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Effects of lipophilic and hydrophilic leaf extracts of *Portulaca oleracea* Linn. (Purslane) on male reproductive parameters in albino rats

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ABSTRACT

Objectives: To supply scientific information/literature on the use of *Portulaca oleracea* in enhancing fertility in males; either to validate or to refute this folkloric use. This study investigated the effect of the lipophilic and hydrophilic leaf extracts of *P. oleracea* on male reproductive parameters.

Methods: The extracts were obtained using two extracting solvents—chloroform and 80% aqueous methanol in succession. Experimental animals were randomly divided into seven groups of 16 rats each. Group A (control) received 0.5 ml 20% Tween 80 (vehicle), groups B, C, and D received 125, 250, and 500 mg/kg of the lipophilic extract, respectively, and groups E, F, and G received 125, 250, and 500 mg/kg of the hydrophilic extract, respectively, for 60 days. On days 14, 28, 42, and 60, four rats from each group were weighed and anesthetized for sample collection. Blood (for testosterone), testes (weight and histomorphology), and sperm cells from caudal epididymis (for semen analysis) were collected.

Results: Both extracts had no significant ($p > 0.05$) effect on testicular weight and histomorphology, and sperm cell characteristics but caused a significant ($p < 0.05$) increase in sperm count. Only the hydrophilic extract produced a significant ($p < 0.05$) reduction in the testosterone level.

Conclusion: Leaf extracts of *P. oleracea* as used in this study improved spermatogenesis irrespective of the decline in testosterone level. This may justify the use of *P. oleracea* leaf extracts in enhancing fertility in males, although further studies are required to elucidate the mechanism and compounds associated with these properties.

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Portulaca oleracea; sperm cell count; testosterone; testis; sperm morphology

Introduction

Portulaca oleracea Linn., known as common purslane, is a member of family Portulacaceae. It is a warm climate green herb, with obovate leaves, small yellow flowers, which open individually at the middle of the leaves for few hours on sunny days, especially in the mornings, and branched succulent stems, which are decumbent near the base [1]. Purslane has a wide cosmopolitan distribution, which gave it different names in various geographical locations. It is known as "Ma-Chi-Xian" in China, "rigla" in Egypt, "pigweed" in England, "Pourpier" in France, and "purslane" in Australia and USA [2,3]. It also has different names in various ethnic groups in Nigeria. It is known as "Ntioke," or "Idiridi" in Igbo, "Esan omode" or "Papasan" in Yoruba, "Babbajibji" or "Halshen saniya" in Hausa, and "Eferemakara" in Efik [4,5].

The use of *P. oleracea* in folk medicine dates back to ancient times and has been given the name, "Global Panacea" by the World Health Organization [6]. The Chinese legend calls it "vegetable for long life," as it has been used in their traditional medicine for many generations [2]. It is one of the indigenous plants used globally for management of health challenges and treatment of diseases [7]. *Portulaca oleracea* has been demonstrated to possess antifungal activity [8], antidiabetic effect [9], hepatoprotective

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activity [10], antioxidant effects [11], analgesic and anti-inflammatory action [12], and wound healing properties [13]. The presence of various biologically active compounds such as flavonoids, alkaloids, phenol, saponin, tannin, terpenoid, omega-3-fatty acids, cardiac glycoside, steroid, phlobatannin, protein, and starch have been reported in the phytochemical screening of *P. oleracea* [14,15].

In folk medicine, *P. oleracea* Linn. is used to treat different health challenges such as scurvy, urinary disorder, hemorrhoids, fever, headache, wounds, and sores [16]. In Nigeria, the plant has been employed by traditional medicine practitioners in the management of infertility in women. In the southern part of Nigeria, that is, the Niger Delta region, the leaves of *P. oleracea* is added to either yam or cocoyam porridge and taken by women in order to enhance their fertility. In the Eastern part of Nigeria, the aerial parts of the plants are crushed to extract the juice which is taken with or without raw egg for the purpose of improving fertility both in males and females.

So far, only one study has been reported on the effect of *P. oleracea* on male reproductive functions. The study demonstrated that the aqueous and methanol extracts of stem and leaves of *P. oleracea* caused a significant reduction in testosterone concentration and percentage sperm motility with an increase in abnormal sperm cells of exposed albino rats, an indication of the harmful potential of *P. oleracea* extracts in male animals [17]. The dearth of literature on the effect of *P. oleracea* on male reproduction prompted this study.

This study was, therefore, designed to investigate the effect of lipophilic (chloroform) and hydrophilic (80% aqueous methanol) leaf extracts of *P. oleracea* on male sex hormone—testosterone, sperm cell count, sperm cell characteristics, and testicular histomorphology. The findings of this study will also serve as a guide to either justify or refute the folkloric use of *P. oleracea* leaf in enhancing fertility in humans.

Materials and Methods

Plant material and authentication

Fresh leaves of *P. oleracea* were gathered from Alakahia axis of Port Harcourt, Nigeria from the months of December 2017 to February 2018. A voucher specimen (UPH/V/1,302) of the plant, authenticated in the Department of Plant Science and Biotechnology, University of Port Harcourt, Port Harcourt, Nigeria, was placed at the University Herbarium.

Preparation of the extract

The collected leaves were dried under a shade at room temperature for a duration of 6 weeks. The dried leaves of *P. oleracea* were weighed and ground to fine powder. Successive solvent extraction by cold maceration was done for 72 hours using two solvents; chloroform and 80% aqueous methanol. In each case, there was a fresh replacement of solvent every 24 hours. Extraction solvents were used in the ascending order of polarity (chloroform before aqueous methanol). All reagents used were of analytical grades.

