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Effects of Ethanolic Extract of *Dioscorea villosa* Tubers on Male Reproductive Parameters in Wistar Rats

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Authors' contributions

This work was carried out in collaboration between all authors. Authors SAS and NJU were involved in concept, design and histopathological analysis. Author AOF was involved in data analysis/interpretation. Author ICE participated in manuscript creation involving critical writing and revising of the content. All authors read and approved the final version of this manuscript.

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Original Research Article

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ABSTRACT

Aim: The current study investigates the effect of the ethanoic extract of *Dioscorea villosa* on male reproductive parameters using wistar rats.
Study Design: Twenty four male rats were randomly sorted into 6 groups (4 rats/group). Group 1 served as the short term control group and Group 2 served as the long term control group. Both groups were given water and rat chow ad libitum with no dose of the extract. Group 3 served as the short term low dose group and Group 4 as the long term low dose group. Both groups were administered 100 mg/kg body weight of *Dioscorea villosa* extract for 14 days and 28 days respectively using distilled water as the medium. Group 6 served as the short term high dose group and group 5 as the long term high dose group. Both groups were administered 400 mg/kg body

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weight of the *Dioscorea villosa* extract for 14 days and 28 days respectively, using distilled water as the medium.
Methodology: After the last day of administration (the 15th for the short term groups and the 29th day for the long term groups), the animals were sacrificed by cervical dislocation, fasting serum samples were obtained for the sex hormone analysis, cauda epididymis were dissected for sperm count, motility and viability, and organ weights of the testis and seminal vesicle were taken. Histopathological changes of the testis were also studied.
Results: *Dioscorea villosa* extract caused significant changes in the sperm count, serum FSH, LH and Testosterone levels of the short term groups and a significant change in the serum Testosterone level of the long term group. There was an increase in the sperm count in the short term groups when compared to the short term control group with that of the high dose being statistically greater than the low dose group. The serum levels of follicle stimulating hormones (FSH), luteinising hormones (LH) and Testosterone decreased in the low dose group but were all increased in the high dose group when compared to the short term control. The long term group recorded an increase in the serum testosterone level in the high dose group when compared with the long term control group. There however was no significant improvement in the sperm motility and viability of the groups given low and high dose of extract when compared to that of the control group. There was no any statistically significant changes in the weight differences of the rats between the experimental and control groups. The control group showed a decrease in weight of -8.30±6.86% while the low dose (100 mg/kg bwt) and high dose (400 mg/kg bwt) groups showed differences of -6.80±3.25% and 1.44±1.73% respectively. There was also no significant weight loss or weight gain of the reproductive organs (testis and seminal vesicles) was noticed, in both the short term low dose (100 mg/kg bwt) and high dose (400 mg/kg bwt) groups when compared to that of the control group.
Conclusion: The result suggests that *Dioscorea villosa* has significant effects on male reproductive parameters both in short term and long term administration. It also showed significant effect both in low and high doses.

Keywords: Dioscorea villosa; rat; sperm parameters; testis; testosterone levels; Follicle Stimulating Hormones (FSH); Luteinizing Hormones (LH).

1. INTRODUCTION

The reproductive system is distinctive amongst the other systems of the body, being the only one that functions for the survival and continuity of the species unlike the others which function for the survival of the individual. It presents other distinctive qualities such as being dormant until puberty; and having great dissimilarity in the male and female species [1].

The male reproductive system is divided into factors that affect male function. These include: Brain centres, which control the release of hormones from the pituitary and sexual behaviour; gonadal structures, which produce sperm and hormones; a system of ducts, which store and transport sperm; and accessory glands, which support viability of the sperm [2].

The structures of the system can be grouped based on function into: Primary sex organs, which specifically are the testes; secondary sex organs, which include the sperm-transporting ducts – the epididymis, ductus deferentia, ejaculatory ducts and urethra, the accessory reproductive glands – the seminal vesicles, prostate and bulbourethral glands, and the copulatory organ – the penis [1].

The system carries out three major functions: Spermatogenic function, which includes the production, maintenance and transportation of the sperm; discharge function, which covers the release of sperm into the female reproductive tract during coition; and hormone production, which consists of the production and secretion of hormones responsible for maintaining the male reproductive system.

The male reproductive system is regulated by the Hypothalamic-Pituitary Gonadal (HPG) axis. Of the three components of the HPG axis, the hypothalamus and the pituitary have sole regulatory functions, mediated by the hormones they produce and secrete. The third component, the testes, produces sperm, and hormones that control male sexual characteristics [3].

The hypothalamus produces Luteinizing Hormone-Releasing Hormone (LHRH), which is secreted in a pulsatile fashion into a system of blood vessels that connect the hypothalamus

and pituitary gland. This causes the pituitary gland to produce two protein hormones – Luteinizing Hormone (LH) and Follicle-Stimulating Hormone (FSH), which are released into the body's general circulation and act primarily at the level of the gonads. LH stimulates the production of testosterone from specialized Leydig cells while FSH functions for sperm maturation in the epididymis. Testosterone helps regulate the production of LH and FSH via negative feedback mechanism. When the system is functioning normally, low testosterone levels result in a rise in pituitary gonadotropins [3].

