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Full Length Research Paper

Antifertility effects of aqueous crude extract of *Ocimum gratissimum* L. leaves in male mice

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***Ocimum gratissimum* Linn. (OG) is widely used in folk medicine for several conditions because of its high medicinal value and therefore calls for its toxicological screening. The present study is designed to investigate the effects of aqueous leaf extract of OG on hormonal and semen parameters of mice. Animals were divided into 3 groups and orally administered OG (11 - 88 mg/kg) daily for 1, 2 and 4 weeks, respectively, while a fourth (control) group received only distilled. They were sacrificed at the end of each treatment period and blood sample was assayed for serum levels of testosterone, FSH and LH. Semen was collected and analyzed for semen parameters, while the testis was evaluated for histological changes. OG caused no significant ($p \leq 0.05$) effect on the serum levels of the hormones studied. However, sperm count and motility were decreased, while the percentages of abnormal sperm cells, sperm debris and primordial cells were increased dose- and time-dependently. Furthermore, OG caused histopathological damages to the seminiferous epithelium of the testes. The results indicate that OG has anti-fertility effect in the male mouse, which may be mediated through a direct deleterious action on the testis without disruption of the testicular endocrine function.**

Key words: Antifertiliy, mice, *Ocimum gratissimum*, seminiferous.

INTRODUCTION

Ocimum gratissimum Linn. (also known as African basil) is a medicinal plant which belongs to the family Lamiaceae. It is a native of Africa and Asia but is now distributed to other parts of the world including the United States of America (Darrah, 1980; Sulistiarini, 1999). The plant is very common in the tropical regions. In Nigeria, *O. gratissimum* is described by different local names, but it is popularly known as “Scent Leaf” in most parts of the country. The plant is used as a condiment and spice in most parts of the world including Nigeria in the preparation of different dishes. It is also used widely in folk medicine for the treatment of several ailments including fever, cough and respiratory disorders (Corrêa, 1932; Oliver, 1980), sore throat, kidney stones, epilepsy and dermatitis (Oliver, 1980; Sofowora, 1993), headache, stress, mental diseases (Osifo, 1989) etc. Because of this popular use of the plant in native medicine, its pharmacological and toxicological screening has been provoked in recent times. Studies had also shown that the leaf extract of *O. gratissimum* contain potent bioactive components (essential oils) made up of eugenol, citral, linalool, charvicol, thymol, gerianol (triterpenoids, saponnins, alkaloids, etc (Darrah, 1980; Sulistiarini, 1999; Leal et al., 2006; Matasyoh et al., 2007). These phytochemicals possess antibacterial (Nakamura et al., 1999; Akinyemi et al., 2005), antifungal (Nwosu and Okafor, 1995), antinoceptive (Rabelo et al., 2003), antihypertensive (Leylliane et al., 2007), antidiarrhoeal (Ilori et al., 1996; Adebolu and Salau, 2005), antioxidant (Odukoya et al., 2005; Aprioku and Obianime, 2008), insecticidal (Eze et al., 2006) and anthelmintic (Pessoa et al., 2002) properties among others which may justify its high medicinal use in folk medicine. Additionally, the toxicological effects of the plant on the hematopoietic system have been studied, showing that it causes reductions in the PCV and Hb values, with proliferation of

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leucocytes (Effraim et al., 2000; Jimoh et al., 2008; Obianime et al., 2010). Furthermore, *Ocimum sanctum* which is in the same genus with *O. gratissimum* had been reported to have antifertility effects in male albino rats (Kantak and Gogate, 1992; Reghunandana et al., 1993; Ahmed et al., 2002), however, not much is known about the reproductive effects of OG prior to this study.

Normal male reproductive function is dependent on the normal functioning of the male reproductive organ and other accessory organs/structures. The male reproductive organ is the testis, which is primarily responsible for the production of spermatozoa. Sperm production occurs in the seminiferous tubules of the testis, which is controlled by testosterone, produced by the Leydig (interstitial) cells of the testis (Dolores and Cheng, 2004). Testosterone production is directly dependent on the concentration (or activity) of leutinizing hormone (LH), in the milieu secreted by the anterior pituitary gland (Ganong, 2001). Follicle stimulating hormone (FSH), released also by the anterior pituitary stimulates the Sertoli cells of the testis which give support and nourishment to developing spermatozoa (Christensen, 1975; Huang et al., 1991; Ganong, 2001). The quality and quantity of spermatozoa produced will therefore depend on normal functioning of the testicular structures and reproductive hormones (Elkington and Blackshaw, 1974; Huang et al., 1991; Ganong, 2001).