Lipophilic extraction

A 4.5 kg portion of dried pulverized *P. oleracea* leaves were soaked in 13.5 l of chloroform for 24 hours. At the expiration of the time, they were stirred and filtered first with muslin fabric and later the resultant solution was further filtered with Whatman's No. 1 filter paper. The marc (residue) was macerated again with the same volume of chloroform for another 24 hours, followed by filtration with muslin fabric and Whatman's No. 1 filter paper. This resulting marc was soaked again for another 24 hours (making a total of 72 hours of maceration) and the filtration procedure repeated. The filtrates were combined and concentrated with a rotary evaporator (Model No: RE-52A) at 45°C *in vacuo* and later transferred to an evaporating dish and dried over a water bath (Digital thermostatic water bath, Jinotech instruments). The chloroform leaf extracts of *P. oleracea* (Lipophilic extract) obtained were stored in a desiccator. The resulting marc was dried to a constant weight for subsequent extraction with the second (hydrophilic) solvent system–80% aqueous methanol.

Hydrophilic extraction

A 2.5 kg portion of marc obtained after the chloroform extraction was soaked in 7.5 l of 80% aqueous methanol (ratio of methanol to distilled water is 4:1) for 24 hours. At the expiration of the time, they were stirred and filtered first with muslin fabric and later the resultant solution was further filtered with Whatman's No. 1 filter paper. The marc (residue) was macerated again with the same volume of 80% methanol for another 24 hours, followed by the filtration with muslin fabric and Whatman's No. 1 filter paper. This resulting marc was soaked again

for another 24 hours (making a total of 72 hours of maceration) and the filtration procedure repeated. The filtrates were combined and concentrated with a rotary evaporator (Model No: RE-52A) at 45°C *in vacuo* and later transferred to an evaporating dish and dried over a water bath (Digital thermostatic water bath, Jinotech instruments). The aqueous methanol leaf extracts of *P. oleracea* (Hydrophilic extract) obtained were stored in a desiccator.

The percentage yield of each extract was calculated as:

$$\frac{\text{Weight of dried extract}}{\text{Weight of plant material}} \times 100\%$$

Preliminary phytochemical screening

This was done on the lipophilic extract for the presence of alkaloids, anthraquinones, triterpenoids/steroids, cardiac glycosides, and carbohydrates following the method of Harborne [18] and Houghton and Raman [19]. Test for tannin (Phenols), flavonoid—Shinoda reduction test, saponins—frothing test, and phlobatannin were not carried out with the lipophilic extract due to its solubility in water. The preliminary phytochemical screening of the hydrophilic extract of *P. oleracea*, which has been reported in our work [20], tested for alkaloid, tannin (phenols), flavonoid, saponins, phlobatannin, carbohydrates, anthraquinones, triterpenoids/steroids, and cardiac glycosides.

Ethical approval

The study protocols were duly approved by the Research Ethics Committee of the Centre for Research Management and Development, University of Port Harcourt with the Ref. No: UPH/CEREMAD/REC/04. All experimental animals were humanely handled in accordance with the Ethics and Regulation guiding the use of research animals as approved by the University.

Acute oral toxicity study

The acute oral toxicity study for chloroform (lipophilic) and aqueous methanol (hydrophilic) leaf extracts of *P. oleracea* were carried out to determine the LD_{50} of the extracts using the method of Lorke [21].

Animals

One hundred and twelve sexually mature male albino rats of about 18 weeks old, weighing an average of 200 g, procured from the Animal House of the Department of Pharmacology, College of Health Sciences, University of Port Harcourt, Nigeria, were used in the study. The rats were acclimatized for 2 weeks before commencing the study. They were fed water and commercially sourced feed (Top Feeds Nigeria Limited) *ad libitum* all through the study.

Experimental procedure

Following acclimatization, the animals were randomly assigned to 7 groups of 16 animals each for treatment as follows:

Group A (Control)—0.5 ml 20% Tween 80 (vehicle).

Group B—125 mg/kg of chloroform (lipophilic) extract

Group C—250 mg/kg of chloroform (Lipophilic) extract

Group D—500 mg/kg of chloroform (Lipophilic) extract

Group E—125 mg/kg of aqueous methanol (Hydrophilic) extract

Group F—250 mg/kg of aqueous methanol (Hydrophilic) extract

Group G—500 mg/kg of aqueous methanol (Hydrophilic) extract

All treatments were by gavage daily for a period of 60 days. Animals' weights were measured weekly and the doses adjusted accordingly. On days 14, 28, 42, and 60, four animals from each of the experimental groups were weighed and anesthetized. Blood samples were collected by cardiac puncture into the sterile plain bottles for testosterone assay. The testes were excised and weighed independently. The epididymides were harvested and the caudal part was used to determine the epididymal sperm characteristics**.** Testicular samples were fixed in Bouin's solution for histomorphology examination. The relative organ (testicular) weight was estimated individually as follows:

$$\text{Relative Organ weight} = \frac{\text{Absolute Testicular weight (g)}}{\text{Absolute Body weight (g)}} \times 100\%$$

Testosterone assay

Serum concentration of testosterone was determined by a microplate enzyme immunoassay using Accu-bind ELISA Microwells (Testosterone Test System Product Code: 3725-300) from Monobind, USA.