Low levels of testosterone in adult men have been associated with a variety of medical problems which include accelerated osteoporosis, decreased muscle and prostate function, anaemia, and decreased reproductive ability [4-7].

Dioscorea is a genus of 600 species flowering plants in the Dioscoreaceae, the family that contains true yams. It is found in Africa, India, Southeast Asia, Australia and tropical America [8-10] and probably originated in South-East Asia, before spreading more than 100 million years ago to Africa and the Americas [11]. They are slender twining annual herbs distributed throughout the tropical, subtropical and warm temperate regions of the world.

Dioscorea villosa is a tuber vegetable commonly called Wild yam or Wild Mexican yam, colic root or rheumatism root [12]. It is one out of the numerous species of the genus Dioscorea. Other synonyms for it as named by The Plant List (2010) include *Dioscorea quaternata* and *Dioscorea hirticaulis* amongst many. It is a woodland herb native from the temperate forests of eastern North America composed of chemicals such as protodioscin, methylprotodioscin, dioscine, prosapogenin, epiafzelechinglu-copyranoside, saponin glycosides, steroidal saponin, diosgenin, alkaloids, tannins and phytoestrogen [13-17]. *Dioscorea villosa* is a deciduous perennial herbaceous twiner that grows counter-clockwise over small and medium-size shrubs. The plants are dioecious and the fruit is a membranous 3-valved capsule with one or two chocolate-coloured winged seeds in each locule. The parts of this plant used are the dried rhizome and roots [12].

Dioscorea villosa has been discovered from previous studies to have a lot of uses mostly medical, which include: Remedy for pains associated with rheumatism and arthritis, colic and intestinal cramps, and being a reliable anti-inflammatory and antispasmodic [18] hence the names "Colic root and Rheumatism root". It is also said to be used to relieve labour pains [19], treat nausea and spasms [20], ease nervous excitement and muscular tension [21], and treat all manner of gut conditions [22]. Also, the roots and rhizomes of the plant have been popularly used as a non-conventional treatment of symptoms of menopause and menstrual complaints [23-25], rheumatoid arthritis and hypoprogesteronemia [13,16] amongst other uses.

Despite all the said effects and functions of wild yam, research has not been carried out on its effects on the male reproductive system. The current study therefore investigates the effect of the ethanoic extract of *Dioscorea villosa* on male reproductive parameters using wistar rats.

2. MATERIALS AND METHODS

2.1 Materials

2.1.1 Instrumentation

Six rat cages, Rat chow, Drinking water, Drinkers, Weighing balance, *Dioscorea villosa* tubers, Knife, Mortar and Pestle, Cloth, Hand gloves, Oral cannula, Dissecting set, Dissecting board, Office pins, Beakers, Syringes, Permanent marker, Teat pipette, Serum bottles, Organ bottles, Test tubes, Centrifuge, Spatula, Microscope, Distilled water, Normal Saline, Formal saline, Bouin's solution

2.1.2 Experimental animals

24 adult Albino Wistar rats weighing 130 – 170, (age 5 to 6 months) were purchased from the Animal House of National Veterinary Research Institute, Jos, Nigeria and kept under standard conditions in the Bingham University Animal Facility. The rats were housed in well ventilated cages and kept under controlled light schedule (12-hour light and 12-hour dark cycle) and were fed standard rat chow and water ad libitum. The rats were allowed to acclimatize for 4 weeks before the start of the administration.

2.1.3 Plant material

Twenty (20) tubers of the plant material were collected from a village in Keffi, Nasarawa State, Nigeria in March 2014.

2.2 Methodology

2.2.1 Preparation of extract

The tubers were chopped into chips and dried under shade at room temperature. They were then pounded with mortar and pestle to obtain a coarse powder which was then subjected to ethanolic extraction by maceration method in the Bingham University Chemistry and Biochemistry Laboratories.

400 g of powdered *Dioscorea villosa* was weighed using an electronic weighing balance and transferred into a 2000 ml conical flask. 50% ethanol was used to soak the sample. The content was left to stand overnight and then it was shaken for 3 hours. The content was filtered followed by several addition of the extracting solvent and subsequent filtration.

The filtrate was concentrated in a rotary evaporator and then evaporated to dryness in a water bath at a control temperature of 78℃. A brown solid substance was then obtained (which was named the "extract"). It was weighed and diluted using distilled water.

2.2.2 Experimental design

The rats were randomly divided into 6 different groups each containing 4 rats.

