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Comparative effects of methanol and oil extracts of *Ocimum gratissimum* on testicular morphology and epididymal sperm reserve of adult male albino rats (Wistar strain)

Ikokide Emmanuel Joseph[a], Ishmael Festus Jaja[b,*], Adamu Hamman Boyi[a], Oyeyemi Mathew Olugbenga[a]

[a] Department of Theriogenology, Faculty of Veterinary Medicine, University of Ibadan, Ibadan, Oyo State, Nigeria
[b] Department of Livestock and Pasture Science, Faculty of Science and Agriculture, University of Fort Hare, Alice, 5700, South Africa

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ABSTRACT

Fertility control with medicinal plants has been reported in the ancient and modern medical literature. In this research, the effects of methanol and oil extracts of *Ocimum gratissimum* (OG) on testicular morphology and epididymal sperm reserve were investigated in adult male Wistar rats. Fifty-five rats divided into 5 groups A-E of 11 rats each was used for this study. Methanol and oil extracts of *Ocimum gratissimum* were administered at two doses 250 and 500 mg/kg for 28 days. Animals in control group A received distilled water for the same duration. At day 14 and 28 of the study, five animals were sacrificed from each group, and their blood and testes samples were collected for the analysis. Spermiogram, testicular biometry, serum testosterone assay, and testicular morphology were assessed from harvested testes. The results revealed that there was no significant difference ($p > 0.05$) in serum testosterone level. There was also no significant difference ($p > 0.05$) in sperm volume, livability and abnormalities but there was significant differences ($p < 0.05$) in the sperm motility and concentration. Testicular biometry and testicular morphology between control and treated groups did not reveal any significant differences ($p > 0.05$). The study showed that methanol and oil extracts of *Ocimum gratissimum* (OG) did not have any inhibitory effect on the reproductive function and fertility of adult male albino rats. Further works should be carried out on isolated active fractions of this plant on male fertility.

1. Introduction

Herbal remedies play an essential role in the wellbeing of many people all over the world. In many African countries, the poor people living in rural and peri-urban areas readily access medicinal plants because of its easily available, affordable and already a well-established practice and culture in such a society. Those needing medical care begin treating themselves using medicinal plants before visiting a herbalist or hospital. In Nigeria for instance, herbal products are popularly used over an extended period by persons with little or no knowledge of science [1]. *Ocimum gratissimum* Linn. is a perennial herb belonging to the family Lamiaceae. The plant which is believed to originate from Asia and Africa is also widely distributed in other regions, occurring as different species. The leaves of the plant contain essential oils, and it is cultivated for various purposes [2,3]. In many parts of West Africa, it is used as a spice and condiment in dishes, because of its high pungent flavour of clove. The whole plant and the essential oils of the plant have many applications in folk medicine. The crude extracts have been used in the treatment of respiratory tract infections diarrhoea rheumatism and haemorrhoids dermatological conditions microbial infections epilepsy, high fever and mental illness [4–7]. Phytochemical characterisation of the leaf extract of *Ocimum gratissimum* (OG) had shown the plant to contain alkaloids, terpenoids, flavonoids, saponins, steroids, tannins, anthraquinone, and cardiac glycosides [4,6,8]. Also, the leaves of the plant have essential oils that are rich in geraniol, citral, eugenol, Linalool 1, 8-cineole, thymol, and ethyl cinnamate. The quality and quantity of these essential oils depend on the method of extraction of the plant, the geographical location where OG is grown and the species [2,9,10].

Furthermore, the use of OG in folk medicine is attributed to its diverse pharmacological properties. These properties include antioxidant, chemotherapeutic, antimutagenic, antidiarrheal, contraceptive, insecticidal, hypotensive and antihelmintic [2,5,6,9–12]. Also, OG has been shown to possess acetyl-cholinesterase (AChE) inhibiting activity

* Corresponding author.
E-mail address: Ijaja@ufh.ac.za (I.F. Jaja).

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Fig. 1. Showing map of Africa and a pointer showing map of Nigeria, where the study is shown.

up to 90% at lower concentrations than that of galatamine [13]. The aqueous and oil extracts of this plant have been reported to possess antifertility properties on adult male Wistar rats [2,14]. Ethanol extract of *Ocimum gratissimum* (OEOG) leaves has been shown to significantly increased the sexual behaviours of normal male albino mice without any gastric ulceration and adverse effects [15]. However, to our knowledge, there is the paucity of information on the effect of methanol extract of *Ocimum gratissimum*(MEOG) on the fertility of male albino rats. Hence this study investigated the comparative effect of methanol and oil extracts of OG on testicular morphology and epididymal sperm reserve of adult male albino rats (Wistar strain).

2. Material and method

2.1. Plant collection and identification

Fresh leaves of OG were harvested from Akinyele Local Government Ibadan, Oyo State in Nigeria. The identification and authentication were done, at Botany Department, University of Ibadan, Nigeria (Fig. 1). A voucher specimen with reference number UIH-22617 was deposited at the Herbarium.