Analysis of epididymal spermatozoa characteristics

Sperm count

The caudal epididymis was dissected out from the testes, lacerated, and carefully pressed on a clean grease free glass slide in order to express the sperms. Two drops of normal saline were added to emulsify it to form a suspension. The suspension was diluted with formal saline in the ratio of 1–20. The new improved Neubauer counting chamber (Hemocytometer) was charged with a drop of the diluted sperm. The counting chamber was mounted on the slide stage of a light microscope and viewed under the magnification of ×40. The count was expressed as million/ml of suspension [22].

Sperm motility

One drop of the caudal epididymal sperm was applied to a clean glass slide and two drops of normal saline were added to emulsify it before it was covered with a cover-slip. The slide was examined under a light microscope at ×40 magnification. The sperm motility was assessed by counting both active progressive movements (motile) and non-progressive movements (immotile) of sperms. The motile and non-motile cells were counted in 10 random fields and the number expressed as a percentage of the total number of sperms [22].

Sperm viability

One drop of sperms from the caudal epididymis was applied to a clean glass slide, with two drops of normal saline added to it to form a suspension. The suspension was mixed with one drop of 0.5% eosin solution. After a few seconds, the slide was examined under light microscope at ×40 magnification. Eosin-stained non-viable sperm was differentiated from unstained viable sperm [22].

Sperm morphology

One drop of the caudal epididymal sperm was applied to a clean glass slide, followed by two drops of normal saline to make a suspension. The suspension was mixed with one drop of 0.5% eosin solution. After a few seconds, the slide was examined under light microscope at ×40 magnification. Morphological appearance of normal and abnormal spermatozoa (abnormality in the head, mid-piece, or tail) was determined and their percentages were estimated [22].

Histological study of the tissues

The fixed testicular samples were then processed using the method of Lillie [23]. The tissues were processed with time by dehydration in ascending grades of alcohol, followed by clearing in xylene. After infiltration and embedding in paraffin, they were sectioned at 4–5 µm, followed by deparaffinization in xylene, dehydration in descending grades of alcohol, and stained with H&E dyes. The processed tissues were mounted on glass slides, covered with cover-slip, and then examined under a standard light microscope. The photomicrographs were captured using Olympus® CX31 digital camera.

Statistical analyses

Statistical analyses were done with SPSS 21; the data were represented as mean ± SEM and assessed using one-way analysis of variance and Tukey post-hoc test. The significance level was set at $p < 0.05$.

Results

Extraction yield

The extraction of *P. oleracea* Linn. leaves using a lipophilic solvent (chloroform) and hydrophilic solvent (80% aqueous methanol) yielded different weights of extracts as shown in Table 1. Extraction of the leaves using the hydrophilic solvent produced the highest yield.

Preliminary phytochemical screening of the plant extracts

Table 2 summarizes the result of the phytochemical screening for both the hydrophilic and lipophilic leaf extracts of *P. oleracea.* The result of the phytochemical screening for the hydrophilic extract of *P.oleracea* as reported in our other work shows the presence of alkaloids, carbohydrates, saponins, steroids, phlobatannins, and cardiac glycosides [20]. The lipophilic extract of *P. oleracea* could not dissolve in water and as such allowed for very few tests, out of which only the test for triterpenoid/steroids was positive. The lipophilic extract examined

Table 1. Percentage yield of *Portulaca oleracea* Linn. leaf extracts.

Solvent type	Weight of plant sample used (kg)	Extract yield (%)
Lipophilic (Chloroform)	4.5	81
Hydrophilic (80% aqueous methanol)	2.5	82

on thin layer chromatography plate sprayed with Dragendorrf's reagent and ferric chloride solution was negative for alkaloids and phenolics compounds, respectively.

Acute toxicity study

Acute toxicity test did not show any mortality, morbidity, or other apparent signs of toxicity at the doses used. This was proof that both extracts were not noxious at the maximum dose of 5,000 mg/kg. Hence, 1/40th, 1/20th, and 1/10th of this maximum dose (5,000 mg/kg) were adopted for the studies which gave rise to 125, 250, and 500 mg/kg doses of lipophilic and hydrophilic leaf extracts of *P. oleracea* treatment groups.

Relative testicular weight

Lipophilic and hydrophilic leaf extracts of *P. oleracea* had no significant ($p > 0.05$) effect on the relative left and right testicular weights of treated rats throughout the 60 days of treatment in comparison with the control (Tables 3 and 4).

Table 2. Phytochemical elements of *P. oleracea* leaf extracts.

Tests	Phytochemical elements	Lipophilic extract of *P. oleracea*	Hydrophilic extract of *P. oleracea*
Wagner's Reagent		–	+
Dragendorrf's Reagent	Alkaloid	–	+
Meyer's Reagent		–	–
Hager's Reagent		–	–
Ferric Chloride	Tannin	ND	–
Shinoda Reduction Test	Flavonoid	ND	–
Molisch Test	carbohydrate	–	+
Fehling's Test			+
Frothing Test	Saponins	ND	+
Phlobatannins Test	Phlobatannins	ND	–
Bontrager's Test	Anthraquinone	–	+
Lieberman's Test Salkowski's Test Keller-Killiani's Test	Triterpenoids/Steroids	+	+
Kedde Test			+
	Cardiac glycoside	–	

(+) means presence and (–) means absence. ND = Not done due to solubility of lipophilic extract of *P. oleracea* (highly lipophilic).

Table 3. Effect of varied doses of lipophilic leaf extract of *P. oleracea* on relative testicular weight.