Group 1 was used as the **short term control group** and was fed with standard rat chow and water ad libitum without any administration of the extract for 14 days.
Group 2 was used as the **long term control group** and was fed with standard rat chow and water ad libitum without any administration of the extract for 28 days.
Group 3 was the **short term low dose group** and was fed 100 mg/kg of body weight of the extract in addition to standard rat chow and water ad libitum for 14 days.
Group 4 was the **long term low dose group** and was fed 100 mg/kg of body weight of the extract in addition to standard rat chow and water ad libitum for 28 days.
Group 5 was the **long term high dose group** and was fed 400 mg/kg of body weight of the extract in addition to standard rat chow and water ad libitum for 28 days.
Group 6 was the **short term high dose** group and was fed 400 mg/kg of body weight of the extract in addition to standard rat chow and water ad libitum for 14 days [26].

2.2.3 Sperm function test

Sperm Function Analysis: The rats were sacrificed 24 hrs after the last day of administration and weighed for the essential reproductive organs, such as testis, and seminal vesicle. The spermatozoa were obtained by making a small incision (1 mm) in the caudal epididymis and evaluated for sperm count, motility and viability. The sperm count was determined with an improved Neubauer haemocytometer and viability was assessed by eosin-nigrosin dye exclusion test. Epididymal sperm motility was assessed by calculating motile spermatozoa per unit area and was expressed as percent motility [26].

2.2.4 Hormonal assay

Three hormones involved in the regulation of the reproductive system were tested for in the short and long term groups of rats and their levels compared with their various control groups. These hormones include testosterone, Luteinizing Hormone (LH) and Follicle Stimulating Hormone (FSH).

Blood samples were spun at 2500 rpm for 10 minutes in a Table top centrifuge. The serum samples obtained were analyzed to determine the concentration of Testosterone, Luteinizing Hormone (LH), and Follicle Stimulating Hormone (FSH).

The analysis was done using stat-fax semi-automatic machine and cobas e411 auto-analyser. The reagent used was AccuBind ELISA reagents. Serum samples were assayed for levels of the three hormones using Microwell enzyme linked immunoassay (ELISA) technique [27].

2.3 Statistical Analysis

The data gotten from the experiments were statistically evaluated using ANOVA (see appendix), and the students t-test with SPSS/14.0 software (SPSS Inc., Chicago, USA) and were expressed as Mean ± Standard Error of Mean (SEM). A value of $P< 0.05$ was considered to indicate a significant difference between groups.

3. RESULTS

3.1 Sperm Function

A significant increase was observed in the sperm count of both the low dose (100 mg/kg bwt)

group (69±2.68x10^6/ml) and high dose (400mg/kg bwt) group (82±1.22x10$^{6/}$/ml) when compared with the control group (54.25±3.90x10$^{6/}$/ml).

There however was no significant improvement in the sperm motility and viability of the groups when compared to that of the control group.

No significant change was seen either in the sperm motility, sperm viability and sperm count of the long term group in both the low dose (100 mg/kg bwt) and high dose (400 mg/kg bwt) groups when compared to that of the control group.

3.2 Organ Weights

No significant weight loss or weight gain of the reproductive organs (testis and seminal vesicles) was noticed, in both the short term and long term low dose (100 mg/kg bwt) and high dose (400 mg/kg bwt) groups when compared to that of the control group.

3.3 Hormonal Assay

Significant changes were seen in the serum levels of FSH, LH, and testosterone in the experimental groups when compared to the control group (3.08±023 miU/ml, 2.24±0.20 miU/ml, 5.13±0.19 ng/ml, respectively). There was a significant decrease of FSH, LH and testosterone in the low dose (100 mg/kg bwt) group (1.79±0.08 miU/ml, 1.35±0.06 miU/ml, 2.64±0.21 ng/ml, respectively) and a significant increase in the high dose (400 mg/kg bwt) group (4.51±0.25 miU/ml, 3.32±0.22 miU/ml, 8.96±0.61 ng/ml, respectively).

A significant increase was seen in the testosterone levels in the high dose (400mg/kg bwt) group (2.65±0.29 ng/ml) when compared to the control group (0.75±0.28 ng/ml). There was however no other significant change in the FSH, LH and testosterone levels of the low dose (100 mg/kg bwt) and high dose (400 mg/kg bwt) groups when compared with the control group.

3.4 Body Weights

No statistically significant changes in the weight differences of the rats between the experimental and control groups for the short term administration. The control group showed a decrease in weight of -8.30±6.86% while the low dose (100 mg/kg bwt) and high dose (400 mg/kg bwt) groups showed differences of -6.80±3.25% and 1.44±1.73% respectively.

No statistically significant change was seen in the weight of different experimental groups for the long term administration. There were however differences between the experimental groups and the control group. The control group showed a weight difference of 11.89±5.27% while the low dose (100 mg/kg bwt) and high dose (400 mg/kg bwt) groups showed reduced weight increments of 6.42±11.33% and 5.48±8.57% respectively.