Although, OG had been reported in previous studies to cause congestion and edema in seminiferous tubules, with increase in abnormal sperm cells (Leigh and Fayemi, 2008), the study failed to show any dose- and time-related effects because only two doses were used and harvesting was done once. Secondly, the study could not give a possible mechanism of OG in the testis. In this study, we investigated the dose- and time-dependent effects of the aqueous crude leaf extract of *O. gratissimum* on the hormonal and semen parameters (sperm count, sperm motility, percentage of abnormal sperm, percentage of sperm debris and percentage of premature sperm cells) in addition to the histopathology of the testis in mice.

MATERIALS AND METHODS

Extraction of plant material

The fresh leaves of *O. gratissimum* were collected in December, 2008 from a local garden within the premises of the University of Port Harcourt, Nigeria. The plant was identified and authenticated by Dr. Goodie Uzo Obute- a senior botanist of the Department of Botany, University of Port Harcourt, Nigeria and a voucher specimen was deposited accordingly at the herbarium of the Department of Plant Science, University of Port Harcourt, Port Harcourt, Nigeria.

Extraction

The fresh leaves of the plant were air-dried, pulverized and extracted exhaustively in distilled water. The filtrate was concentrated and evaporated to dryness in vacuo at 40°C, using rotary evaporator. The yield was calculated and the dry extract was stored in a refrigerator at -4°C until use for the experiments. During the experiment, the crude extract was dissolved in distilled water and administered to the animals at 0.7 ml/kg orally.

Animals

The animals used in this study were male mice weighing between 30 - 35 g. The animals were obtained from the animal house of the Department of Pharmacology, University of Port Harcourt, Nigeria. The animals were randomly distributed into cages and allowed to acclimatize for 10 days in a well ventilated room at a room temperature of 28.0 ± 2.0°C under natural lighting condition. The animals were fed with standard mouse chow and allowed free access to water daily. All animals used in this study were handled in accordance with the international, national and institutional guidelines for Care and Use of Laboratory Animals in Biomedical Research as promulgated by the Canadian Council of Animal Care (2009).

Experimental protocol

Animals were divided into three main groups- Weeks 1, Week 2, Week 4 and control. Each group was further subdivided into 4 groups (n = 5) and orally administered with single doses of 11, 22, 44 and 88 mg/kg of the aqueous extract of *O. gratissimum* daily for 1, 2 and 4 weeks respectively. A fourth group containing 5 animals which was used as the control received only distilled water daily for 4 weeks. At the end of each treatment course, the animals were sacrificed by decapitation under pentobarbital anesthesia at 50 mg/kg, ip (Erhardt et al., 1984).

Assay of hormonal parameters

Blood was collected into lithium heparinized bottle, centrifuged for 15 min at 3,000 rpm and serum was separated and assayed for testosterone, FSH and LH using enzyme linked immunoassay (EIA) technique (Amballi et al., 2007).

Measurement of semen parameters

The caudal epididymis was carefully isolated and then placed in a Petri dish containing 3.0 ml of $NaHCO_3$ buffered Tyrodes's Lactate solution. Several incisions (1 mm) were made on it and semen was gently drawn into plastic transfer pipette and transferred into 5 ml test tubes for analysis. Semen analysis was carried out immediately using the new improved Neubauer counting chamber for determination of the concentration of spermatozoa, sperm motility, percentages of abnormal sperm cells (sperm morphology) and debris using standard laboratory techniques (WHO, 1999).

Histopathological studies

Testis was carefully isolated, weighed, washed in buffered saline and fixed in 10% formalin. Testis sections (5 - 6 µm) were routinely processed by standard histological techniques, stained with hematoxylin and eosin (H and E), and examined by light microscope (Nikon Eclipse E400) to assess histopathological changes among control and experimental animals.

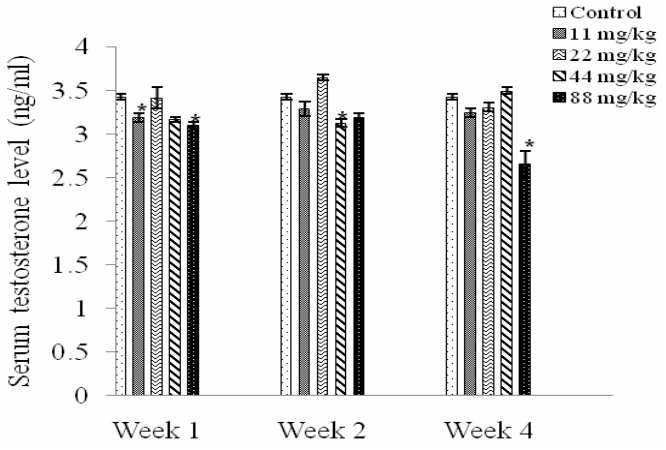

Figure 1a. The effects of aqueous *O. gratissimum* leaf extract on serum testosterone levels of male mice. Data given as mean ± SEM. *Indicates a significant difference from control at $p < 0.05$. **Indicates a significant difference from control at $p < 0.01$. ***Indicates a significant difference from control at $p \leq 0.001$.

