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Lipophilic and hydrophilic leaf extracts of *Portulaca oleracea* (Purslane) disrupts female sex hormones in albino rats (*Rattus norvegicus*)

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ABSTRACT

Background and aim: Decoctions and infusions from the aerial parts of *Portulaca oleracea* Linn., especially the leaves and stems, are used by traditional medicine practitioners in Nigeria to enhance fertility in humans. The scarcity of literature on the use of this plant for the said purpose as well as its efficacy prompted this research. Study investigated effect of lipophilic and hydrophilic leaf extracts of *Portulaca oleracea* on oestrous cycle, female sex hormones at various phases of oestrous cycle and ovarian and uterine histomorphology in albino rats.

Experimental procedure: Experimental animals were randomly divided into 7 groups of 5 rats each. Group A (control) received 0.5 ml 20% Tween 80 (vehicle), groups B, C & D received 125, 250 & 500 mg/kg of the lipophilic extract respectively and E, F & G received 125, 250 & 500 mg/kg of the hydrophilic extract respectively for 21 days. Oestrous cycle was assessed daily. At the end, blood samples (for hormones) and ovarian & uterine sections (histoarchitecture) were collected.

Results and conclusion: Both extracts had no significant ($p > 0.05$) effect on oestrous cycle, ovarian & uterine histoarchitecture and female sex hormones except at proestrus phase where significant ($p < 0.05$) decrease in LH and FSH was recorded. *P.oleracea* as used in this study may have deleterious effect on female reproductive system as shown by the disruption of the hormones at proestrus phase. This can form a basis to refute the use of *P.oleracea* leaf extracts in enhancing fertility as it has been shown to affect the gonadotropins involved in folliculogenesis.

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1. 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 some hours on sunny days especially

Abbreviations: LH, luteinizing hormone; FSH, follicle stimulating hormone; LD₅₀, lethal dose 50; NaCl, Sodium chloride; ELISA, enzyme linked immunosorbent assay; CLEPO, Chloroform (Lipophilic) leaf extract of *Portulaca oleracea*; MLEPO, Aqueous methanol (Hydrophilic) leaf extract of *Portulaca oleracea*; SEM, standard error of mean.

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in the mornings, and branched succulent stems which are decumbent near the base.¹ Purslane has 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 *Portulaca oleracea* in folk medicine dates back to ancient times and has been given the name, 'Global Panacea' by the World Health Organization (WHO).⁶ All parts of the plant, especially the leaves and stems, are useful as remedies for many ailments and they are usually used in fresh or dried state. In Nigeria, the plant is used by traditional medicine practitioners in the management of infertility in women where about 500 ml of the decoction or juice is usually administered twice daily for a minimum period of two weeks. 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.

Previous studies had reported that the methanol extract of the aerial parts of *P. oleracea* had no significant effect on oestrous cycle and histomorphology of the ovarian sections⁷ while the chloroform extract had antifertility effect on ovulation by decreasing the number of ova in ovary and also increasing the uterine weight.⁸ The effect of *P. oleracea* on female sex hormones especially at different phases of oestrous cycle is lacking.

This study was therefore designed to investigate the effect of lipophilic and hydrophilic leaf extracts of *Portulaca oleracea* on oestrous cycle and female sex hormones at various phases of oestrus cycle using two extracting solvents - chloroform (lipophilic) and aqueous methanol (hydrophilic) in succession. The findings of this study will serve as guide to either justify or refute the folkloric use of *Portulaca oleracea* leaf in enhancing fertility in humans.

2. Materials and methods

2.1. Plant material and authentication

Fresh leaves of *Portulaca oleracea* were collected from Alakahia axis of Port Harcourt, Nigeria from the months of December 2017 to February 2018. The plant, authenticated by Dr. Chimezie Ekeke of Plant Science Biotechnology Department, University of Port Harcourt, Port Harcourt, Nigeria was placed at the University Herbarium with the voucher no: UPH/V/1302.

2.2. Preparation of the extracts

The collected leaves were dried under a shade at room temperature for a duration of six weeks. The dried leaves of *P. oleracea* were weighed and ground to fine powder. Successive solvent extraction by cold maceration was done for 72 h using two solvents, Chloroform and 80% aqueous methanol. In each case, there was fresh replacement of solvent every 24 h. Extraction solvents were used in the ascending order of polarity (chloroform before aqueous methanol). Briefly, a 4.5 kg portion of dried pulverized *P. oleracea* leaves were soaked in 13.5 Litres of chloroform for 24 h with intermittent stirring. At the expiration of the time, they were stirred and filtered first with a 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 h, followed by filtration with muslin fabric and Whatman's No. 1 filter paper. This resulting marc was soaked again for another 24 h (making a total of 72 h of maceration) and the filtration procedure repeated. The filtrates were combined and concentrated with rotary evaporator (Model No: RE-52A) at 45 °C *in vacuo* and later transferred to an evaporating dish and dried over a water bath set at 45 °C (Digital thermostatic water bath, Jinotech instruments). The chloroform leaf extracts of *Portulaca 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 in a similar manner to obtain the hydrophilic extract. All reagents used were of analytical grades.