Table 1. Effect of oral administration of *Dioscorea villosa* extract on the sperm function tests for the short term (14 days) groups

Groups (14 Days)	Count (x10^6/ml)	Motility (%)	Viability (%)
Group 1 – Control	54.25±3.90	62.5±8.54	70±7.07
Group 3 – 100 mg/kg	69±2.68^a	63.25±6.24	66.5±2.36
Group 6 – 400 mg/kg	82±1.22^{ab}	80±4.08	76.5±2.36^b

Values expressed as mean±sem, P<0.05, n=4. ^{a} represents values that are statistically significant when compared to control group, ^{b} represents values that are statistically significant when compared with the low dose (100 mg/kg bwt) group

Table 2. Effect of oral administration of *Dioscorea villosa* extract on the sperm function tests for the long term (28 days) groups

Groups (28 Days)	Count (x10^6/ml)	Motility (%)	Viability (%)
Group 2 – Control	81.5±3.28	67.5±4.79	82.5±4.79
Group 4 – 100 mg/kg	68.75±4.29	77.5±4.79	80±4.08
Group 5 – 400 mg/kg	77.00±4.29	77.5±6.29	70±0.01

Values expressed as mean ± sem, P<0.05, n=4, ^{a} represents values that are statistically significant when compared to control group, ^{b} represents values that are statistically significant when compared with the low dose (100 mg/kg bwt) group

4. DISCUSSION

The main function of the male reproductive system is spermatogenesis, which results in the formation of the spermatozoa. Local regulation of spermatogenesis is carried out by extra-testicular stimuli provided by the hypothalamus and pituitary gland [28] which together with the testis form the hypothalamic-pituitary-gonadal-axis. Spermatogenesis is regulated by the pulsatile release of Gonadotropin-releasing Hormone (GnRH) from the arcuate nucleus of the hypothalamus. This then stimulates the release of Follicle-Stimulating Hormone (FSH) and Luteinizing Hormone (LH) from the anterior pituitary. The LH stimulates the Leydig cells to produce testosterone, which acts locally on the interstitium and seminiferous tubules, resulting in sperm production and maturation. This effect was manifested by very high intra-testicular testosterone compared with the bloodstream. FSH acts directly on the Sertoli cells to promote spermatogenesis [29]. Testosterone and estradiol (converted through aromatase in the testis interstitium) are direct negative feedback modulators of GnRH, LH and FSH. Aromatase inhibition increases FSH levels indicating that FSH regulation is more dependent on estradiol than testosterone [30,31].

Table 3. Effect of oral administration of *Dioscorea villosa* extract on reproductive organ weights for the short term (14 days) groups

Groups (14 days)	Testis (g)	Seminal vesicle (g)
Group 1 – Control	0.96±0.13	0.97±0.23
Group 3 – 100 mg/kg	0.99±0.09	1.01±0.19
Group 6 – 400 mg/kg	1.12±0.03	1.10±0.16

Values expressed as mean±sem, P<0.05, n=4. [a] represents values that are statistically significant when compared to control group, [b] represents values that are statistically significant when compared to the low dose (100 mg/kg bwt) group and high dose (400 mg/kg bwt)

Table 4. Effect of oral administration of *Dioscorea villosa* extract on reproductive organ weights for the long term (28 days) groups

Groups (28 days)	Testis (g)	Seminal vesicle (g)
Group 2 – Control	1.29±0.12	1.53±0.13
Group 4 – 100 mg/kg	1.21±0.11	1.09±0.14
Group 5 – 400 mg/kg	1.32±0.09	1.32±0.19

Values expressed as mean±sem, P<0.05, n=4. [a] represents values that are statistically significant when compared to control group, [b] represents values that are statistically significant when compared to the low dose (100 mg/kg bwt) group

4.1 Effect on the Sperm Count, Motility, and Viability

The increase in the sperm count might be as a result of an increase in the secretion of the reproductive hormones which are controlled in negative feedback fashion by the hypothalamic-pituitary-gonadal-axis (HPG-axis). That is to say that there could have been an increase in the secretion of GnRH which would then caused an increase in the level FSH, and LH which would stimulate the Leydig cells to produce testosterone, which acts locally on the interstitium and seminiferous tubules, resulting in sperm production and maturation [30]. The components of the *Dioscorea villosa* extract may have triggered a secretion of GnRH from the arcuate nucleus of the hypothalamus, which could then trigger the rest of the actions of the HPG-axis. This can also be seen from the histology of the testes which showed a reduction in the occlusion of the lumen seen in the control group, with that of the high dose group being the most open. The histology of the high dose group also showed an increase in the number of spermatogonia present compared to the low dose and control groups, confirming the increase in the sperm count. There was also a statistically significant change between the values of the sperm count and sperm viability indicating that the effect mediated by the extract is dose dependent.