2.2. Preparation of extracts

The plant samples were selected into a blender for crushing and blending into smaller pieces. Blending enhances the penetration of the extracting solvents into the plant cells, thus facilitating the release of the active particles. The cold maceration method was used as described elsewhere [16,17]. Briefly, 2 kg of powdered OG (leaf) were weighed using a weighing balance into two 1000 ml capacity conical flask. The solvents (Hexane, and Methanol) were added to each of the samples respectively. The mixture in a conical flask placed on a shaker for 24 h. After shacking and mixing for 24 h, it was filtered using a muslin cloth. The filtrates were filtered again through suction pressure using a vacuum pump. The concentration of the filtered extract was done using the rotary evaporator (model 801, Fisatom; Brazil), and dried on an evaporating dish at a 50°C to 60°C to a semi-solid form. A greenish sticky semi-solid substance was obtained for both samples. The extracts were stored in a well-corked universal bottle-20 °C till their usage in the different tests. The solutions were prepared as 250 and 500 mg/ml.

2.3. Experimental animals

The experimental animals used for this study were locally bred male Wistar rats from the experimental animal house of Faculty of Veterinary Medicine University of Ibadan, Nigeria. Andrological examination was carried out to determine the sex of the rat and remove from the study female rats and rats with any sexual abnormality. They were fed with commercial rat pellet and allowed unrestricted access to clean, freshwater. The rats were managed as per recommendations of the University of Ibadan Animal Care and Use Research Ethics Committee. All protocols were appraised and approved by the committee with assigned number UI-ACUREC/18/0033.

2.4. Experimental design

Fifty-five rats (*Wistar strain*) weighing between 140 g–200 g were randomly assigned to five groups of eleven rats per group (Fig. 2). The

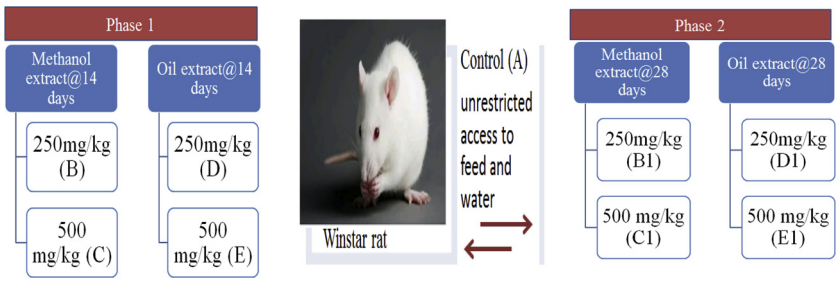

Fig. 2. Experimental design of study showing phase 1 and 2 of the study with treatment groups.

Table 1
Semen characteristic at 14 and 28 days post-treatment with methanol extract of *Ocimum gratissimum.*

Groups	Sperm motility(%)	Sperm livability(%)	Semen volume(ml)	Sperm concentration × 10(ml^{-1})	Sperm abnormalities(%)
A	93.00 ± 1.23[a]	97.40 ± 0.60[a]	5.18 ± 0.02[a]	154.00 ± 3.23[a]	4.60 ± 0.25[a]
B	78.00 ± 2.00[ab]	96.80 ± 0.74[a]	5.18 ± 0.02[a]	116.60 ± 3.97[ab]	7.60 ± 0.68[a]
C	73.33 ± 6.67[ab]	91.00 ± 5.54[a]	5.17 ± 0.03[a]	111.00 ± 11.02[ab]	6.00 ± 0.45[a]
B1	74.00 ± 2.45[ab]	78.90 ± 17.61[a]	5.18 ± 0.02[a]	106.80 ± 5.83[ab]	6.67 ± 0.33[a]
C1	74.00 ± 2.45[ab]	96.40 ± 0.74[a]	5.16 ± 0.03[a]	122.00 ± 4.85[ab]	6.60 ± 0.60[a]

Values are expressed as mean ± SEM. [ab] significantly different from the control at $p < 0.05$.
Group A: control, Group B: 250 mg/kg methanol extract (OG) at 14 days treatment, Group C: 500 mg/kg methanol extract (OG) at14 day's treatment, Group B1: 250 mg/kg methanol extracts (OG)) at 28 days treatment, Group C1: 500 mg/kg methanol at 28 days treatment.

rats were grouped and treated with methanolic (MEOG) and oil (OEOG) extract of OG in two phases. In the first phase of the study (phase 1), animals in the control group (Group A) were allowed unrestricted access to feed and water alone for 14 days. In group B and C, rats were dosed with 250 mg/kg, and 500 mg/kg of the MEOG leaves for 14 days respectively. In group D and E, rats were dosed with 250 mg/kg, and 500 mg/kg of the OEOG leaves for 14 days respectively. In the second phase of the study (phase 2), Animals in the control group (Group A) were allowed unrestricted access to feed and water alone for 28 days. In group B1 and C1, rats were dosed with 250 mg/kg, and 500 mg/kg of the MEOG leaves for 28 days, respectively. In group D1 and E1, the wister rats were dosed with 250 mg/kg, and 500 mg/kg of the OEOG leaves for 28 days respectively.