2.3. Acute oral toxicity study

The acute oral toxicity study for the lipophilic and hydrophilic leaf extracts of *Portulaca oleracea* were carried out to determine the

LD₅₀ of the extracts using the method of Lorke.⁹

2.4. Animals

Thirty – five (35) female rats weighing an average of 170g and showing regular cycles were used for this study. The animals were procured from the Animal House of Department of Pharmacology, College of Health Sciences, University of Port Harcourt, Nigeria and acclimatized for two (2) weeks before commencing the study. Commercially sourced feed (Top Feeds Nigeria Limited) and clean drinking water were given to the animals, *ad libitum* throughout the study period.

Following acclimatization, the animals were randomly assigned to seven (7) groups of five (5) 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 rats consumed the same volume of 20% tween 80. The treatments were by oral gavage daily for 21 days and were started on the oestrus phase of the cycle.

2.5. Vaginal cytology

The phases of the oestrous cycle of the experimental animals were assessed daily (in the mornings) using the pipette smear technique described by OECD¹⁰ and Obinna & Kagbo.¹¹ Few drops of physiologic saline (0.9% NaCl) contained in a dropping pipette was inserted into the vagina of the animals and used to wash the vaginal walls. The lavage containing cells lining the vaginal wall was released on a grease-free microscope slide and observed under light microscope at 10x objective lens. The phases of oestrous cycle were established depending on the various distinct cells ranging from round and nucleated cells (epithelial cells), irregular anucleated cells (cornified cells) to the small round cells (leucocytes). Proestrus phase was identified by the predominance of epithelial cells; oestrus phase was mainly characterized by cornified cells; metestrus phase described by the presence of leucocytic cells; cornified and/or epithelial cells, and diestrus by mainly leucocytes (Fig. 1).

2.6. Blood collection for hormone analyses

From day 21, blood samples were collected by orbital bleeding technique^{11,12} according to the phases of oestrous cycle in the seven groups. This was done by introducing a microhaematocrit plain tube into orbital plexus of the medial canthus of the eye until a contact was made with the orbital bone; the tube was slightly twisted and removed a little to allow the blood to stream through the tube into the sterile plain bottles. The blood samples were kept stationary to settle for 30–45 min, for coagulation to take place after which they were centrifuged for 15 min at 3000 rev/min in order to harvest the sera. The harvested sera were then put into separate clean bottles, kept in tightly covered microcentrifuge tubes, and stored at –20 °C until analysis. The sera were later subjected to hormonal analysis by ELISA for evaluation of LH, FSH, progesterone and oestrogen levels.