Table 5. Effect of oral administration of *Dioscorea villosa* extract on the hormonal levels (FSH, LH, and testosterone) for the short term (14 days) groups

Groups (14 Days)	FSH (miU/ml)	LH (miU/ml)	Testosterone(ng/ml)
Group 1 – Control	3.08±0.23	2.24±0.20	5.13±0.19
Group 3 – 100mg/kg	1.79±0.08[a]	1.35±0.06[a]	2.64±0.21[a]
Group 6 – 400mg/kg	4.51±0.25[a,b]	3.32±0.22[a,b]	8.96±0.61[a,b]

Values expressed as mean ± sem, P<0.05, n=4, [a] represents values that are statistically significant when compared to control group, [b] represents values that are statistically significant when compared to the low dose (100 mg/kg bwt) group

Table 6. Effect of oral administration of *Dioscorea villosa* extract on the hormonal levels (FSH, LH, and Testosterone) for the long term (28 days) groups

Groups (28 Days)	FSH (miU/ml)	LH (miU/ml)	Testosterone (ng/ml)
Group 2 – Control	0.60±0.04	0.33±0.05	0.75±0.28
Group 4 – 100 mg/kg	0.68±0.08	1.05±0.36	0.68±0.13
Group 5 – 400 mg/kg	0.75±0.06	0.73±0.29	2.65±0.29 [ab]

Values expressed as mean±sem, P<0.05, n=4. [a] *represents values that are statistically significant when compared to control group,* [b] *represents values that are statistically significant when compared to the low dose (100 mg/kg bwt)*

Table 7. Body weight change of the short term groups

Groups (14 Days)	Initial body weights (g)	Final body weights (g)	Weight diff. (%)
Group 1 – Control	217±06.86	198.25±12.15	-8.30±6.86
Group 3–100 mg/kg	229.5±11.51	215±17.41	-6.80±3.25
Group 6–400 mg/kg	215±14.73	218±15.10	1.44±1.73

Values expressed as mean±sem, P<0.05, n=4. [a] *represent values that are statistically significant when compared to control group,* [b] *represents values that are statistically significant when compared to the low dose (100 mg/kg bwt)*

Table 8. Body weight change of the long term groups

Groups (28 Days)	Initial body weights (g)	Final body weights (g)	Weight diff. (%)
Group 2 – Control	223.25±20.84	247.25±14.97	11.89±05.27
Group 4 – 100 mg/kg	215.75±14.94	227±21.09	6.42±11.33
Group 5 – 400 mg/kg	201.25±15.61	208.5±06.38	5.48±08.57

Values expressed as mean±sem, P<0.05, n=4. [a] *represent values that are statistically significant when compared to control group,* [b] *represents values that are statistically significant when compared to the low dose (100 mg/kg bwt)*

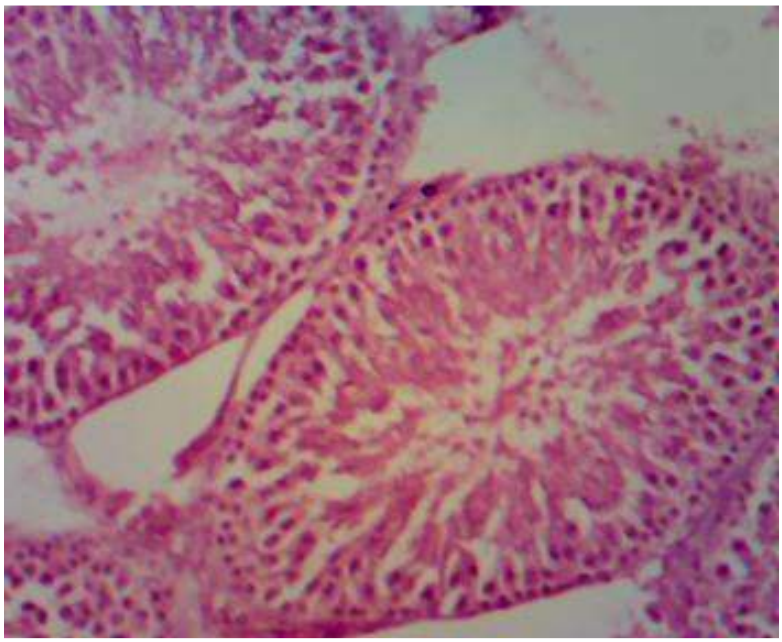

Fig. 1. Histological appearance of the testis for the short term control group (H&E X100 magnification), showing normal spermatogonia in the seminiferous tubule with no pathological damage, however, with slight occlusion of the seminiferous tubule