Parameter	Left testis (g)				Right testis (g)			
Duration treatment	**14 days**	**28 days**	**42 days**	**60 days**	**14 days**	**28 days**	**42 days**	**60 days**
Group A (control)	0.58 ± 0.02	0.59 ± 0.02	0.56 ± 0.03	0.51 ± 0.24	0.58 ± 0.02	0.57 ± 0.02	0.57 ± 0.03	0.49 ± 0.02
Group B (125 mg/kg)	0.63 ± 0.04	0.66 ± 0.03	0.55 ± 0.04	0.55 ± 0.05	0.62 ± 0.04	0.65 ± 0.02	0.54 ± 0.03	0.54 ± 0.06
Group C (250 mg/kg)	0.50 ± 0.01	0.58 ± 0.01	0.53 ± 0.02	0.51 ± 0.01	0.50 ± 0.03	0.57 ± 0.01	0.52 ± 0.01	0.52 ± 0.01
Group D (500 mg/kg)	0.64 ± 0.01	0.57 ± 0.02	0.53 ± 0.00	0.52 ± 0.03	0.60 ± 0.02	0.58 ± 0.02	0.52 ± 0.00	0.50 ± 0.02

Results are given as mean ± SEM for four rats in each group. Experimental groups are compared with Group A (control). No significant variation exists across the table at 95% confidence interval ($p > 0.05$).

Table 4. Effect of varied doses of hydrophilic leaf extract of *P. oleracea* on relative testicular weight.

Paramester	Left testis (g)				Right testis (g)			
Duration treatment	**14 days**	**28 days**	**42 days**	**60 days**	**14 days**	**28 days**	**42 days**	**60 days**
Group A (control)	0.58 ± 0.02	0.59 ± 0.02	0.56 ± 0.03	0.51 ± 0.24	0.58 ± 0.02	0.57 ± 0.02	0.57 ± 0.03	0.49 ± 0.02
Group E (125 mg/kg)	0.56 ± 0.07	0.61 ± 0.02	0.57 ± 0.22	0.38 ± 0.13	0.58 ± 0.06	0.61 ± 0.02	0.58 ± 0.01	0.37 ± 0.12
Group F (250 mg/kg)	0.61 ± 0.04	0.66 ± 0.03	0.63 ± 0.04	0.59 ± 0.01	0.60 ± 0.02	0.64 ± 0.02	0.63 ± 0.04	0.58 ± 0.01
Group G (500 mg/kg)	0.55 ± 0.09	0.60 ± 0.03	0.63 ± 0.01	0.60 ± 0.03	0.54 ± 0.08	0.58 ± 0.02	0.63 ± 0.02	0.60 ± 0.03

Results are given as mean ± SEM for four rats in each group. Experimental groups are compared with Group A (control). No significant variation exists across the table at 95% confidence interval ($p > 0.05$).

Sperm cell count and characteristics

The result in Tables 5–8 and Figures 1 and 2 summarized the effects of lipophilic and hydrophilic leaf extracts of *P. oleracea* on sperm count, sperm motility, viability, and morphology on days 14, 28, 42, and 60 of treatment. Both extracts had no significant ($p > 0.05$) effect on the sperm motility, viability, and morphology relative to the control (Tables 4–7). The hydrophilic extract caused a significant ($p < 0.05$) increase in the sperm count at 250 mg/kg dose on day 28, while the lipophilic extract also caused a marked ($p < 0.05$) increase in the sperm count at the dose of 250 mg/kg on days 14 and 28 in relation to control (Figs. 1 and 2). The mean sperm count in the other treated groups did not differ significantly ($p > 0.05$) from the control group throughout the 60-day duration of treatment.

Testosterone level

Figures 3 and 4 show the effects of lipophilic and hydrophilic leaf extracts of *P. oleracea* on testosterone levels on days 14, 28, 42, and 60 of treatment. The lipophilic extract caused no significant ($p > 0.05$) variation in the testosterone level throughout the 60-day duration of treatment relative to control.

The hydrophilic extract had no significant ($p > 0.05$) effect on testosterone level in all the test groups on days 14 and 42, and also in the 250 mg/kg treated rats (group F) on days 28 and 60 in comparison with the control. However, it caused a significant ($p < 0.05$) decrease in testosterone level on days 28 and 60 in rats treated with 125 (group E) and 500 mg/kg (group G) of the extract (Fig. 4).

Testicular sections

Photomicrographs of testicular sections of rats from lipophilic and hydrophilic leaf extracts of *P. oleracea* treatment groups are not different from that of the control. No obvious change was observed (Plates 1 and 2).

Discussion

From this study, *P. oleracea* leaf extracts caused no significant change in the relative testicular weights of the experimental animals on days 14, 28, 42, and 60 of treatment. This indicates that the plant

Table 5. Effect of varied doses of lipophilic leaf extract of *P. oleracea* on sperm motility.

Parameter	Active sperms (%)				Sluggish sperms (%)				Dead sperms (%)			
Durationtreatment	14 days	28 days	42 days	60 days	14 days	28 days	42 days	60 days	14 days	28 days	42 days	60 days
Group A (control)	52.50 ± 6.61	66.25 ± 4.27	67.50 ± 3.23	77.50 ± 3.23	8.75 ± 1.25	11.25 ± 1.25	12.50 ± 1.44	10.00 ± 2.04	38.75 ± 5.91	22.50 ± 3.23	20.00 ± 4.08	12.50 ± 2.50
Group B (125 mg/kg)	55.00 ± 7.36	70.00 ± 4.56	78.75 ± 4.27	76.25 ± 5.54	10.00 ± 2.04	10.00 ± 2.04	8.75 ± 1.25	10.00 ± 2.04	35.00 ± 6.45	20.00 ± 4.08	12.50 ± 3.23	13.75 ± 3.75
Group C (250 mg/kg)	68.75 ± 6.57	73.75 ± 5.54	78.75 ± 6.57	80.00 ± 4.08	8.75 ± 1.25	7.50 ± 1.44	7.50 ± 1.44	8.75 ± 1.25	22.50 ± 6.29	17.50 ± 4.79	13.75 ± 5.54	11.25 ± 3.15
Group D (500 mg/kg)	45.00 ± 4.56	72.50 ± 4.79	77.50 ± 5.95	76.25 ± 5.54	11.25 ± 1.25	10.00 ± 0.00	8.75 ± 2.39	10.00 ± 2.04	43.75 ± 3.75	17.50 ± 4.79	13.75 ± 3.75	13.75 ± 3.75