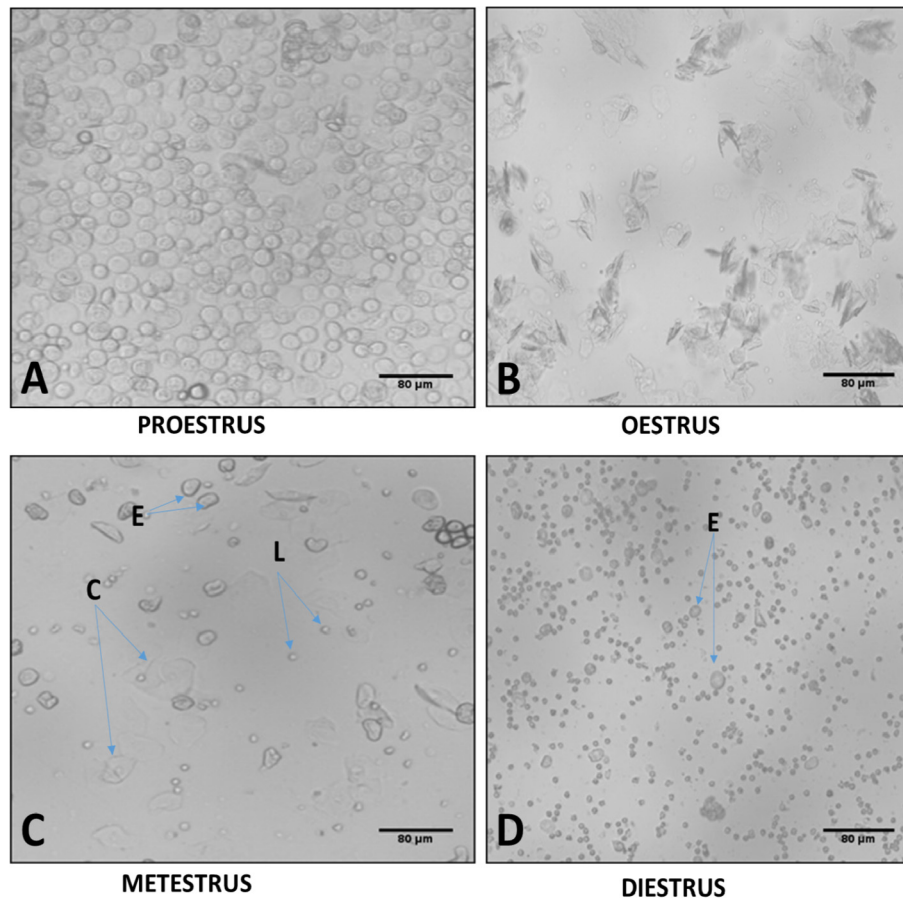


Fig. 1. Photomicrographs of unstained vaginal cells of experimental rats showing different phases of the oestrous cycle. Original magnification, 100x, scale bar = 80 μ m. **A** - Proestrus phase characterized by nucleated epithelial cells - rounded cells. **B** - Oestrus characterized by large non-nucleated cornified cells. **C** – Metestrus characterized by combination of leucocytes (L), epithelial cells (E) and cornified cells (C). **D** – Diestrus characterized by mainly leucocytes with small number of epithelial cells (E).

2.7. Hormonal assay

2.7.1. LH

Serum concentration of Luteinizing Hormone (LH) was determined by a Microplate Enzyme Immunoassay, Colorimetric using Accu-bind ELISA Microwells (Luteinizing Hormone (LH) Test System Product Code: 625–300) from Monobind Inc. Lake Forest, CA 92630, USA.

2.7.2. FSH

Serum concentration of Follicle Stimulating Hormone (FSH) was determined by a Microplate Immunoassay (IEMA/ELISA) using Accu-bind ELISA Microwells (Follicle Stimulating Hormone (FSH) Test System Product Code: 6425–300) from Monobind Inc. Lake Forest, CA 92630, USA.

2.7.3. Progesterone

Serum concentration of progesterone was determined by a Microplate Enzyme Immunoassay, Colorimetric using Accu-bind ELISA Microwells (Progesterone Test System Product Code: 4825–300) from Monobind Inc. Lake Forest, CA 92630, USA.

2.7.4. Oestrogen (Estradiol)

Serum concentration of Estradiol was determined by a Microplate Enzyme Immunoassay using Accu-bind ELISA Microwells (Estradiol (E2) Test System Product Code: 425–300) from Monobind Inc., USA.

2.8. Harvesting of tissue for histomorphology

On the final day of experiment, the animals were anaesthetized with chloroform in a desiccator and dissected. The uterine horns and ovaries were harvested, cleaned and fixed in Bouin's solution for histomorphology.

2.9. Histological study of the tissues

The fixed ovarian and uterine samples were then processed using the method of Lillie.¹³ The tissues were processed with time by dehydration in ascending grades of alcohol, followed with clearing in xylene. After infiltration in paraffin, they were embedded and sectioned at 4–5 μ m, followed by deparaffination 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.

2.10. Statistical analyses

Statistical analyses were done with SPSS 21; the data were represented as mean \pm SEM, and assessed using one-way Analysis of Variance (ANOVA) and Tukey post-hoc test. The significance level was set at $p < 0.05$.

3. Results

3.1. Acute toxicity study

Acute toxicity test did not show any mortality, morbidity or other apparent signs of toxicity at the doses used. With this in mind, 1/40th, 1/20th and 1/10th of this maximum dose (5000 mg/kg) was adopted for the study which gave rise to 125, 250 and 500 mg/kg doses of the extracts used in the treatment groups.

3.2. Effects of *Portulaca oleracea* leaf extracts on oestrous cycle – vaginal cytology

The charts in Fig. 2 summarized the effects of lipophilic and hydrophilic leaf extracts of *Portulaca oleracea* on phases of oestrous cycle of female albino rats treated for 21 days. Both extracts had no significant effect on the phases of the oestrous cycle (Fig. 1) of treated rats in comparison with the control.

3.3. Effects of *Portulaca oleracea* leaf extracts on female sex hormones according to the phases of oestrous cycle

3.3.1. Proestrus phase

Fig. 3A and B shows that treatments with Lipophilic and Hydrophilic leaf extracts of *Portulaca oleracea* resulted in a decline in the mean serum levels of LH at proestrus. However, only the decrease in the 250 mg/kg lipophilic extract treated rats (group C) was significant ($p < 0.05$) in relation to the control. A significant ($p < 0.05$) decrease in mean serum levels of FSH was indicated in the 250 and 500 mg/kg lipophilic extract treated rats (groups C and D respectively) in relation to control. No significant ($p > 0.05$) change was observed in the oestrogen and progesterone levels of both extract treated groups relative to the control at proestrus [Fig. 3C and D)].

Results are given as mean \pm SEM for 5 rats in each group. Experimental groups are compared with Group A (control). * indicates significant difference at $p < 0.05$.