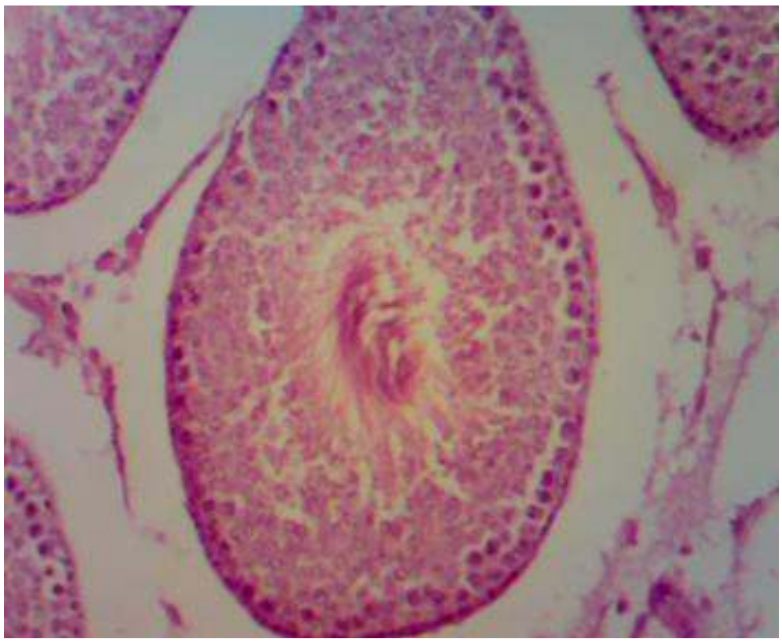

Fig. 2. Histological appearance of the testis for the short term low dose (100 mg/kg bwt) group (H&E X100 magnification), showing normal spermatogonia in the seminiferous tubule, with no necrotic change but with a slight occlusion of the seminiferous tubule

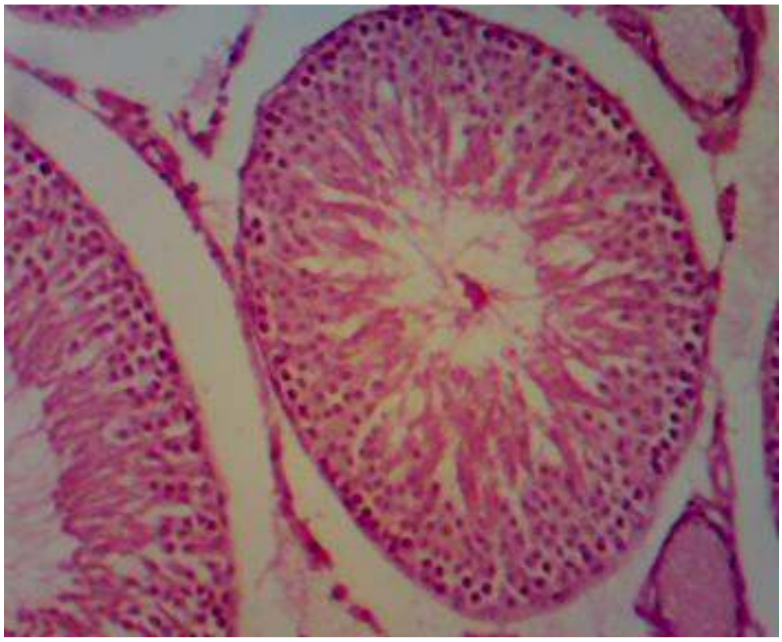

Fig. 3. Histological appearance of the testis for the short term high dose (400 mg/kg bwt) group (H&E X100 magnification). Showing an increased number of spermatogonia compared to the control group. There is also no occlusion of the seminiferous tubule lumen

The long term groups – low dose (100 mg/kg bwt) and high dose (400 mg/kg bwt) – did not show any significant change in any of the parameters (sperm count, sperm motility and sperm viability tested for. There was a general increments in the sperm function tests compared

with the control, and a general decrease was seen in the sperm count and sperm viability of both the low dose (100 mg/kg bwt) and high dose (400 mg/kg bwt) groups with an increase in the sperm motility, none of which was statistically significant. This was also depicted in the histology, which showed vacuoles in the seminiferous epithelium and a greatly reduced number of spermatogonia for the low dose group as reflected in the sperm count. The histology for the high dose group showed hyperplasia of the cells, with occlusion of the lumen, being reflected as a decrease in the sperm count and other sperm parameters.

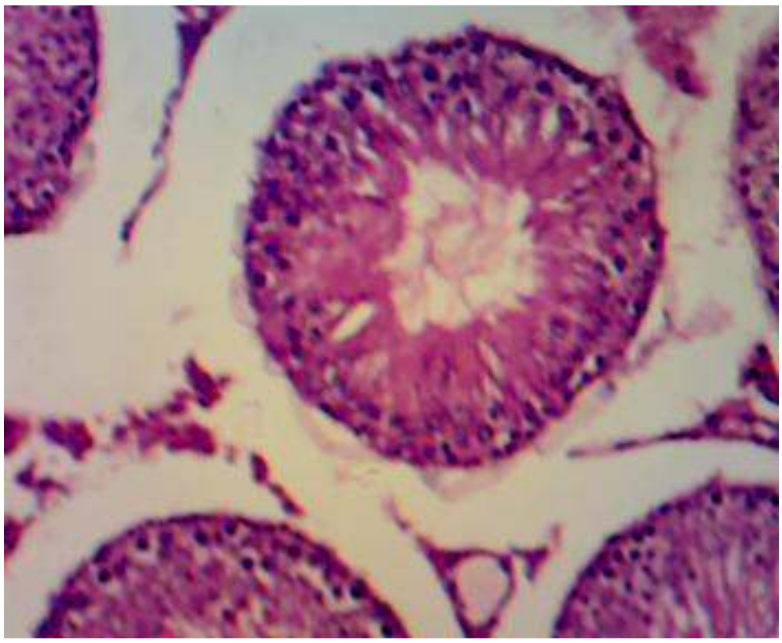

Fig. 4. Histological appearance of the testis for the long term control group (H&E X100 magnification), showing normal spermatogenesis