2.5. Testosterone assay

At the end of each phase, five rats were anaesthetized with chloroform for about 5 min. Blood was collected through the medial canthus of the eye and allowed to clot. Serum was harvested for testosterone assay (TA) using commercial ELISA kits (Abcam scientific, South Africa). TA was carried out based on the instructions of the kits manufacturers and expressed as ng/ml [18].

2.6. Spermiogram

The anaesthetised rats were placed on dorsal recumbency, and a lower abdominal incision to remove the testicles was made. The epididymis was trimmed off the testes, and the samples were collected using a Pasteur pipette from the cauda through an incision made with a scalpel. Semen analysis to evaluate spermatozoa properties such as sperm concentration and morphology sperm motility, live-dead ratio, were done using methods described elsewhere [19,20]. Briefly, motility was evaluated with one drop each of semen sample and 2.9% sodium citrate buffer under a cover-slip and viewed under X 40 of microscope. Semen smears were stained with Eosin-nigrosin for live-dead ratio and Wells and Awa stain for morphology. The spermatozoa concentration was evaluated using the improved Neubauer Haemocytometer (Deep 1/10 mm, LABART, Germany) as described elsewhere [20].

2.7. Testicular and epididymal biometry

The weights of the rats were taken before anaesthetising the animal. The testes with the epididymis were immediately collected after anaesthetization. Each epididymis was carefully separated from the testis. Testicular and epididymal weight was measured by putting the testes and epididymis on a sensitive electronic weighing scale. The testicular diameter was also measured around the widest point at an area that is equidistant to the testicular poles. The testicular length was measured using a sensitive electronic vernier calliper along the longitudinal axis of the testis starting from one pole of the testis to the other pole [19,20].

2.8. Histological study

Testes from all animals were fixed in 10% buffered formalin in labelled bottles. They were processed routinely by embedding in wax (paraffin). A 5 µm thick sections were then cut, stained with haematoxylin and eosin (H&E) and examined under a light microscope (Olympus CH; Olympus, Tokyo, Japan) and photomicrographs were taken with a Leica DM 750 camera at x 100 and 400 magnification [21].

2.9. Statistical analysis

The results were expressed as mean ± standard error of the mean (M ± SEM). Data obtained from the study was analyzed using one-way analysis of variance (ANOVA) for comparison between means for treated groups and control group for statistical difference using SPSS version 24. The values of $p < 0.05$ were considered significant.

3. Result

3.1. Spermiogram

Tables 1 and 2 shows the difference in mean of sperm motility was significant ($p < 0.05$) in groups B, C, B1 and C1 when compared to A (93.00 ± 1.23). Also, there was a significant difference in mean ($p < 0.05$) in groups D, D1 and E1 when compared with A (93.00 ± 1.23), D (84.00 ± 2.45), D1 (74.00 ± 4.00), E1 (77.50 ± 2.50) respectively. The difference in mean obtained for

Table 2
Semen characteristic at 14 and 28 days post-treatment with oil extract of *Ocimum gratissimum.*

Groups	Sperm Motility(%)	Sperm Livability(%)	Semen volume(mls)	Sperm Concentration × 10(ml^{-1})	Sperm Abnormalities(%)
A	93.00 ± 1.23[a]	97.40 ± 0.60[a]	5.18 ± 0.02[a]	154.00 ± 3.23[a]	4.60 ± 0.25[a]
D	84.00 ± 2.45[ab]	98.00 ± 0.00[a]	5.1 8 ± 0.02[a]	134.60 ± 4.55[ab]	5.40 ± 0.25[a]
E	78.00 ± 2.00[ab]	94.20 ± 2.40[a]	5.20 ± 0.00[a]	120.00 ± 3.05[ab]	4.60 ± 0.25[a]
D1	74.00 ± 4.00[ab]	94.20 ± 2.40[a]	5.18 ± 0.02[a]	94.40 ± 5.24[ab]	5.00 ± 0.41[a]
E1	77.50 ± 2.50[ab]	96.50 ± 0.87[a]	5.15 ± 0.03[a]	105.50 ± 7.60[ab]	5.20 ± 0.37[a]

Values are expressed as mean ± SEM. [ab] significantly different from the control at $p < 0.05$.
A: control, D: 250 mg/kg oil extract (OG) at 14 days treatment, E: 500 mg/kg oil extract (OG) at14 day's treatment, D1: 250 mg/kg oil extract (OG) at 28 days treatment, E1: 500 mg/kg oil extracts (OG) 28 days treatment.

Table 3
Testicular biometry at 14 and 28 days post-treatment with methanol extract of *Ocimum gratissimum*.