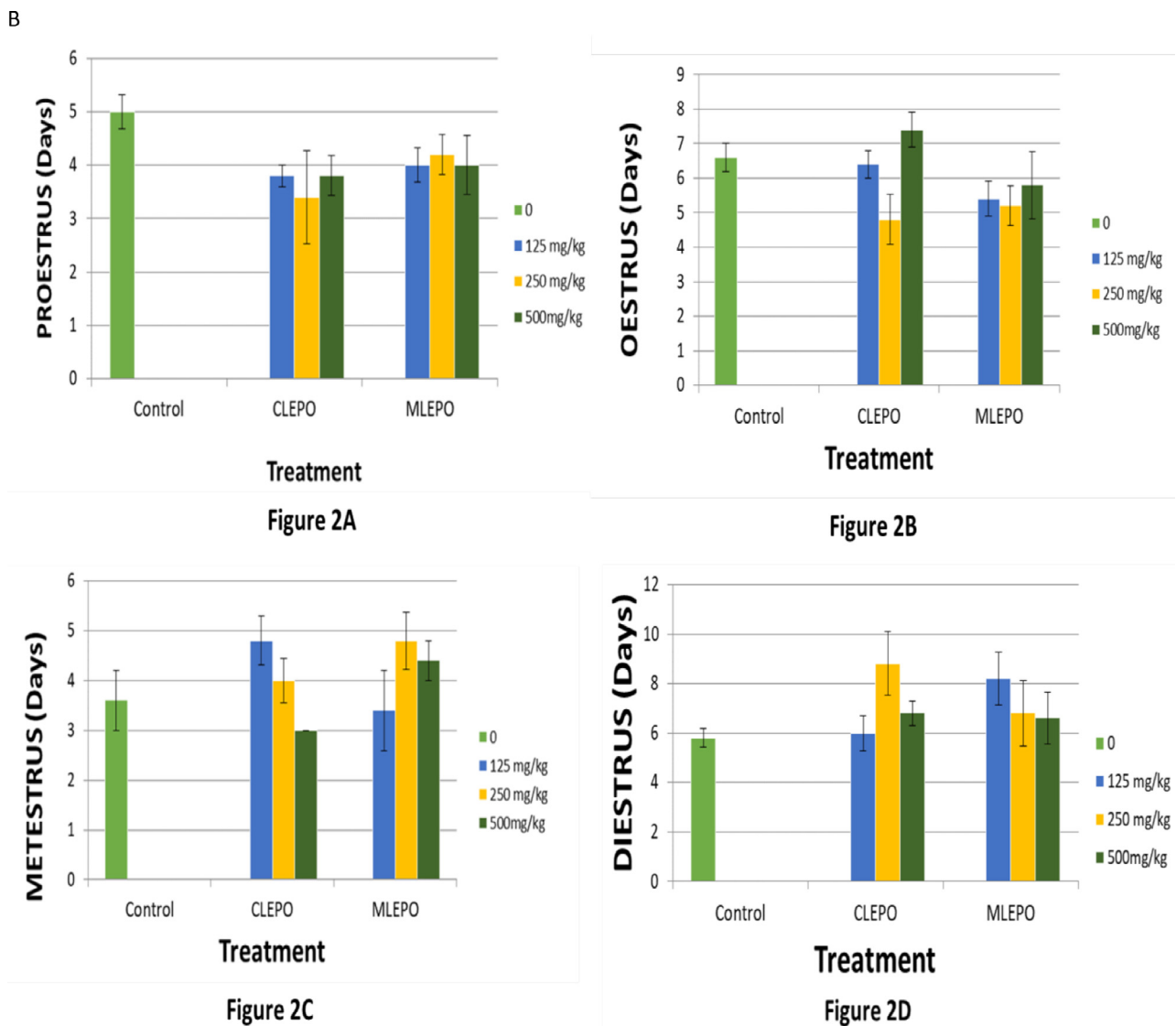


Fig. 2. Effect of Lipophilic and Hydrophilic leaf extracts of *Portulaca oleracea* on phases of oestrous cycle – Proestrus (2A), Oestrus (2B), Metestrus (2C) and Diestrus (2D). Results are given as mean \pm SEM for 5 rats in each group. Experimental groups are compared with Group A (control). No significant difference at 95% confidence interval ($P > 0.05$). **CLEPO** – Chloroform (Lipophilic) leaf extract of *Portulaca oleracea*; **MLEPO** – Aqueous methanol (Hydrophilic) leaf extract of *Portulaca oleracea*.