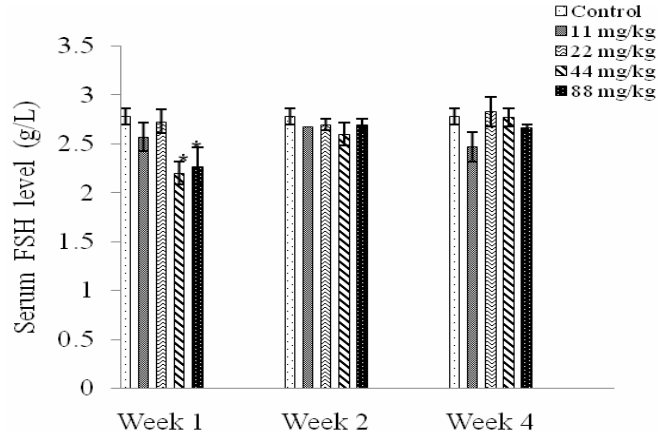

Figure 1b. The effects of aqueous *O. gratissimum* leaf extract on serum FSH levels of male mice. Data given as mean ± SEM. *Indicates a significant difference from control at $p < 0.05$. **Indicates a significant difference from control at $p < 0.01$. ***Indicates a significant difference from control at $p \leq 0.001$.

Statistical analysis

Data were expressed as means ± standard errors of mean. Comparisons between data of control and treated groups of guinea-pigs were performed with one-way analysis of variance (ANOVA). Statistical significance was set at $p \leq 0.05$.

RESULTS

Hormonal parameters

O. gratissimum (11 - 88 mg/kg), administered for 4 weeks caused no significant ($p \leq 0.05$) effects on the basal serum levels of testosterone, FSH and LH (Figures 1a, b and c). Daily administration of the agent for 1 week caused significant decrease in testosterone at some doses, but failed to cause clear dose-dependent effects (Figure 1a).

Semen parameters

O. gratissimum caused significant ($p \leq 0.05$) dose-dependent decreases in sperm count and motility over 1 week (Figures 2a and b). It also dose-dependently increased the percentages of abnormal sperm cells, primordial sperm cells and sperm debris (Figures 2c, d and e). These effects were also time-dependent over the 4 weeks period of administration (Figures 2a, b, c, d and e). Administration of 88mg/kg of the extract for 4 weeks significantly ($p \leq 0.05$) decreased sperm count from 69.50 ± 0.5 × 10^6 to 44.33 ± 3.5 × 10^6/ml and sperm motility from 67.50 ± 2.5 to 26.67 ± 3.3% (Figures 2a and b). Furthermore, the percentage of abnormal sperm cells (morphology) was increased from 18.0 ± 0 to 36.0 ± 4.9; percentage of sperm debris from 18.25 ± 1.2 to 35.0 ± 2.9 and percentage of primordial cells from 20.0 ± 0 to 33.33 ± 3.3 at 88 mg/kg of extract (Figures 2c, d and e).

Histopathology of testes

One week administration of OG caused significant ($p \leq 0.05$) dose-dependent decrease in testicular weight. However, there were no significant changes in testicular weight in animals that received OG for 2 and 4 weeks, compared to the control animals (Table 1). Furthermore, 1 week exposure to OG caused damages to the seminiferous epithelium, characterized by varying degrees of edema within the tubules and the interstitial cells, reduced spermatogenesis (maturation arrest) and collapse of the tubules under the tunica albunigea, compared to normal testicular structures in the control animals (Figures 3a - e). However, animals that were treated with OG for 2 and 4 weeks showed apparently normal testicular histology with mild histological changes.