Parameters	Groups A	B	C	B1	C1
Body weight(kg)	154.65 ± 7.3[a]	119.52 ± 3.4[a]	148.7 ± 13.78[a]	139.66 ± 8.35[a]	151.49 ± 4.49[a]
Left testes weight(kg)	1.16 ± 1.09[a]	0.93 ± 0.05[a]	1.09 ± 0.07[a]	0.98 ± 0.03[a]	1.15 ± 0.08[a]
Right testes weight(kg)	1.10 ± 0.05[a]	0.90 ± 0.06[a]	1.08 ± 0.06[a]	0.95 ± 0.02[a]	1.12 ± 0.07[a]
Left testes length(cm)	17.03 ± 0.35[a]	16.07 ± 0.44[a]	17.54 ± 0.35[a]	11.89 ± 0.33[ab]	16.21 ± 1.13[a]
Right testes length(cm)	17.23 ± 0.30[a]	15.27 ± 0.38[a]	16.94 ± 0.54[a]	12.04 ± 0.43[ab]	14.99 ± 1.33[a]
Left testes diameter(cm)	7.41 ± 0.49[a]	8.71 ± 0.18[a]	9.80 ± 0.20[a]	2.78 ± 0.42[ab]	5.53 ± 1.19[ab]
Right testes diameter (cm)	7.09 ± 0.27[a]	8.77 ± 0.77[a]	10.13 ± 0.17[a]	3.11 ± 0.28[ab]	5.88 ± 1.07[ab]
Epididymal weight(kg)	0.44 ± 0.03[a]	0.31 ± 0.03[a]	0.37 ± 0.06[a]	0.36 ± 0.02[a]	0.41 ± 0.03[a]

Values are expressed as mean ± SEM. [ab] significantly different from the control at $p < 0.05$.
Group A: control, Group B: 250 mg/kg methanol extract (OG) at 14 days treatment, Group C: 500 mg/kg methanol extract (OG) at14 day's treatment, Group B1: 250 mg/kg methanol extracts (OG)) at 28 days treatment, Group C1: 500 mg/kg methanol at 28 days treatment.

spermatozoa livability and spermatozoa volume of groups B–E were not significant ($p > 0.05$) when compared with the control A.

There was significant difference ($p < 0.05$) in sperm concentration of groups B, C, B1 and C1 (116.60 ± 3.97, 111.00 ± 11.02, 106.80 ± 5.83, and 122.00 ± 4.85) respectively, and groups D, E, D1 and E1 (134.60 ± 4.55, 120.00 ± 3.05, 94.40 ± 5.24, and 105.50 ± 7.60) respectively, when compared to the control group A (154.00 ± 3.23). The difference in mean of the morphological sperm abnormalities was not significant ($p > 0.05$) across the treatment groups in phase 1 (B, C, B1, C1), and phase 2 (D, E, D1 and E1) (Tables 1 and 2).

3.2. Testicular biometry

The results showed that administration of extract of MEOG leaves, at the doses of 250 and 500 mg/ kg/day, for 14days and 28days, caused no significant difference ($p > 0.05$) in the body weight, epididymal weight, left and right testes weight compared with the control group (Table 3). However in the left and right testicular length, and right testicular diameter there was a significant difference ($p < 0.05$) in groups, B1 and C1 compared with the control group A (Table 3). OEOG was administered in groups D, E, D1 and E1 but there was no significant difference ($p > 0.05$) in body weight, epididymal weight, left & right testicular weight, left & right testicular diameter compared to the control group A (Table 4). However, in the left and right testicular length, there was a significant difference ($p < 0.05$) in groups E1 compared with the control group A (Table 4).

3.3. Testosterone assay

The administration of extract MEOG leaves, at the doses of 250 and 500 mg/ kg/day, for 14days and 28days, caused no significant difference ($p > 0.05$) in testosterone level in groups B (2.88 ± 1.68), C (0.69 ± 0.20), B1 (1.49 ± 0.39) and C1(0.85 ± 0.41) when compared with the control group A(1.49 ± 0.33) (Fig. 3). Also, there was no significant difference ($p > 0.05$) in the mean testosterone level of groups D, E, D1 and E1 when compared to the control group.

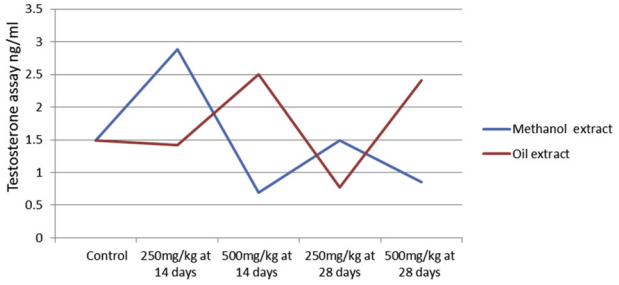

Fig. 3. Group A: control, Group B and D: 250 mg/kg methanol and oil extract (OG) at 14 days treatment, Group C and E: 500 mg/kg methanol and oil extract (OG) at14 day's treatment, Group B1 and D1: 250 mg/kg methanol and oil extracts (OG) at 28 days treatment, Group C1 and E1: 500 mg/kg methanol and oil at 28 days treatment.