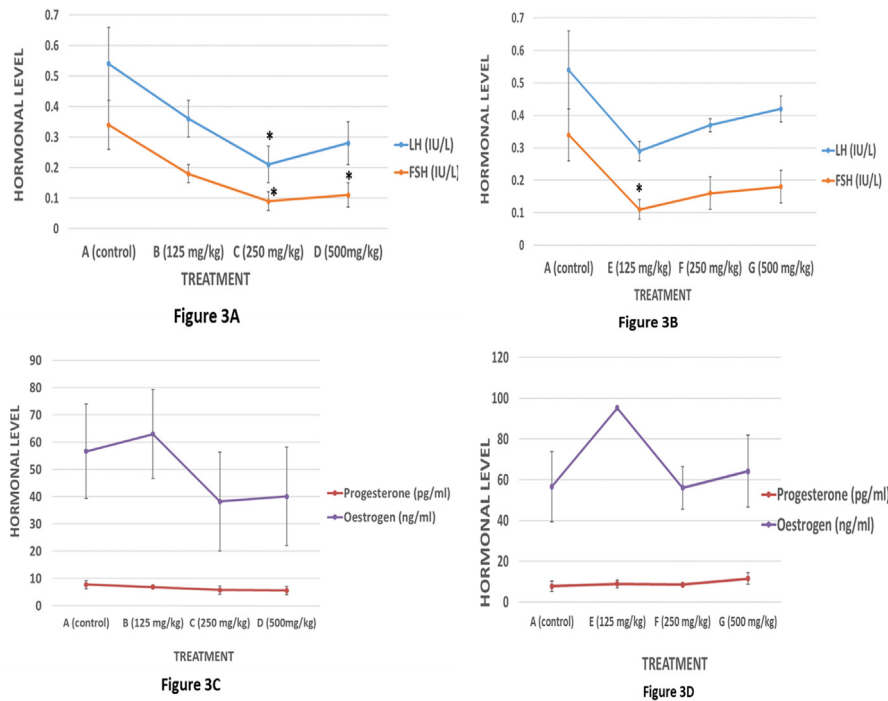


Fig. 3. Effect Lipophilic and Hydrophilic leaf extracts of *Portulaca oleracea* on female sex hormones at proestrus phase of Oestrous Cycle. 3A: Effect of the lipophilic extract on LH and FSH levels. 3B: Effect of the hydrophilic extract on LH and FSH levels. 3C: Effect of the lipophilic extract on progesterone and estrogen levels. 3D: Effect of the hydrophilic extract on progesterone and estrogen levels.

3.3.2. Oestrus phase

There was no variation in the mean serum levels of LH, FSH, progesterone and oestrogen during the oestrus phase in all the Lipophilic and Hydrophilic extracts test groups in comparison with control (Tables 1 and 2).

3.3.3. Metestrus phase

Lipophilic and Hydrophilic leaf extracts of *Portulaca oleracea* had no significant ($p > 0.05$) effect on mean serum levels of LH, FSH, progesterone and oestrogen during the metestrus phase in comparison with the control (Tables 1 and 2).

3.3.4. Diestrus phase

The mean serum levels of LH, FSH, progesterone and oestrogen of all the Lipophilic and Hydrophilic extracts treatment groups showed no significant ($p > 0.05$) change during the diestrus phase

when compared with the control (Tables 1 and 2).

3.4. Effects of *Portulaca oleracea* leaf extracts on ovarian sections

The photomicrographs of ovarian sections from all the lipophilic (B, C and D) and hydrophilic (E, F and G) extract treated rats showed no obvious histological change in comparison with control (A). However, the ovarian sections from 125 mg/kg hydrophilic extract treated rats (group E) showed hypertrophied ovarian follicles (Fig. 4).

3.5. Effect of *Portulaca oleracea* leaf extracts on uterine sections

Photomicrographs of uterine sections of rats from lipophilic and hydrophilic extracts treatment groups showed no pathology relative to those of the control (Fig. 5). From the photomicrographs, the

Table 1

Effect of Lipophilic leaf extract of *Portulaca oleracea* on Female Sex Hormones during the Oestrus, Metestrus and Diestrus Phases of Oestrous Cycle.

Phases of oestrous cycle	Hormones	Groups			
		A (Control)	B (125 mg/kg)	C (250 mg/kg)	D (500 mg/kg)
Oestrus	Luteinizing Hormone (IU/L)	0.46 ± 0.02	1.00 ± 0.61	0.42 ± 0.06	0.46 ± 0.05
	Follicle Stimulating Hormone (IU/L)	0.22 ± 0.06	0.38 ± 0.22	0.19 ± 0.07	0.24 ± 0.08
	Progesterone (pg/ml)	9.00 ± 1.08	7.07 ± 0.90	6.89 ± 1.04	7.18 ± 0.61
	Oestrogen (ng/ml)	55.88 ± 10.94	32.78 ± 8.61	61.34 ± 7.28	89.74 ± 10.06
Metestrus	Luteinizing Hormone (IU/L)	0.41 ± 0.08	0.31 ± 0.02	0.37 ± 0.05	0.26 ± 0.11
	Follicle Stimulating Hormone (IU/L)	0.26 ± 0.05	0.17 ± 0.03	0.20 ± 0.03	0.14 ± 0.06
	Progesterone (pg/ml)	7.86 ± 0.80	9.71 ± 1.08	8.20 ± 0.23	4.01 ± 1.70
	Oestrogen (ng/ml)	44.00 ± 15.52	70.84 ± 11.07	55.82 ± 16.10	39.16 ± 20.37
Diestrus	Luteinizing Hormone (IU/L)	0.36 ± 0.03	0.33 ± 0.04	0.45 ± 0.09	0.36 ± 0.06
	Follicle Stimulating Hormone (IU/L)	0.19 ± 0.02	0.17 ± 0.03	0.26 ± 0.08	0.20 ± 0.04
	Progesterone (pg/ml)	8.25 ± 0.56	8.32 ± 1.02	7.89 ± 1.38	7.15 ± 1.33
	Oestrogen (ng/ml)	47.52 ± 13.49	34.38 ± 8.69	45.82 ± 11.73	34.56 ± 15.22

Results are given as mean ± SEM for 5 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 2
Effect of Hydrophilic leaf extract of *Portulaca oleracea* on Female Sex Hormones during the Oestrus, Metestrus and Diestrus Phases of Oestrous Cycle.