Results are given as mean ± SEM for four rats in each group. Experimental groups are compared with Group A (control). No significant variation exists across the table at 95% confidence intervalsss ($p > 0.05$).

Table 6. Effect of varied doses of hydrophilic leaf extract of *P. oleracea* on sperm motility.

Parameter	Active sperms (%)				Sluggish sperms (%)				Dead sperms (%)			
Durationtreatment	14 days	28 days	42 days	60 days	14 days	28 days	42 days	60 days	14 days	28 days	42 days	60 days
Group A (contssrol)	52.50 ± 6.61	66.25 ± 4.27	67.50 ± 3.23	77.50 ± 3.23	8.75 ± 1.25	11.25 ± 1.25	12.50 ± 1.44	10.00 ± 2.04	38.75 ± 5.91	22.50 ± 3.23	20.00 ± 4.08	12.50 ± 2.50
Group E (125 mg/kg)	57.50 ± 7.22	58.75 ± 2.39	73.75 ± 8.51	78.33 ± 4.41	10.00 ± 2.04	12.50 ± 1.44	10.00 ± 2.04	8.33 ± 1.67	32.50 ± 6.29	28.75 ± 3.15	16.25 ± 8.00	13.33 ± 3.33
Group F (250 mg/kg)	57.50 ± 8.29	72.50 ± 9.24	68.75 ± 7.18	75.00 ± 2.89	12.50 ± 1.44	10.00 ± 2.04	8.75 ± 1.25	10.00 ± 2.04	30.00 ± 7.07	17.50 ± 7.50	22.50 ± 6.29	15.00 ± 2.04
Group G (500 mg/kg)	50.00 ± 5.40	61.25 ± 7.47	63.75 ± 3.75	72.50 ± 3.23	11.25 ± 2.39	13.75 ± 1.25	11.25 ± 1.25	12.50 ± 1.44	38.75 ± 6.57	25.00 ± 6.45	25.00 ± 2.89	15.00 ± 2.89

Results are given as mean ± SEM for four rats in each group. Experimental groups are compared with Group A (control). No significant variation exists across the table at 95% confidence interval ($p > 0.05$).

Table 7. Effect of varied doses of lipophilic leaf extract of *P.leracea* on sperm viability and morphology.

Parameter	Sperm viability (%)				Normal sperms (%)				Abnormal sperms (%)			
Durationtreatment	**14 days**	**28 days**	**42 days**	**60 days**	**14 days**	**28 days**	**42 days**	**60 days**	**14 days**	**28 days**	**42 days**	**60 days**
Group A (control)	63.75 ± 5.54	75.00 ± 5.40	72.50 ± 4.33	80.00 ± 4.08	68.75 ± 3.15	70.00 ± 2.04	72.50 ± 3.23	75.00 ± 3.54	31.25 ± 3.15	30.00 ± 2.04	27.50 ± 3.23	25.00 ± 3.54
Group B (125 mg/kg)	58.75 ± 4.27	72.50 ± 4.33	83.75 ± 2.39	81.25 ± 5.15	57.50 ± 5.20	73.75 ± 5.54	80.00 ± 2.04	78.75 ± 5.15	42.50 ± 5.20	26.25 ± 5.54	20.00 ± 2.04	21.25 ± 5.15
Group C (250 mg/kg)	77.50 ± 6.29	80.00 ± 5.40	81.25 ± 5.15	82.50 ± 4.33	73.75 ± 6.57	75.00 ± 2.04	80.00 ± 5.40	82.50 ± 3.23	26.25 ± 6.57	25.00 ± 2.04	20.00 ± 5.40	17.50 ± 3.23
Group D (500 mg/kg)	57.50 ± 3.23	77.50 ± 6.29	85.00 ± 3.54	81.25 ± 4.27	55.00 ± 4.56	78.75 ± 4.73	83.75 ± 2.39	78.75 ± 4.27	45.00 ± 4.56	21.25 ± 4.73	16.25 ± 2.39	21.25 ± 4.27

Results are given as mean ± SEM for four rats in each group. Experimental groups are compared with Group A (control). No significant variation exists across the table at 95% confidence interval ($p > 0.05$).

Table 8. Effect of varied doses of hydrophilic leaf extract of *P. oleracea* on sperm viability and morphology.