DISCUSSION

The male reproductive system consists of the testis as the main reproductive organ and other accessory structures, with a primary responsibility of sperm production. Agents (especially oxidative agents) that alter testicular function will affect the quality and quantity of spermatozoa, which depends on several reproductive factors. In this study, the effects of aqueous leaf extract of *O. gratissimum* on the reproductive hormones and semen parameters of mice were investigated. The reproductive hormones studied were testosterone, FSH

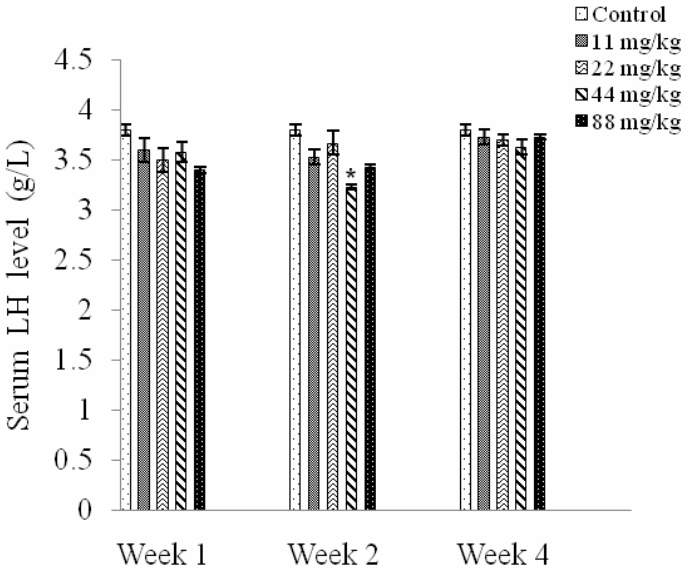

Figure 1c. The effects of aqueous *O. gratissimum* leaf extract on serum leutinizing hormone (LH) levels of male mice. Data given as mean ± SEM. *Indicates a significant difference from control at $p < 0.05$. **Indicates a significant difference from control at $p < 0.01$. ***Indicates a significant difference from control at $p \leq 0.001$.

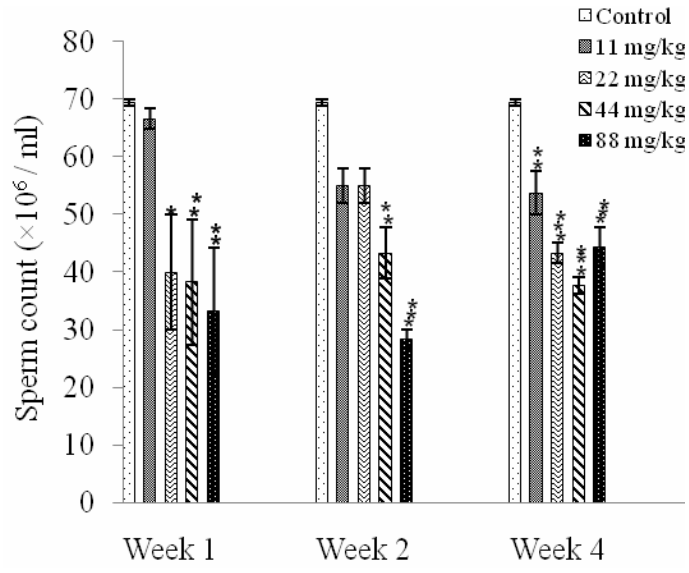

Figure 2a. The effects of aqueous *O. gratissimum* leaf extract on sperm count of male mice. Data given as mean ± SEM. *Indicates a significant difference from control at $p < 0.05$. **Indicates a significant difference from control at $p < 0.01$. ***Indicates a significant difference from control at $p \leq 0.001$.

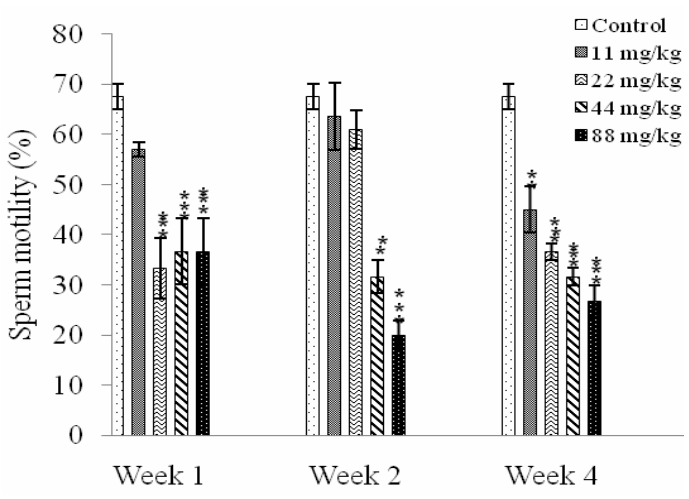

Figure 2b. The effects of aqueous *O. gratissimum* leaf extract on sperm motility male mice. Data given as mean ± SEM. *Indicates a significant difference from control at $p < 0.05$. **Indicates a significant difference from control at $p < 0.01$. ***Indicates a significant difference from control at $p \leq 0.001$.

