99	2.96±1.18	2.88±1.34	0.854	3.72±1.95	3.35±0.68	3.12±1.95	0.817
Prolactin (ng)	6.30±2.01	5.92±3.79	6.12±3.27	0.978	6.22±2.11	4.92±2.55	5.21±2.96	0.661
Testosterone (pg/ml)	3.44±1.30	4.62±0.10	5.08±1.00	0.025*	3.35±1.20	5.35±1.20	5.44±1.30	0.016*
Estrogen (pg)	14.18±9.51	11.84±5.96	12.73±2.41	0.828	5.75±9.05	14.35±3.0	13.32±3.88	0.048*
Progesteron (ng)	3.38±2.81	3.88±1.30	4.42±1.32	0.658	4.98±1.86	3.26±1.60	3.44±1.12	0.144
Testosterone/estrogen ratio	0.24	0.39	0.40		0.21	0.37	0.41	

*Statistically significant at $P < 0.05$. FSH - Follicle stimulating hormone, LH - Luteinizing hormone

Table 4: Semen parameters of rats at 4 weeks

Parameter	Four weeks control group (group A2)	Four weeks treatment group		P value (ANOVA)
		Group B1 (500 mg/kgbw)	Group B2 (1000 mg/kgbw)	
Sperm count ($\times 10^6$ /ml)	55.11±7.68	61.21±10.20	72.27±11.11	0.025*
Morphology (percentage of abnormal)	5.30±1.28	6.80±1.16	6.40±1.71	0.193
Motility ($\times 10^6$)	39.36±7.18	48.32±6.13	55.10±11.24	0.108
Livability (%)	88.3	91.2	84.7	

*Statistically significant at $P < 0.05$. ANOVA - Analysis of variance

Table 5: Semen parameters of rats at 8 weeks

Parameter	Eight weeks control group (group A2)	Eight weeks treatment group		P value (ANOVA)
		Group C1 (500 mg/kgbw)	Group C2 (1000 mg/kgbw)	
Sperm count ($\times 10^6$ /ml)	58.21±11.17	69.25±16.28	83.54±17.50	0.036*
Morphology (percentage of abnormal)	6.32±1.80	4.40±1.02	6.02±0.98	0.051
Motility ($\times 10^6$)	41.54±8.63	53.55±11.52	64.22±13.08	0.018*
Livability (%)	92.6	86.7	90.2	

*Statistically significant at $P < 0.05$. ANOVA - Analysis of variance

DISCUSSION

Conflicting reports on the effects of extracts of various components of *T. occidentalis* on male fertility have appeared in the literature in recent times. Some of these reports claimed that the aqueous leaf extract caused decreased spermatogenesis with distortion of the histological features of the seminiferous tubules and alteration in sperm characteristics (Adisa *et al.*, 2014). Other researchers claimed an enhancing effect with low doses (200 mg/kgbw) while higher doses (400 mg/kgbw and 800 mg/kgbw) decreased sperm count with associated testicular damage or without significant effects on testicular histology (Saalu *et al.*, 2010; Akang *et al.*, 2010). These researchers either used aqueous or seed oil extract and at dosages between 200 mg/kgbw and 800 mg/kgbw for their experiments. The present study used the aqueous leaf extract which is closest to the traditional method by which *T. occidentalis* leaf is consumed as a vegetable in soups or squeezed and drunk as concoction either alone or as a mixture with milk or tomato paste. It also used higher dosages of 500 and 1000 mg/kgbw < 5244.04 mg/kg – The LD₅₀ of *T. occidentalis* in Wistar rats (Ekpeyong *et al.*, 2012) and administered the extract for short and long durations (4 weeks and 8 weeks, respectively).

The present study showed increased body and organ weight gain among the experimental animals. This corroborates previous studies which showed that *T. occidentalis* is associated with weight gain because of its high protein and vitamin content (Fasuyi, 2006). The histological studies showed normal testicular architecture with histology of the seminiferous tubules and epididymis well preserved throughout the study period. There was an evidence of increased spermatozoa in the lumina of the seminiferous tubules and epididymis among the treated rats.

The sperm parameters showed that *T. occidentalis* had an enhancing effect on spermatogenesis. There was increased sperm count, motility, and livability with increasing doses and duration of treatment. The hormone profiles studied also showed significant rise in testosterone and testosterone/estrogen ratios among the treated rats with associated increase in the levels of gonadotropins (LH and FSH).

Spermatogenesis in mammals requires the action of a complex assortment of hormones, each playing an important role in the normal functioning of the seminiferous epithelium. These hormone messengers require male germ cell development and also the proliferation and functioning of somatic cell types required for proper development of the testis (Sharpe, 1994; McLachlan *et al.*, 2002). FSH and LH act directly on the testes to stimulate

somatic cell function in support of spermatogenesis. The secretion of FSH and LH is regulated by the release of gonadotrophin releasing hormone produced in the hypothalamus (Pierce and Parsons, 1981; Pinilla *et al.*, 2012). In males, FSH receptor expression is limited to the testicular sertoli cells while LH receptors are found primarily in the Leydig cells, although receptor staining was also observed in spermatogenic cells (Rannikki *et al.*, 1995; Eblen *et al.*, 2001; Lei *et al.*, 2001). Whereas LH is critical for the stimulation of Leydig cells to secrete testosterone, FSH stimulates the sertoli function and regulation of spermatogenesis (Mendis-Handagama, 1997). Testosterone is absolutely required for the long meiotic prophase and entry into the final meiotic division. Without testosterone, no haploid spermatids are formed. The progression of haploid spermatids through spermiogenesis also relies on testosterone and in the absence of androgens, spermatogenic arrest occurs. The final release of spermatids during the process of spermiation is also sensitive to androgen and gonadotropin inhibition (De Gendt *et al.*, 2004; Chang *et al.*, 2004; Abel *et al.*, 2008; Lim *et al.*, 2009; O'Donnell *et al.*, 1999; Holdcraft and Braun, 2004; O'Donnell *et al.*, 2011). The result of the present study showed that the administration of aqueous leaf extract of the leaves of *T. occidentalis* at doses of 500 mg and 1000 mg/kgbw for 4 and 8 weeks duration progressively increased testosterone and testosterone estrogen ratio.

The increased levels of testosterone in this study, correlates with the sperm characteristics and histological findings in which there was increased spermatogenesis and maintenance of normal testicular architecture.

Put together, the findings from the histological, sperm parameters and hormonal studies support increased spermatogenesis following administration of the aqueous extract of the leaves of *T. occidentalis* to adult male Wistar rats. It can, therefore, be concluded that the aqueous extract of *T. occidentalis* has pro-fertility potentials.

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Nil.

Conflicts of Interest

There are no conflicts of interest.

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