3.4. Histology

Histology sections of the testes of the adult rats (Fig. 3) in the control group showed normal seminiferous tubules and interstices. The morphology of the testes of the male rats that were given MEOG and OEOG did not appear different from those of the control rats. No overt pathological lesions were apparent in the seminiferous epithelia or the interstitial spaces of the testes (Figs. 4–8) of the male rats that received the different doses of methanol and oil extracts of OG.

Table 4
Testicular biometry at 14 and 28 days post-treatment with oil extract of *Ocimum gratissimum*.

Parameters	Groups A	D	E	D1	E1
Body weight(kg)	154.65 ± 7.33[a]	145.50 ± 5.60[a]	140.50 ± 7.76[a]	169.83 ± 9.88[a]	151.05 ± 5.08[a]
Left testes weight(kg)	1.16 ± 1.09[a]	1.11 ± 0.07[a]	0.81 ± 0.17[a]	1.00 ± 0.15[a]	0.93 ± 0.18[a]
Right testes weight(kg)	1.10 ± 0.05[a]	1.09 ± 0.07[a]	0.79 ± 0.16[a]	0.99 ± 0.14[a]	0.88 ± 0.17[a]
Left testes length(cm)	17.03 ± 0.35[a]	16.76 ± 0.53[a]	15.55 ± 1.22[a]	17.20 ± 1.16[a]	6.89 ± 2.12[ab]
Right testes length(cm)	17.23 ± 0.30[a]	17.45 ± 0.40[a]	14.92 ± 1.27[a]	16.81 ± 0.95[a]	6.46 ± 2.07[ab]
Left testes diameter(cm)	7.41 ± 0.49[a]	9.43 ± 0.36[a]	8.66 ± 0.59[a]	8.24 ± 0.81[a]	9.07 ± 1.96[a]
Right testes diameter(cm)	7.09 ± 0.27[a]	8.88 ± 0.28[a]	8.74 ± 0.51[a]	8.08 ± 0.62[a]	8.17 ± 1.75[a]
Epididymal weight (kg)	0.44 ± 0.03[a]	0.40 ± 0.04[a]	0.25 ± 0.06[a]	0.40 ± 0.06[a]	0.35 ± 0.05[a]

Values are expressed as mean ± SEM. [ab] significantly different from the control at $p < 0.05$.
Group A: control, Group B: 250 mg/kg methanol extract (OG) at 14 days treatment, Group C: 500 mg/kg methanol extract (OG) at14 day's treatment, Group B1: 250 mg/kg methanol extracts (OG)) at 28 days treatment, Group C1: 500 mg/kg methanol at 28 days treatment.

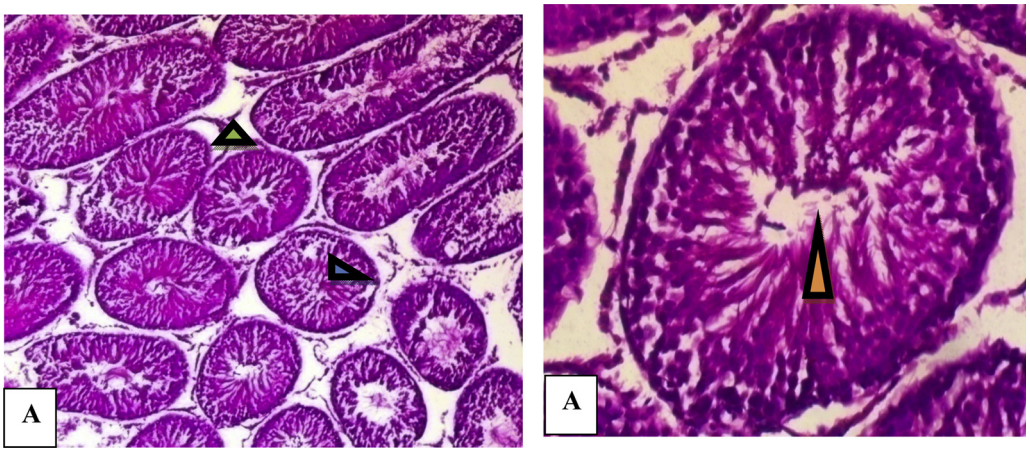

Fig. 4. Histology of the testis of an adult male rat of the control group A showing normal testicular histology. The active seminiferous tubules (orange triangle) and interstitial spaces (green triangle). The seminiferous epithelium (blue triangle) showing active spermatogenesis. — Photomicrograph ×100 and ×400.