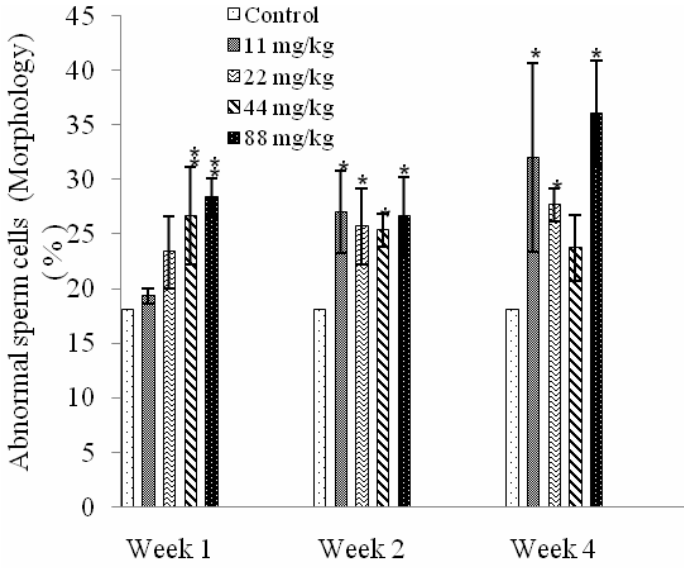

Figure 2c. The effects of aqueous *O. gratissimum* leaf extract on abnormal sperm cells (sperm morphology) of male mice. Data given as mean ± SEM. *Indicates a significant difference from control at $p < 0.05$. **Indicates a significant difference from control at $p < 0.01$. ***Indicates a significant difference from control at $p \leq 0.001$.

and LH, while the semen parameters were sperm count, sperm motility, sperm morphology, sperm debris and primordial sperm count. Oral administration of OG over 4 weeks, caused no significant ($p \leq 0.05$) effects on the serum levels of testosterone, LH and FSH, but caused significant ($p \leq 0.05$) decline in epididymal sperm number and motility in dose and duration dependent manners. In addition, the percentages of abnormal sperm cells, premature sperm cells and sperm debris were significantly increased dose- and time-dependently. This is consistent with the anti-fertility effects reported on *Ocimum sanctum* (Reghunandana et al., 1993).

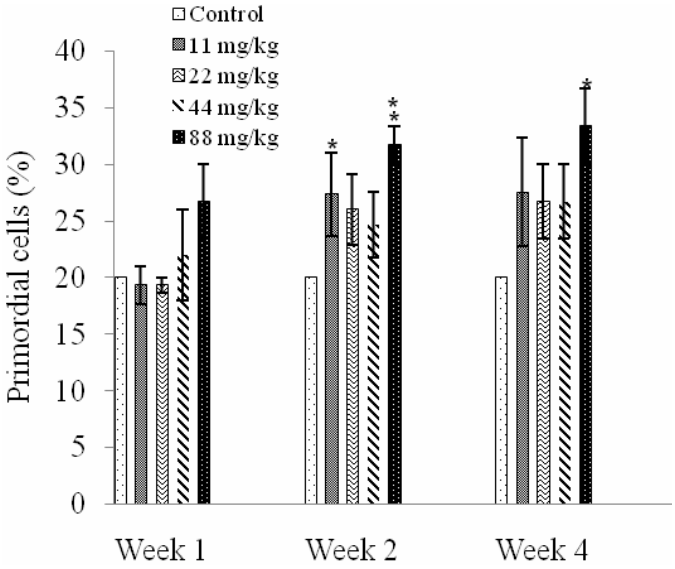

Figure 2d. The effects of aqueous *O. gratissimum* leaf extract on primordial sperm cells of male mice. Data given as mean ± SEM. *Indicates a significant difference from control at $p < 0.05$. **Indicates a significant difference from control at $p < 0.01$. ***Indicates a significant difference from control at $p \leq 0.001$.