Phases of oestrous cycle	Hormones	Groups			
		A (Control)	E (125 mg/kg)	F (250 mg/kg)	G (500 mg/kg)
Oestrus	Luteinizing Hormone (IU/L)	0.46 ± 0.02	0.44 ± 0.09	0.51 ± 0.05	0.38 ± 0.07
	Follicle Stimulating Hormone (IU/L)	0.22 ± 0.06	0.20 ± 0.07	0.23 ± 0.07	0.18 ± 0.08
	Progesterone (pg/ml)	9.00 ± 1.08	5.77 ± 0.65	8.71 ± 0.19	8.75 ± 0.67
Metestrus	Oestrogen (ng/ml)	55.88 ± 10.94	45.42 ± 7.20	76.16 ± 15.11	94.74 ± 42.42
	Luteinizing Hormone (IU/L)	0.41 ± 0.08	0.39 ± 0.06	0.51 ± 0.03	0.43 ± 0.05
	Follicle Stimulating Hormone (IU/L)	0.26 ± 0.05	0.20 ± 0.04	0.31 ± 0.03	0.24 ± 0.05
Diestrus	Progesterone (pg/ml)	7.86 ± 0.80	9.68 ± 1.54	8.32 ± 1.14	11.51 ± 1.94
	Oestrogen (ng/ml)	44.00 ± 15.52	55.42 ± 11.49	26.62 ± 7.93	62.88 ± 13.88
	Luteinizing Hormone (IU/L)	0.36 ± 0.03	0.45 ± 0.09	0.32 ± 0.09	0.30 ± 0.05
	Follicle Stimulating Hormone (IU/L)	0.19 ± 0.02	0.23 ± 0.06	0.18 ± 0.06	0.15 ± 0.03
	Progesterone (pg/ml)	8.25 ± 0.56	5.99 ± 0.73	6.65 ± 1.73	8.80 ± 0.84
	Oestrogen (ng/ml)	47.52 ± 13.49	33.26 ± 8.12	32.66 ± 10.80	47.24 ± 6.90

Results are given as mean ± SEM for 5 rats in each group. Experimental groups are compared with Group A (control). No significant variation exists across the table at 95% confidence interval ($P > 0.05$).