Parameter	Sperm viability (%)				Normal sperms (%)				Abnormal sperms (%)			
Durationtreatment	**14 days**	**28 days**	**42 days**	**60 days**	**14 days**	**28 days**	**42 days**	**60 days**	**14 days**	**28 days**	**42 days**	**60 days**
Group A (control)	63.75 ± 5.54	75.00 ± 5.40	72.50 ± 4.33	80.00 ± 4.08	68.75 ± 3.15	70.00 ± 2.04	72.50 ± 3.23	75.00 ± 3.54	31.25 ± 3.15	30.00 ± 2.04	27.50 ± 3.23	25.00 ± 3.54
Group E (125 mgs/kg)	65.00 ± 5.40	68.75 ± 4.27	77.50 ± 5.95	78.33 ± 4.41	63.75 ± 6.57	66.25 ± 3.75	77.50 ± 4.33	76.67 ± 3.33	36.25 ± 6.57	33.75 ± 3.75	22.50 ± 4.33	23.33 ± 3.33
Group F (250 mg/kg)	57.50 ± 6.61	78.75 ± 6.57	76.25 ± 6.25	76.25 ± 3.75	61.25 ± 5.54	82.50 ± 5.95	75.00 ± 6.12	77.50 ± 3.23	38.75 ± 5.54	17.50 ± 5.95	25.00 ± 6.12	22.50 ± 3.23
Group G (500 mg/kg)	58.75 ± 4.27	65.00 ± 6.12	72.50 ± 4.79	80.00 ± 4.08	53.75 ± 4.27	67.50 ± 5.20	67.50 ± 3.23	78.75 ± 3.15	46.25 ± 4.27	32.50 ± 5.20	32.50 ± 3.23	21.25 ± 3.15

Results are given as mean ± SEM for four rats in each group. Experimental groups are compared with Group A (control). No significant variation exists across the table at 95% confidence interval ($p > 0.05$).

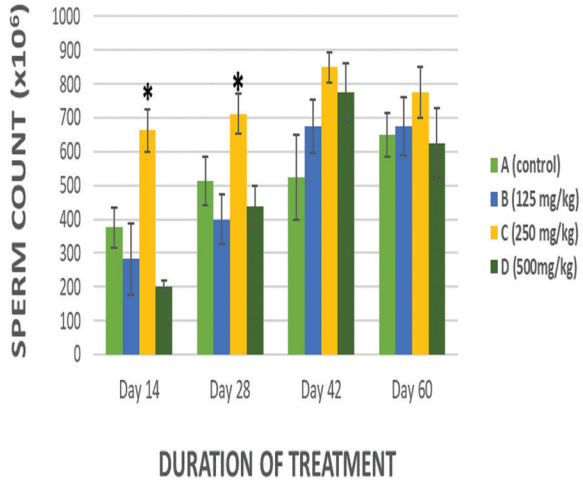

Figure 1. Effect of varied doses of lipophilic leaf extract of *P. oleracea* on sperm count. Results are given as mean ± SEM for four rats in each group. Experimental groups are compared with Group A (control). * indicates a significant difference at $p < 0.05$

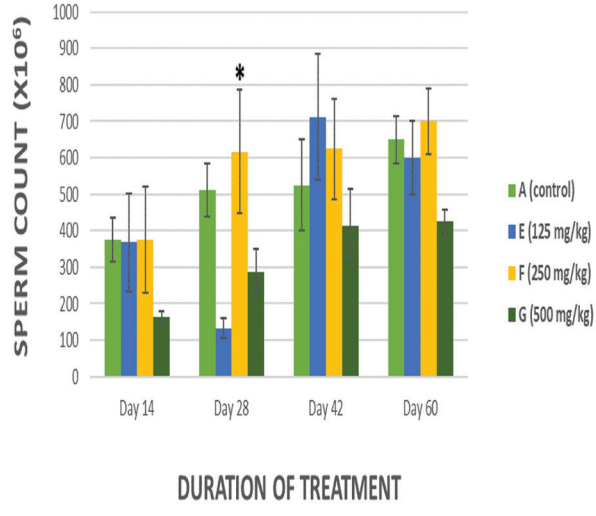

Figure 2. Effect of varied doses of hydrophilic leaf extract of *P. oleracea* on sperm count. Results are given as mean ± SEM for four rats in each group. Experimental groups are compared with Group A (control). *indicates a significant difference at $p < 0.05$.

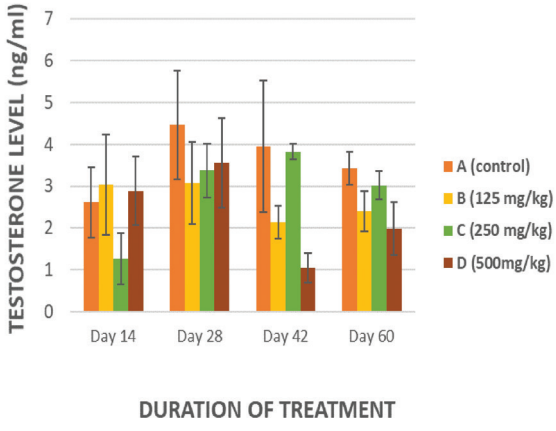

Figure 3. Effect of varied doses of lipophilic leaf extract of *P. oleracea* on testosterone level. Results are given as mean ± SEM for five rats in each group. Experimental groups are compared with Group A (control). No significant difference at 95% confidence interval ($p > 0.05$).

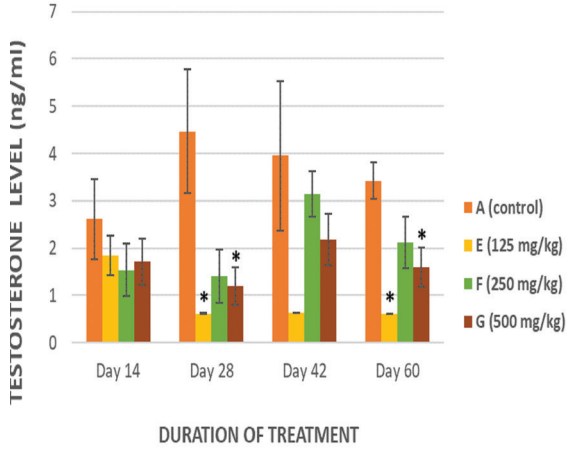

Figure 4. Effect of varied doses of hydrophilic leaf extract of *P. oleracea* on testosterone level. Results are given as mean ± SEM for four rats in each group. Experimental groups are compared with Group A (control). * indicates a significant difference at $p < 0.05$.