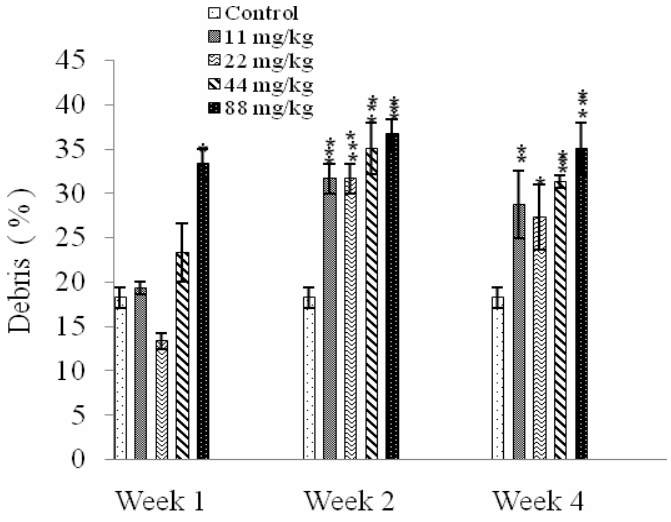

Figure 2e. The effects of aqueous *O. gratissimum* leaf extract on sperm debris of male mice. Data given as mean ± SEM. *Indicates a significant difference from control at $p < 0.05$. **Indicates a significant difference from control at $p < 0.01$. ***Indicates a significant difference from control at $p \leq 0.001$.

Furthermore, OG caused distortion/destruction of the architecture and structure of the testicular histology, characterized by edema, reduced spermatogenesis and maturation arrest of spermatozoa at different stages of germ cell developments mostly at the first week of OG administration, which is equally consistent with the results

Table 1. The effects of aqueous *O. gratissimum* leaf extract on testicular weight in mice. Data given as mean ± SEM. *Indicates a significant difference from control at $p \leq 0.05$. ** Indicates a significant difference from control at $p \leq 0.01$. ***Indicates a significant difference from control at $p \leq 0.001$.

Dose mg/kg)	Weight of testes (g)		
	Week 1	Week 2	Week 4
Control	0.27±0.03	0.27±0.03	0.27±0.03
11	0.18±0.01**	0.23±0.02	0.24±0.02
22	0.17±0.02**	0.23±0.33	0.25±0.06
44	0.13±0.01***	0.22±0.01	0.23±0.02
88	0.15±0.02**	0.17±0.03**	0.20±0.05

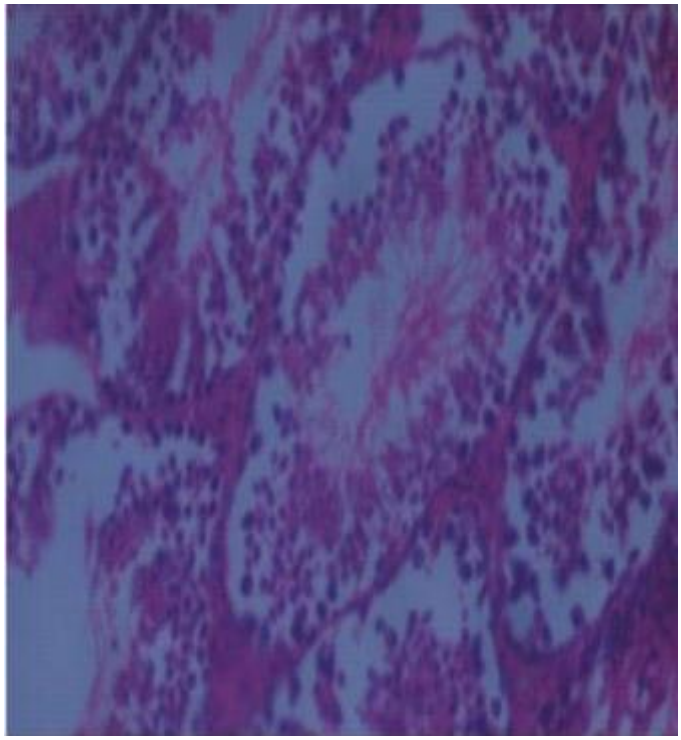

Figure 3a. Control, showing normal spermatogenesis in the seminiferous tubules, normal interstitial cells of the Leydig and normal spermatozoa.

of Leigh and Fayemi (2008). The histopathological result was corroborated by a significant reduction in the mean testicular weight of treated animals. From the results, it shows that the aqueous extract of *O. gratissimum* impairs reproductive function in the male guinea pig. Since, *O. gratissimum* had no significant effects on the reproductive hormonal profile but adversely affected semen parameters and testicular histology, it clearly indicates that the effects of *O. gratissimum* may mainly be due to direct deleterious effects on the seminiferous tubules, without significant effect on the hypothalamo-pituitary