4. Discussion

4.1. Spermiogram

The MEOG and OEOG administered at 250 mg/kg and 500 mg/kg for 14 days & 28 days respectively did have a significant difference ($p < 0.05$) in sperm motility, sperm livability and volume. The difference seen in sperm motility is dose-dependent across the treated groups when compared to the control group. This is in agreement with studies conducted in Iran and Nigeria, where a marked reduction in sperm motility was observed with benzene and aqueous extract of *Ocimum sanctum* leaves [2,22]. On the contrary, it was observed in another study that the crude aqueous extract of OG leaves did not have any significant effect on the sperm motility and liveability of rats [23]. The variance could be due to the type of extracts used for the study [24,25]. Furthermore, the aqueous extract of OG leaves is rich in flavonoids and phenolic compounds [4,6,8]. Many studies have reported the protective action of flavonoids and phenols against oxidative stress-induced cellular damage [8,24]. Flavonoids and phenols can exert their anti-inflammatory and antioxidative activities by various mechanisms, e.g. by inhibiting enzymatic systems responsible for the free radical generation or by scavenging or quenching free radicals, by chelating metal ions [26,27]. The anti-inflammatory and anti-oxidative properties of OG can also be due to the presence of alkaloids, terpenoids, saponin, glycoside and alkaloids present in this plant [4,6,8].

The sperm concentration of the treated groups that received MEOG and OEOG extracts were significantly different ($p < 0.05$) to the control group A. A similar reduction in sperm count was reported in a study conducted elsewhere [28]. However, an increased sperm concentration was observed in a related study by [29]. The percentage of sperm morphological abnormalities in this study also showed no significant difference ($p > 0.05$) across the treated groups when compared to the control. This is at variance with one study of benzene extract of *Ocimum sanctum* leaves, where an increase in sperm anomalies was reported [22]. Moreover, significant morphological abnormalities were also observed with crude aqueous extract of OG [23]. This variance the result from this study to those elsewhere could be due to the type of extracts, and the effect geographical location on plant constituents [2,9,10]. The semen parameter observed showed that methanol and the oil extract OG have no deleterious effect on semen volume, concentration and liveability. The current study contradicts other studies where the oil extract of OG at 300 mg/kg for 60days [14], OG *and G. latifolium* [28] and benzene extract of *Ocimum sanctum leaves* [22] were dosed to rats and significant deleterious effect on total sperm concentration, sperm motility, livability and increase in sperm anomalies were observed. In the present study, there was a significant difference $p < 0.05$ in the sperm motility and concentration, however, these differences judging from standards set by the World Health Organisation were not s significant enough to affect fertility (WHO, 2010).

4.2. Testicular biometry and testosterone assay

The testicular biometry revealed that administration of methanol (MEOG) and oil (OEOG) extracts of OG leaves, at the doses of 250 and

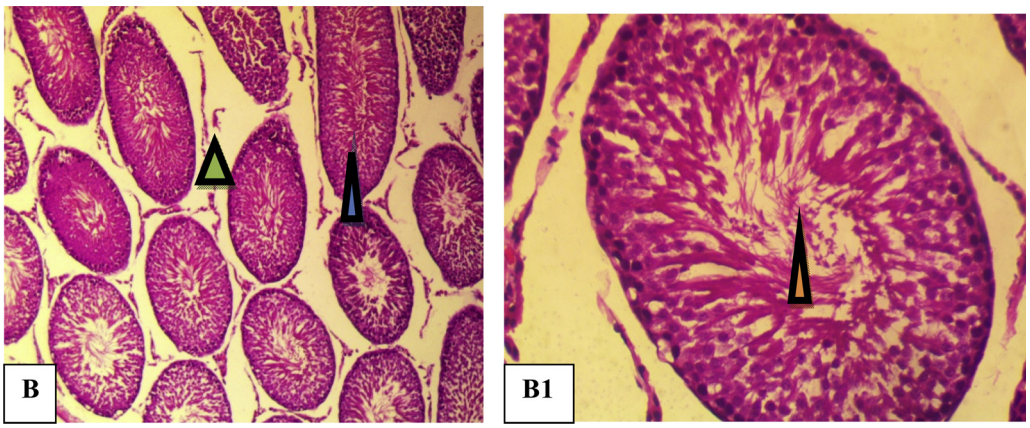

Fig. 5. Histology of the seminiferous tubule of an adult male of group B and B1 showing no overt pathological lesions. The active seminiferous tubules (orange triangle) and interstitial spaces (green triangle).The seminiferous epithelium (blue triangle) showed active spermatogenesis — Photomicrograph ×100 and ×400.