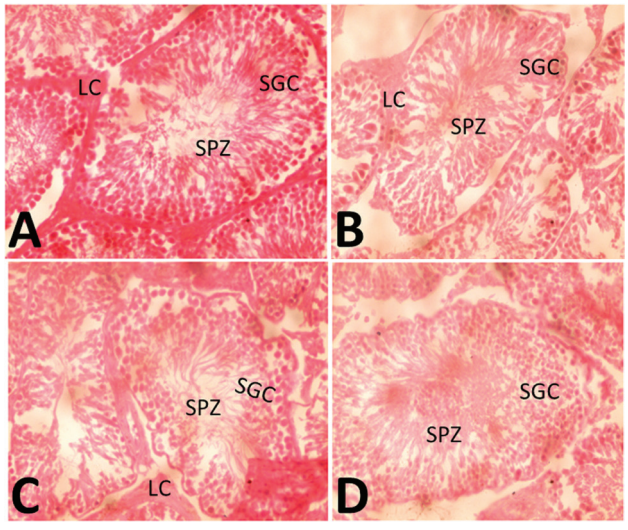

Plate 1. Photomicrographs of testicular sections of rats from Control (A) and lipophilic treated groups B, C, and D (125, 250, and 500 mg/kg doses, respectively) on day 60 of treatment stained with H&E (×400). No abnormality in the testes of treated rats when compared with the control. The interstitial spaces contain the Leydig cells (LC); the seminiferous tubules are filled with spermatogenic cells (SPG) and the lumen contains spermatozoa (SPZ).

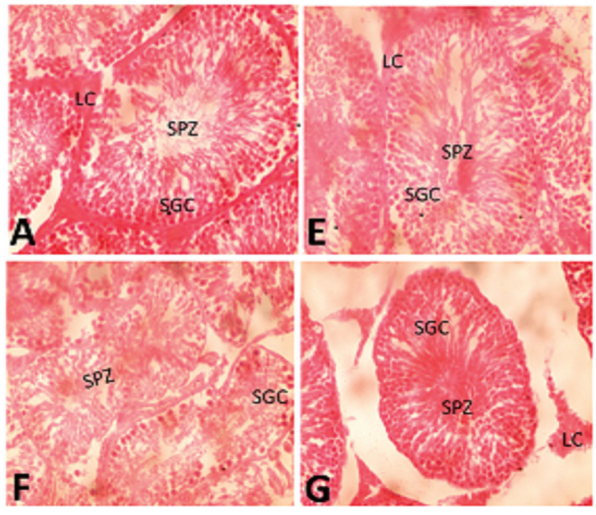

Plate 2. Photomicrographs of testicular sections of rats from Control (A) and hydrophilic extract treatment groups E, F, and G (125, 250, and 500 mg/kg doses, respectively) on day 60 of treatment stained with H&E (×400). No obvious histological change in the testes of treated rats, relative to the control. The interstitial spaces contain the Leydig cells (LC); the seminiferous tubules are filled with spermatogenic cells (SPG) and the lumen contains spermatozoa (SPZ).

extracts are not gonadotoxic even after prolonged exposure of 60 days. Absence of any form of pathology in the testes of treated rats, even on day 60 of treatment, also supports this claim. Both extracts did not significantly alter the sperm motility, viability, and morphology throughout the 60-day duration of treatment. This indicates that the extracts did not evoke any adverse effect on sperm cell characteristics.

Both extracts exhibited the propensity to enhance sperm count at the same dose but at different durations of exposure. From this finding, it can be deduced that the effective dose for both extracts in improving sperm count may be at the dose of 250 mg/kg. The marked rise in the sperm count shows the potential of *P. oleracea* extracts in the management of male fertility, especially as it relates to the sperm count and characteristics. Sperm count and sperm characteristics such as sperm motility, viability, and morphology are key measures of male fertility, as they are the major indicators of testicular spermatogenesis and epididymal sperm maturation [24].

The reduction in testosterone level as recorded in this study may be associated with the hampering of the enzymes/the pathway necessary for its production or the mimicking of the hormone by the extracts. This shows that the extracts likely exerted their effects on the Leydig cells and/or the hypothalamus-pituitary-gonadal axis by inhibiting the secretion of the hormones or altering their regulation [25]. Deformation of Leydig cells can also affect their efficiency in synthesizing testosterone [26].

Correlating testosterone and spermatogenesis, it has been reported that testosterone is essential in males for germ cells maturation, sperm production, and regulation of male fertility in general [27]. From an earlier report, spermatogenesis is known to be essentially dependent on high intra-testicular testosterone concentration stimulated by gonadotropins from the anterior pituitary gland [28]. This intra-testicular testosterone acts on the Sertoli cells to create an environment that enables the normal progression of germ cells through stage VII of the spermatogenic cycle [29]. Thus, testosterone is said to maintain spermatogenesis by aiding the meiotic process [30]. In the absence of testosterone from the Leydig cells or the androgen receptor expressed by the Sertoli cells, spermatogenesis does not progress further than the meiosis stage [31].