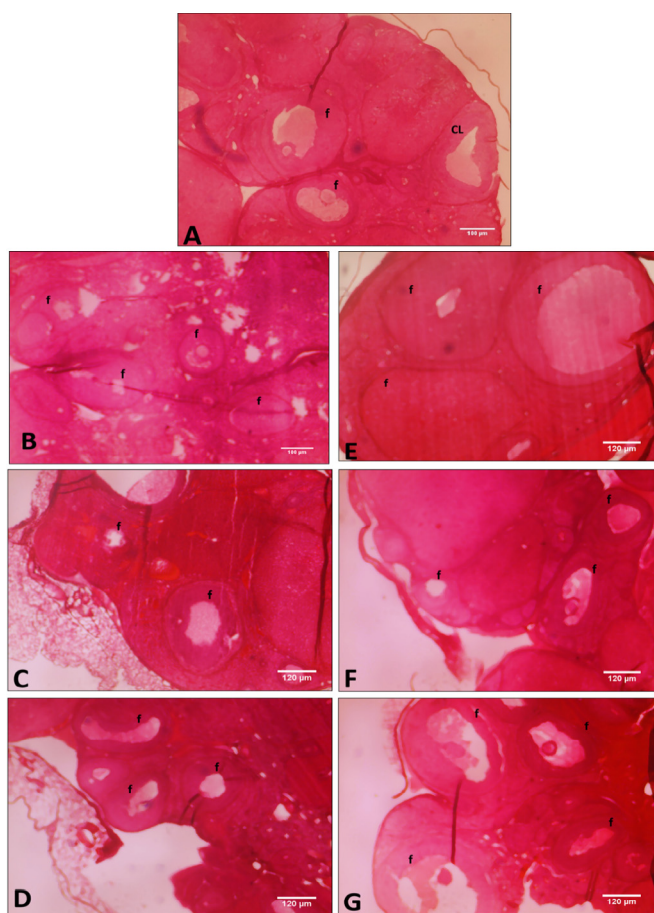


Fig. 4. Photomicrographs of ovarian sections of rats from Control (A), lipophilic extract treatment groups B, C and D (125, 250 and 500 mg/kg doses respectively) and hydrophilic extract treatment groups E, F and G (125, 250 and 500 mg/kg doses respectively) after 21 days of treatment. Original magnification, x100, scale bar (A & B) = 100 μ m and (C, D, E, F & G) = 120 μ m. **A** - normal histoarchitecture of a cycling ovary characterized by ovarian follicles (f) at different stages of development with corpus luteum (CL). **B** - numerous ovarian follicles (f) in the cortical region. **C** - some ovarian follicles (f) in the cortical region. **D** - several ovarian follicles (f) in the cortical region; some of which are corpus luteum. **E** - ovarian cortical region showing hypertrophied ovarian follicles (f). **F** - ovarian cortical region contains some ovarian follicles (f). **G** - Presence of numerous ovarian follicles (f) in the ovarian cortex.

simple columnar epithelial cells were seen lining the luminal border of the uterine cavity.

No obvious histological change in the uterus of treated rats, relative to the control. Presence of uterine glands (UG) and simple columnar epithelial cells (EC) lining the luminal border of the uterine cavity.

4. Discussion

This study shows that the normal pattern of oestrous cycle was not significantly altered by both lipophilic and hydrophilic extracts of *P.oleracea*. This result agrees with the findings of Oyedeji & Bolarinwa⁷ which reported that methanol extract of stem and leaves of *P. oleracea* had no significant effect on oestrus cycle of rats during a 21-day treatment at the dose of 75 mg/kg. It however, contrasted with the report of Nayaka et al.⁸ who demonstrated that the chloroform extract of aerial parts of *P. oleracea* produced a dose dependent significant increase in diestrus stage of treated rats at the doses 250 and 500 mg/kg for 14 days.

The uterine histopathological examination did not present any overt abnormalities in all the groups. The histoarchitecture of ovaries of rats in different groups, which indicated the presence of follicle in their different stages of development and the corpora lutea, did not show any abnormality in these organs. However, ovaries of rats from hydrophilic extract group (low dose) were hypertrophied. This suggests that the hydrophilic extract may have a stimulatory effect on the ovarian follicles than the lipophilic extract.

This study also showed that both lipophilic and hydrophilic extracts of *P.oleracea* had no significant effect on the serum sex hormones during the different phases of oestrous cycle except on the LH and FSH concentrations where significant decrease was detected only at proestrus. The reduction observed in the serum concentration of these gonadotropins (FSH and LH) in the treated rats shows that these extracts of *P.oleracea* may have likely exerted their effect on the anterior pituitary, the site of gonadotropin synthesis and secretion. From this finding, the decline in the serum level of FSH by the extracts may inhibit folliculogenesis and maturation of the follicles, an activity associated with proestrus phase of oestrous cycle. Similarly, the decline in serum LH concentration may possibly inhibit ovulation since it has been reported that a pre-ovulatory surge of LH is a pre-requisite for ovulation to occur in a normal cycling animal.¹⁴ Sequel to these findings, it can be inferred that the significant decrease in FSH concentration during the proestrus phase gave rise to a corresponding reduction in LH, oestrogen and progesterone levels at that phase; although