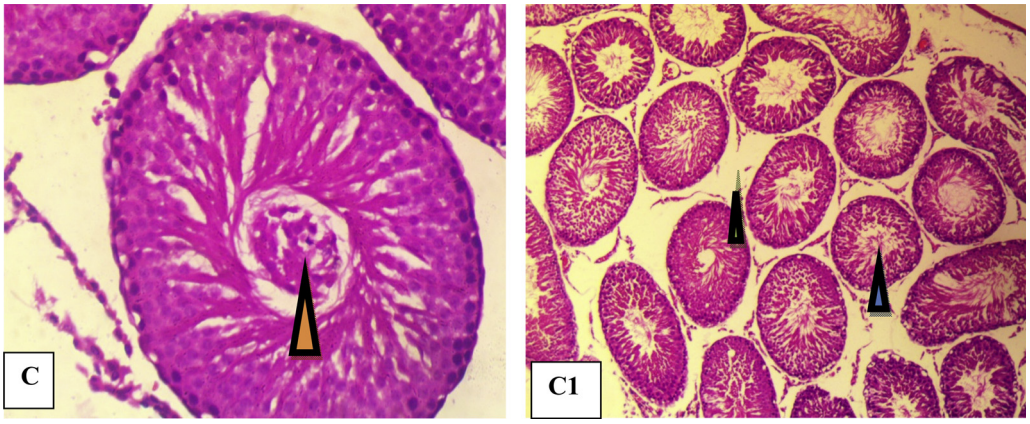

Fig. 6. Histology of the seminiferous tubule of an adult male of group C and C1 showing no overt pathological lesions. The active seminiferous tubules (orange triangle) and interstitial spaces (green triangle). The seminiferous epithelium (blue triangle) showed active spermatogenesis — Photomicrograph ×100 and ×400.

500 mg/ kg/day, for 14 days and 28 days, caused no significant difference ($p > 0.05$) in body weight, epididymal weight, left and right testes weight compared to the control (Tables 3 and 4). This finding correlates with the testicular function parameter observed in this study. Although, the left and right testicular length, right and right testicular diameter groups B1, C1 and E1 there was a significant difference ($p < 0.05$) compared with the control group A but it did not correlate with testicular functions. One study reports a decrease in testes weight/ body weight only after 60 days of administration of OG oil extract at 300 mg/kg and testicular functions [14]. Hence, the duration of study in this regard could play a major role in the difference observed between both studies. More importantly is the finding by a Nigerian study where an apparent and significantly increase weight of the epididymis was noted. The study further acknowledged the anabolic effect of testosterone specifically its role in enhancing the growth and development of other accessory sexual organs [30].

There was no significant difference $p > 0.05$ in the testosterone level across the treated groups compared to the control group. However, there was a noticeable increase in testosterone level when MEOG was given at 250 mg/kg for 14 days. Likewise, a similar increase in testosterone was observed when the OEOG was administered at 500 mg/kg for 14 days. The differences seen between 250 mg/kg MEOG at 14 days and 500 mg/kg OEOG at 14 days could be the method of extraction and dose of the OG. In one study, dosing rats with OG *and G. latifolium* caused no visible reduction in testosterone level [28]. However, increased testosterone level was observed when *Ocimum basilicum* was given to rats [29]. A visibly reduced testosterone level was reported only after the administration of oil extract of OG at 300 mg/kg for 60 days [14]. The differences in these studies could be attributed to the duration of the study and type of extracts [24,25]. Previous studies have shown the administration of herbal substances, coupled with a longer period of administration triggered varying levels of the severely degenerated testis, severe testicular oxidative status, and deranged sperm parameters [20]. Many of these defects could be associated with the existence of phytochemical components in the extract like saponins, tannins, and alkaloids. Tannins alkaloid and saponins at high doses may become pro-oxidant, effectively increasing lipid peroxidation in the testis [31]. The testosterone level obtained in study animals dosed with methanol and oil extracts of OG given for 14 and 28 days, does not have an inhibitory effect on the reproductive function and fertility of adult male rats.

4.3. Histology

The histology of the testes revealed normal architecture of the seminiferous tubules of the testes with no visible pathologic lesions and sequential arrangement of spermatogenic cells. To our knowledge, studies comparing the effect of methanol and oil extract of OG are scanty, hence comparing the histological outcome of our study with others will be difficult. Notwithstanding the dearth of information regarding histological changes associated with the administration OG, one Nigerian study reported that the testicular histoarchitecture was preserved and there was no sign of inflammatory signs even after the administration of OG at 10, 100 and 100 mg/kg bodyweight and 1600, 2900 and 5000 mg/kg body weight in a two-phase study [30]. A similar study on the effect of combined therapy of *Moringa oleifera* and OG testicular parameters of diabetic rats show that the combination therapy reversed testicular damage in diabetic rats [27]. This study further buttresses the important and beneficial role OG place in reproductive health. Studies elsewhere observed germinal tissue erosion