It is, however, important to note that the decrease in the testosterone level observed in this study did not impair spermatogenesis, rather it was found that the sperm cell count, sperm characteristics, testicular histomorphology, and relative testicular weights of the test rats were not altered. The reason for this is that the testosterone concentration required to drive spermatogenesis is not usually very high so long as it is within the physiologic range. This explains the fact that even with the significant variation in the testosterone level recorded in this study, spermatogenesis progressed normally. This finding is supported by Castro et al. [32] who found that the lack of association between testosterone level and indices for spermatogenic activity in rabbits agrees with the possibility that "testosterone concentrations need only meet or exceed some minimal threshold level for spermatogenesis to proceed normally." They also found that in spite of the crucial role of testosterone in maintaining the normal spermatogenic process, the increased concentration of testosterone above basal levels had no influence on the spermatogenic efficiency. According to their report, "additional testosterone, under normal conditions, apparently is not capable of improving spermatogenic efficiency in adult rabbit." The present finding is also consistent with that of Zhang et al. [28] who reported that spermatogenesis can be sustained by testosterone concentrations which are obviously below the physiological range and produced constitutively without LH stimulation since very small residual concentration of testosterone in the absence of LH signaling is essential for the post-meiotic stage of spermatogenesis, from round to elongated spermatids. According to McLachlan et al. [33], spermatogenesis was maintained in gonadotropin-depleted rats with low doses of exogenous testosterone.

This finding, however, contrasts those of Abu et al. [34], Madan [35], Reuben et al. [36], and Obinna and Agu [37] where low testosterone levels in different test groups impaired spermatogenesis by causing a decrease in sperm count and motility, increase in the percentage of defective sperm cells, and altered histomorphology of testis and/or epididymis. The reason being that spermatogenesis may have been hampered as a result of reduced testosterone level which may be below the basal level necessary to maintain spermatogenesis in the animal species of those studies. Contrary to our findings, Oyedeji and Bolarinwa [17] demonstrated that aqueous and methanol extracts of stem and leaves of *P. oleracea* suppressed both sperm production and testosterone secretion in test rats. These effects were attributed to the number of mature Leydig cells which according to Gupta et al. [38] directly influences spermatogenesis.

Among the two extracts used in the study, only the hydrophilic extract decreased the testosterone level. This reduction in the testosterone level may be linked with the presence of triterpenoids/steroids in the extract. Plant steroids (phytosteroids) have been implicated in endocrine-disruption in some laboratory animals [39]. They are known to interact with steroid receptors either as an agonist,

antagonist, or mixed agonist/antagonist depending on the compound, the concentration, and the tissue involved in order to interfere with the endocrine system. This role of phytosteroids in endocrine disruption is possible since testosterone is a steroid hormone. Report has shown that the feeding of phytosteroids to male Japanese quail (*Coturnix coturnix japonica*) decreased the production of testosterone by altering gonadotropin-releasing hormone (GnRH) and gonadotropin-inhibitory hormone (GnIH) expression in the brain and gonads [40]. Reduced testosterone concentration and number of Leydig cells, as well as lower expression of 17 β-hydroxysteroid dehydrogenase in the testes have been reported in male Japanese quail fed with phytosteroids [39]. Some phytosteroids impede steroid metabolizing enzymes, through a change in the concentration of endogenous steroid level, resulting in a biological effect [41]. According to Ogbe et al. [42], phytosteroids can alter testosterone metabolism by inhibiting 5-alpha reductase that converts testosterone to dihydrotestosterone. In line with our findings, Awad et al. [43] found that phytosteroids caused a reduction in serum testosterone concentration with no effect on the testes.

Although triterpenoids/steroids were found to be present in both the lipophilic and hydrophilic extracts as seen in the phytochemical screening result (Table 1), their structural modification and the state in which they exist in the two extracts are not the same, a factor which may have contributed to the marked decrease in testosterone level in the hydrophilic but not the lipophilic extract treated rats. *In vivo*, triterpenoids exist in both free state and in bound state, in combination with sugars to form glycosides and esters. Precisely, triterpenoid saponins are glycosides containing a triterpenoid component (aglycone) and a sugar moiety (glycone) [44]. The triterpenoids/steroids in the hydrophilic extract are in their bound form as more polar saponins and cardiac glycoside, which have both hydrophilic glycone and lipophilic aglycone moieties. The presence of these moieties made them amphiphilic compounds [45]. This amphiphilic character of triterpenoids/steroids glycoside derivatives in the hydrophilic extract likely enhanced their systemic circulation, plasma availability, and the delivery to the active site for binding with the receptor, which in turn disrupted the testosterone level.

On the contrary, the triterpenoids/steroids in the lipophilic extract are in the free aglycone state with no amphiphilic property, and as such the transportation and plasma availability of this more lipophilic free aglycone to the active site may have been impeded relative to the more polar saponins and cardiac glycoside in the hydrophilic extract. This offers a rationale for the absence of a significant effect on testosterone concentration.

Saponins are known as surface acting principles in plants, while cardiac glycosides are the cardiotonic principles in plants. However, both have steroids and non-steroidal triterpenoids as their aglycone moiety which made them good natural sources of phytosteroids [46,47].

In conclusion, although there was a decline in testosterone level, lipophilic and hydrophilic leaf extracts of *P. oleracea* improved spermatogenesis by increasing the sperm count at the doses used in this study. We, therefore, recommend that further studies be carried out to identify the mechanism for the reduced serum concentration of testosterone by the extracts.

Acknowledgments

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