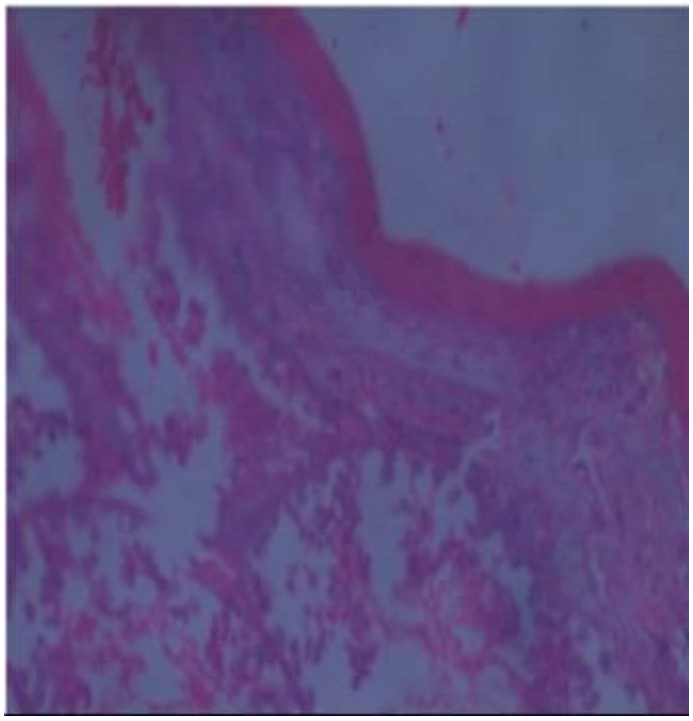

Figure 3b. 11 mg/kg of OG, showing reduced spermatogenesis with collapse of the tubules under the tunica.

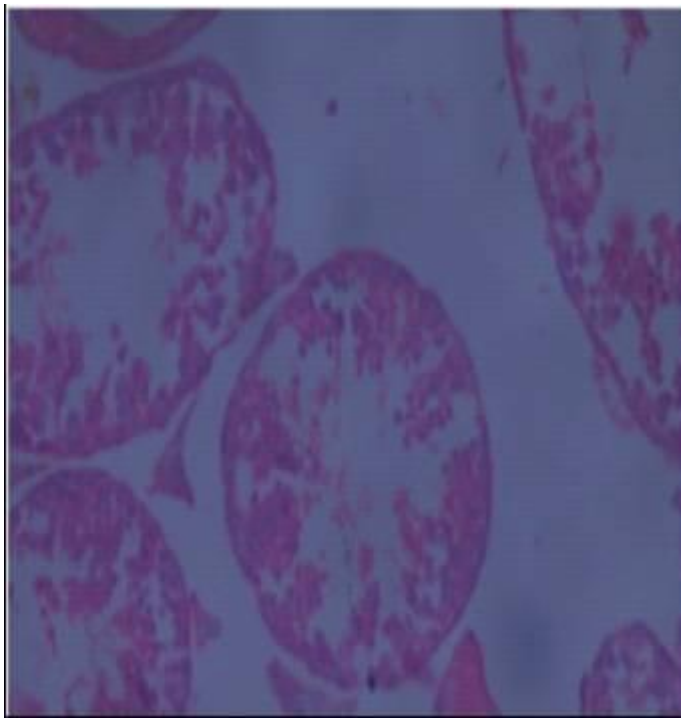

Figure 3d. 44 mg/kg of OG, showing marked collapse of the tubules beneath the tunica albuginea, marked reduction in spermatozoa (maturation arrest) and pronounced oedema between the tubules.

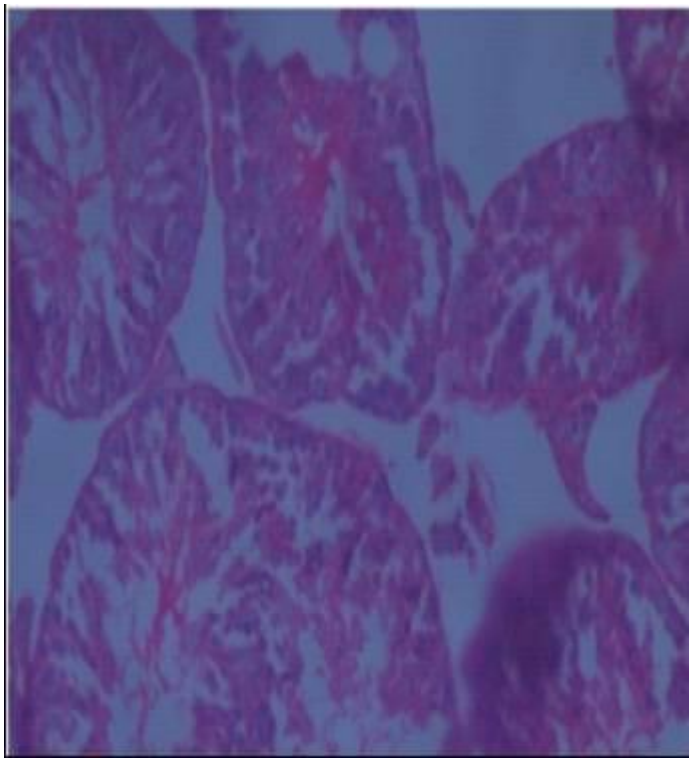

Figure 3c. 22 mg/kg of OG, showing oedema between the tubules in the interstitial area, reduction in spermatozoa and collapse of the tubules under the *Tunica albuginea.*