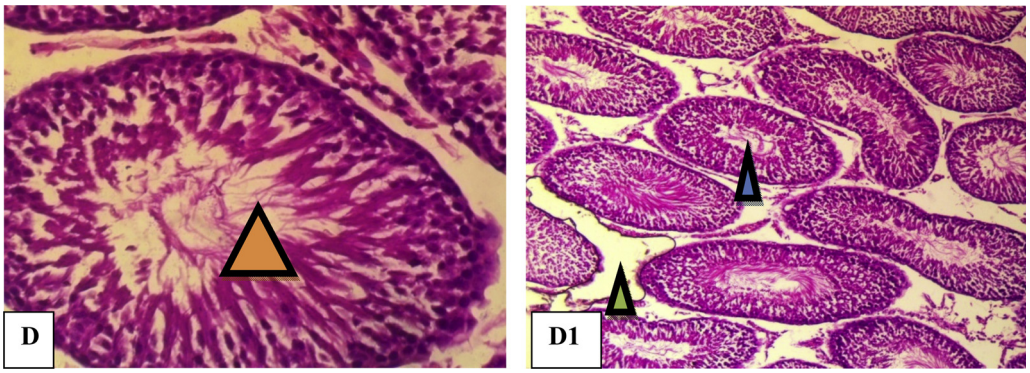

Fig. 7. Histology of the seminiferous tubule of an adult male of group D and D1 showing no overt pathological lesions. The active seminiferous tubules (orange triangle) and interstitial spaces (green triangle).The seminiferous epithelium (blue triangle) showed active spermatogenesis. — Photomicrograph ×100 and ×400.

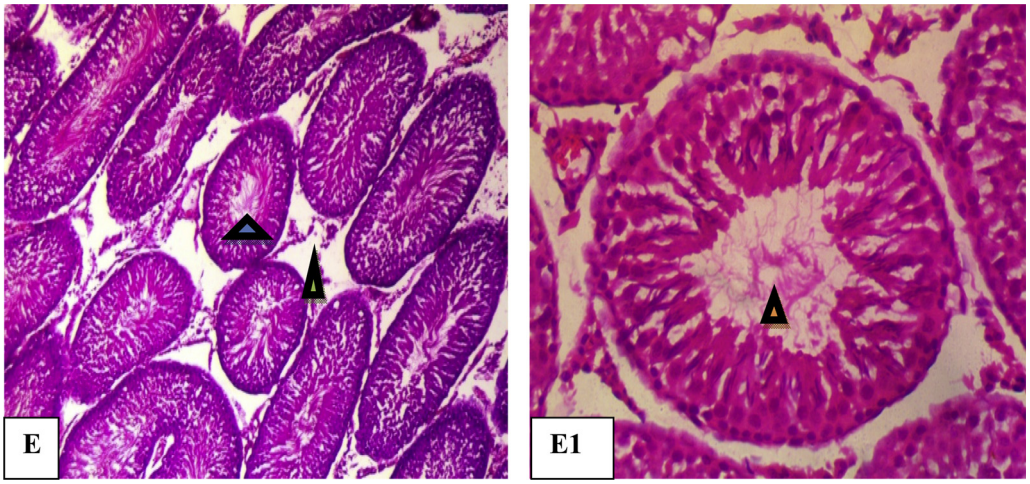

Fig. 8. Histology of the seminiferous tubule of an adult male of group E and E1 showing no overt pathological lesions. The active seminiferous tubules (orange triangle) and interstitial spaces (green triangle). The seminiferous epithelium (blue triangle) showed active spermatogenesis — Photomicrograph ×100 and ×400.

of the seminiferous tubules and interstitial oedema in rats treated with crude aqueous extract of OG [2,23]. The type of extracts and duration of the study play a crucial role in the outcome of study findings and could be responsible for the difference in the histology of the testis. The histological integrity of the entire testes is fundamental to the production of fertile spermatozoa and therefore any factor which damages the testicles such as infection, toxic agents, malnutrition or heat will result in the production of subfertile spermatozoa [20]. The structure of the testes plays a vital role in the production and the quality of spermatozoa produced and as seen in this work the testicular architecture and testosterone level was unaltered. The absence of any overt pathological lesions in the histology of the testes of the adult male rats that received varying doses of OG suggests that the extracts did not have any direct toxic effect on the testes. It also further explains why there was no significant effect on sperm motility, livability, and volume as well as testosterone levels. The study outcomes highlight how important histological integrity of the testis is to the production of quality spermatozoa.

5. Conclusion

The methanol extract of *Ocimum gratissimum* leaves administered at 250 and 500 mg/kg for 14 & 28days has no deleterious effect on serum testosterone level, sperm livability, sperm volume, sperm concentration, testicular biometry and testicular morphology. The oil extract of *Ocimum gratissimum* at 250 mg/kg and 500 mg/kg at 14 & 28 days does not also have any effect on serum testosterone level, sperm livability, sperm volume, sperm concentration, testicular biometry and testicular morphology. Hence methanol and oil extracts of *Ocimum gratissimum* given at 250 mg/kg & 500 mg/kg for 14 & 28 days has no deleterious influence on the reproductive function of the adult male rats. Although there were significant difference $p < 0.05$ in the sperm motility and concentration in this study, these differences were not statistically significant to affect fertility (WHO, 2010). Further research isolating specific active ingredient of this plant which affects the sperm concentration, testicular biometry as well as other reproductive parameters in male animals should be conducted.

Author contribution statement

Ikokide Emmanuel Joseph conceived and carried out the study including the drafting of the manuscript. Ishmael Festus Jaja, Adamu Hamman Boyi, and Oyeyemi Mathew Olugbenga reviewed the manuscript and supervised the study at different stages of the study.

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Declaration of Competing Interest

The authors declare no conflict of interest.

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