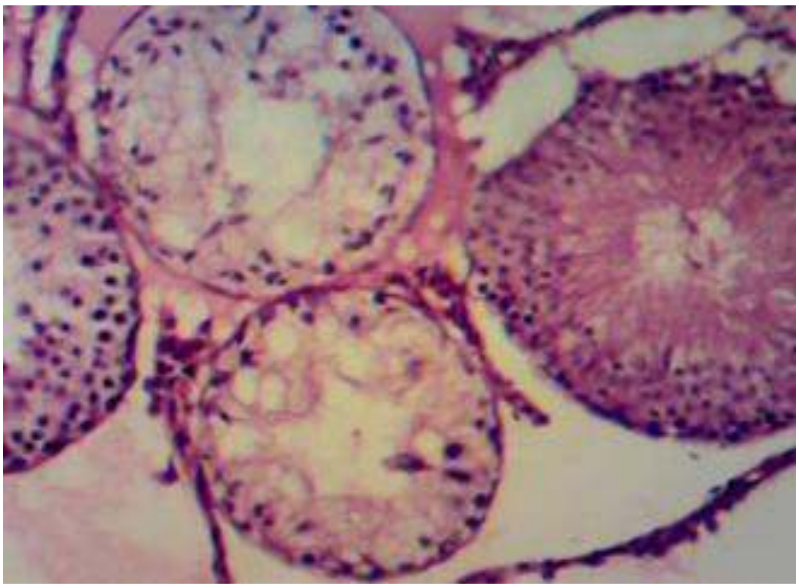

Fig. 5. Histological appearance of the testis for the long term low dose (100mg/kg bwt) group (H&E X100 magnification), showing a reduced number of spermatogonia and vacoulations in the seminiferous epithelium

4.2 Effect on the Weights of the Reproductive Organs

The short term (14 days treatment) groups – both the low dose (100 mg/kg bwt) and high dose (400 mg/kg bwt) – showed increments in the weights of the reproductive organs, compared to the control group. The differences showed however, were not statistically significant. The long term (28 days) groups not following the same trend with the short term groups as seen in the case of the sperm function tests, showed a general decrease in the weights of the reproductive organs compared to the control group, except for the increase seen in the weights of the testes in the high dose (400mg/kg bwt) group. These differences however were still not statistically significant, indicating that the *Dioscorea villosa* extract might not have any serious effect on the weight of the reproductive organs (testis and seminal vesicle).

4.3 Effect on the Hormonal Levels of FSH, LH and Testosterone

The results of the hormonal assay for the short term (14 days administration) groups, there were statistically significant changes in the serum levels of follicle stimulating hormone (FSH), luteinizing hormone (LH) and Testosterone between the experimental and control groups. There was significant decrease in the serum levels of FSH, LH and Testosterone in the low dose (100 mg/kg body weight) group when compared to the control group. However in the high dose (400 mg/kg body weight) group, there was significant increase in the serum levels of all the hormones (FSH, LH and Testosterone) when compared to the control group. This increase seen in the serum levels of the hormones in the high dose (400 mg/kg body weight) group would be as a result of an increase in the secretion of hypothalamic gonadotropin hormone-releasing hormone (GnRH) as secretion of FSH and LH (which then stimulates secretion of Testosterone) is controlled by GnRH [32] and a GnRH pulse precedes each elevation of serum LH as seen in experiments carried out in sheep [33]. The increases in the hormonal levels are reflected in the increased sperm count level of the same group as shown in Table 4. This increase in the sperm count level would occur as FSH and LH act only in the testes [32], FSH acting on the Sertoli and germ cells to stimulate spermatogenesis and LH promoting spermatogenesis indirectly by increasing intra-testicular testosterone [34]. The changes in the serum levels of the three hormones (FSH, LH and Testosterone) between low dose (100 mg/kg body weight) and high dose (400 mg/kg body weight) groups were also significant depicting that the effects of the extract might be dose dependent.