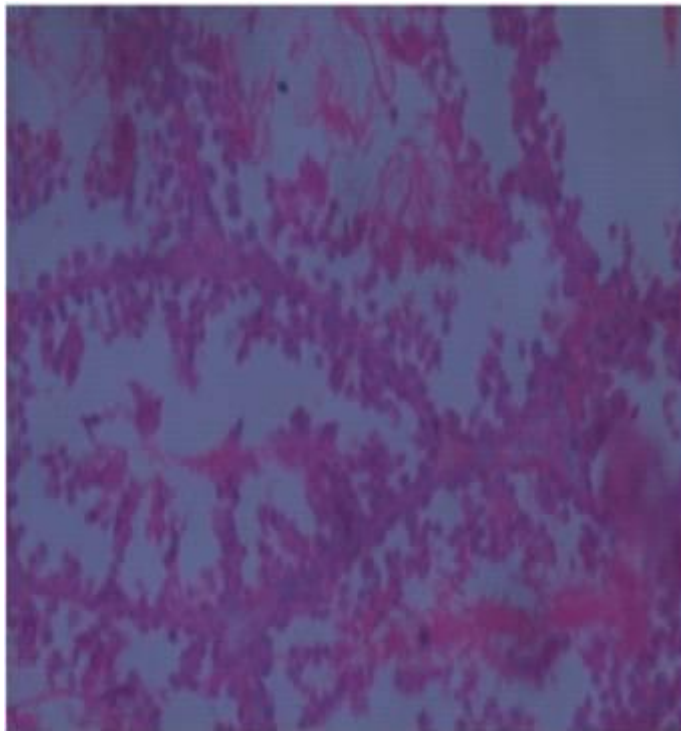

Figure 3e. 88 mg/kg of OG, showing slight reduction in spermatozoa.

(endocrine) function. The decrease in sperm count in this study is likely due to direct damages to the Leydig and Sertoli cells which are directly involved in the production of spermatozoa. In addition, the increase in premature sperm cells may be due to stimulation of mitosis of germinal cells with an inhibition of the meiotic divisions of the sperm cells, resulting in maturation arrest of sperm cells at different stages of development. This is consistent with the works on *O. sanctum* producing juvenile sperm cells (Reghunandana et al., 1993). Furthermore, the reduction in testicular weight by *O. gratissimum* mostly at the first week of OG administration positively correlates to the histopathological effects of OG, and therefore corroborating the toxicity of OG in the testis (Simmons and Berman, 1995). The reduced testicular histopathological effects after one week of OG administration may be due to reduced sensitivity of the testicular structures to OG during prolonged administration of OG.

In our previous studies with guinea-pigs, it was shown that the aqueous leaf extract of *O. gratissimum* inhibited heavy metal-induced serum elevations of some biochemical parameters and also protected the testicular tissues/organs against damage by cadmium-induced oxidative stress (Aprioku et al., 2009; Aprioku and Obianime, 2009). The present study in mice however has shown that 4 weeks administration of the plant is toxic on testicular structures/function. The different actions of *O. gratissimum* may be attributed to the effects of different components of the plant under the different conditions. *O. gratissimum* contains several pharmacologically active components with both oxidative and antioxidative properties, including eugenols, citral, thymol, triterpenes, charvicol, gerianol, saponnins, alkaloids etc (Darrah, 1980; Sulistiarini, 1999; Matasyoh et al., 2007). The spermicidal effect of OG in this study may be due to the effects of triterpenes and saponnins components in the plant which are deleterious to sperm cells (Farnsworth and Wallerm, 1982). The toxicity of the saponnins may be related to their adstringent actions on the cell surfaces of sperm cells, causing a disruption of the cell membrane, which could result in the reduction in sperm motility, as well as the inhibition of specific enzymes (e.g. hyaluronidase and acrosin) necessary for sperm synthesis (Farnsworth and Wallerm, 1982). However, the antioxidant effects of the plant in other studies had been attributed to eugenols, citral and other antioxidant components of the plant.

Conclusion

The study reveals that the aqueous leaf extract of *O. gratissimum* has anti-fertility property when administered alone. The toxic effects of the plant on the testis may be due to direct deleterious effects on the seminiferous tubules which are important testicular structures involved in spermatogenesis, without significant effect on the endocrine function.

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