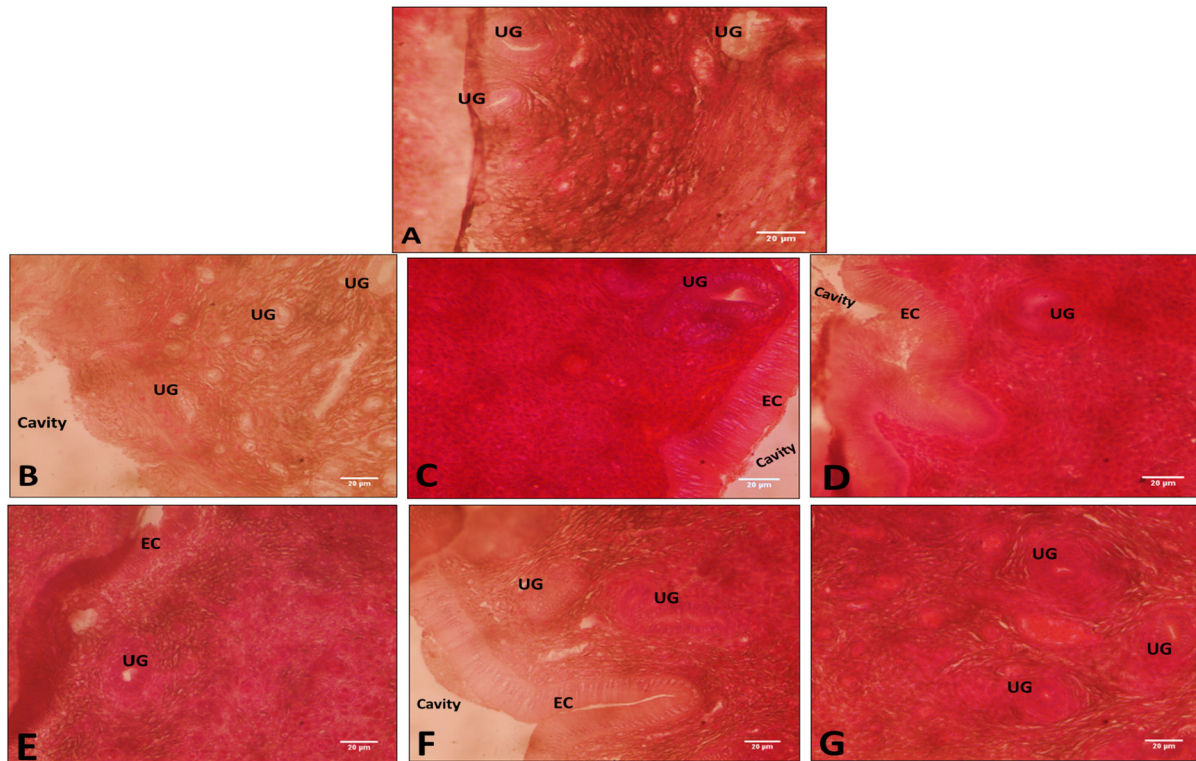


Fig. 5. Photomicrographs of uterine sections of rats from Control (A), lipophilic extract treatment groups B, C and D (125, 250 and 500 mg/kg doses respectively) and hydrophilic extract treatment groups E, F and G (125, 250 and 500 mg/kg doses respectively) after 21 days of treatment. Original magnification, 400x, scale bar = 20 µm.

the decrease in oestrogen and progesterone concentrations were not significant.

Generally, during the growth and maturation of follicles which occurs at proestrus, there is usually a rise in FSH concentration which triggers the development and maturation of follicles in animals. The theca cells of these follicles synthesize androgens which are converted to the oestrogen in the granulosa cells by the enzyme, aromatase. As the level of oestrogen increases, inhibition of FSH occurs with an attendant stimulation of LH release.¹⁵ The residual FSH together with the pre-ovulatory surge of LH triggers ovulation, after which the LH also stimulates the development of corpus luteum from the ruptured follicle by luteinization of the granulosa (a process by which the granulosa is converted from oestrogen secretion to progesterone secretion). The cells of the corpus luteum secrete large quantity of progesterone and little of oestrogen which accounts for the high level of progesterone associated with the luteal phase.

In our previous studies where the preliminary phytochemical screening of lipophilic and hydrophilic leaf extracts of *P. oleracea* were carried out, it was found that the lipophilic extract could not dissolve in water due to its highly lipophilic nature, and as such allowed for very few tests, out of which only the test for triterpenoids/steroids was positive.¹⁶ The hydrophilic extract, on the other hand, showed the presence of alkaloids, carbohydrates, saponins, triterpenoids/steroids, anthraquinone, and cardiac glycosides.¹⁷ The decline in the concentration of gonadotropins as recorded in this study may not be unconnected with the presence of triterpenoids/steroids in the extracts, which result is similar to our earlier finding where the hydrophilic leaf extract of *P. oleracea* decreased the testosterone level in male albino rats.¹⁶ According to Qasimi et al.,¹⁸ phytoosteroids (plant steroids) have been shown to be associated with endocrine-disruption in laboratory animals.

Contrary to our findings on the female sex hormones,

Ahangarpour et al.¹⁹ demonstrated that ethanol extract of aerial parts of *P. oleracea* did not significantly affect sex hormones of female rats although the phase of oestrous cycle when the samples were collected was not stated.

5. Conclusion

Lipophilic and hydrophilic leaf extracts of *P. oleracea* did not disrupt the oestrous cycle, the ovarian and uterine histo-architecture as well as the ovarian hormones (oestrogen and progesterone) all through the study. The anterior gonadotropins (FSH and LH) were decreased only at proestrus with no variation in their concentration during oestrous, metestrus and diestrus phases. This shows that the extracts may have affinity for the anterior pituitary which secretes the gonadotropins. This action on the anterior pituitary may be transient such that the exposure duration of 21 days which had a significant effect on the anterior pituitary hormones may be rather too short to show any significant effect on the ovarian follicles and duration of oestrous cycle.

From the findings of this study, it may be concluded that the lipophilic and hydrophilic leaf extracts of *P. oleracea* may have deleterious effect on female reproductive system evidenced by the disruption of the hormonal interplay at proestrus. This can form a basis to refute the use of *P. oleracea* leaf extracts in enhancing fertility as it has been shown to affect the gonadotropins involved in folliculogenesis. Further studies are however recommended to identify the mechanism for the disruption in the secretion of the gonadotropins at proestrus and to isolate the active compound(s) implicated in the leaf extracts of this plant in this regard.

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Authors' contribution

V.C. Obinna designed and carried out the study, performed the statistical analysis and wrote the manuscript. H.D. Kagbo and G.O. Agu supervised the study, managed the analyses of the study and proofread the manuscript. All Authors read and approved the final manuscript.

Declaration of interest

None.

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