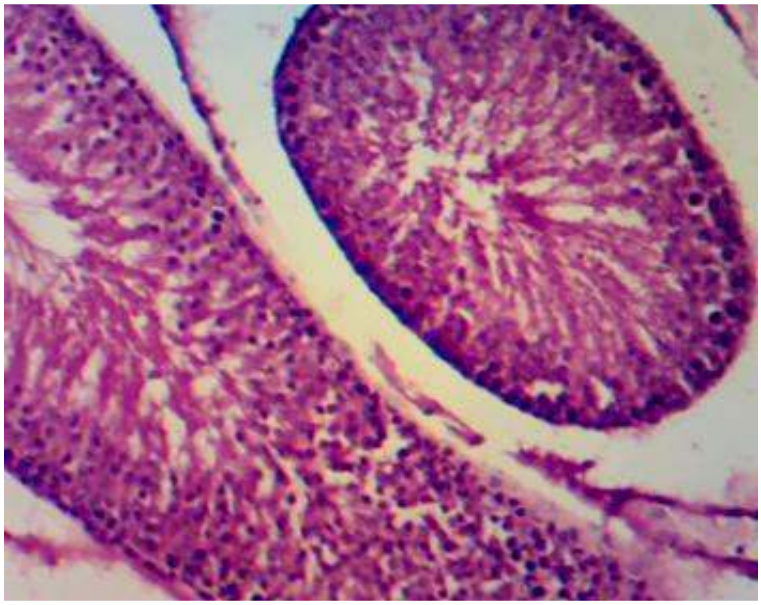

Fig. 6. Histological appearance of the testis for the long term high dose (400 mg/kg bwt) group (H&E X100 magnification), showing hyperplasia of the cells with occlusion of the lumen

In the long term groups, although there were differences between the serum levels of FSH, LH and testosterone between the control and experimental groups, only the difference between the testosterone levels of the high dose (400 mg/kg body weight) group and control group was statistically significant. The high dose group (400 mg/kg body weight), showed a statistical increase in the level of testosterone from that of the control group, rising to 2.65±0.29 ng/ml as against the control level of 0.75±0.28 ng/ml. This however was not replicated in the sperm function of the long term groups, where there was no statistically significant change in any of the parameters.

From the results, it can be inferred that the effect of the extract wears off after some time even with continued administration or that the animals habituated to the extract, as there were no significant changes in the parameters checked except for the serum testosterone level.

From the general results obtained, it can be proposed that short term administration of the extract causes changes in the male reproductive parameters which after some time begin to reverse, despite chronic administration, as in the case of kisspeptin (which has a role in the neuro-endocrine feedback pathway controlling the male reproductive system) [29]. Studies have shown that kisspeptin administration increases GnRH secretion in neuronal cell lines [35]. Other studies have also shown that although acute administration of kisspeptin seems to increase LH, FSH and testosterone secretion, chronic administration lowers serum LH levels in monkeys [36,37]. *Dioscorea villosa* however may have negative or harmful effects on the male reproductive system when used chronically for a long period of time, as seen from the results of the histology, which showed reduced number of spermatogonia, vacuoles and closed lumen in the long term experimental groups.

As interesting as this result may appear to be, attention has been drawn to the low number of test subjects per group, which may have inflated the error within groups. It is noted, and therefore recommended that a high number of subjects be used for similar research to back up the claims of the current one, particularly to reduce such error associated with low number.

5. CONCLUSION

The study suggests that a high dose of *Dioscorea villosa* might have potential positive effects on the male reproductive system, enhancing the production of the reproductive hormones and increasing sperm count when used for a short time, and negative or harmful effects when used for a long period of time. Even though animal studies cannot be directly extrapolated to humans, the results from this investigation give a clue to the possible effects on *Dioscorea villosa* on the male reproductive system in human.

CONSENT

It is not applicable.

ETHICAL APPROVAL

All authors hereby declare that the study was approved by the ethics of Bingham University, Karu, Nigeria.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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APPENDIX

The ANOVA table gives a detail comparison within groups, giving insight to some differences that the students' t-test could not bring out

		Sum of squares	df	Mean square	F	Sig.
Sperm motility	Between groups	1236.875	5	247.375	1.735	.178
	Within groups	2566.750	18	142.597		
	Total	3803.625	23			
Sperm viability	Between groups	2864.875	5	572.975	3.569	.020
	Within groups	2889.750	18	160.542		
	Total	5754.625	23			
Sperm count	Between groups	2199.333	5	439.867	6.393	.001
	Within groups	1238.500	18	68.806		
	Total	3437.833	23			
Testes weight	Between groups	.450	5	.090	2.177	.102
	Within groups	.744	18	.041		
	Total	1.194	23			
Seminal vesicle weight	Between groups	.899	5	.180	1.429	.261
	Within groups	2.265	18	.126		
	Total	3.163	23			
Serum FSH level	Between groups	50.997	5	10.199	113.346	.000
	Within groups	1.620	18	.090		
	Total	52.617	23			
Serum LH level	Between groups	24.232	5	4.846	23.394	.000
	Within groups	3.729	18	.207		
	Total	27.961	23			
Serum testosterone level	Between groups	197.711	5	39.542	93.230	.000
	Within groups	7.634	18	.424		
	Total	205.345	23			
Initial body weight	Between groups	5137.375	5	1027.475	1.140	.375
	Within groups	16219.250	18	901.069		
	Total	21356.625	23			
Final body weight	Between groups	5679.500	5	1135.900	1.227	.337
	Within groups	16664.500	18	925.806		
	Total	22344.000	23			

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