

Evaluation of the Reproductive Functions of *Portulaca Oleracea* Extracts in Female Albino Rats

¹K.O. Oyedeji and ²A.F. Bolarinwa

¹Department of Physiology, Faculty of Basic Medical Sciences,
Ladoke Akintola University of Technology, P.M.B. 4000, Ogbomosho, Nigeria

²Department of Physiology, College of Medicine,
University of Ibadan, Ibadan, Nigeria

Abstract: The aim of this study was to investigate the effects of aqueous and methanolic extracts of *Portulaca oleracea* designated as AEPO and MEPO, respectively on estrous cycle and histopathology of the ovaries and uteri in female Albino rats. Treatments of rats for 21 days with 75 mg kg⁻¹ BW AEPO produced no significant ($p>0.05$) change in the duration of all the phases of estrous cycle. Likewise, treatment of rats for 21 days with 75 mg kg⁻¹ BW MEPO produced no significant ($p>0.05$) change in the duration of all the phases of estrous cycle. Treatment of rats for 25 days with 75 mg kg⁻¹ BW AEPO and MEOP produced no significant ($p>0.05$) change in the ovarian and uterine weights of the treated rats relative to the control. Treatment of rats for 25 days with 75 mg kg⁻¹ BW AEPO and MEPO induced no pathologic lesions or effects in both the ovaries and uteri of the treated rats. These findings indicate that AEPO and MEPO have no deleterious effects on the reproductive functions of female Albino rats.

Key words: *Portulaca oleracea*, estrus cycle, ovaries, uteri, Nigeria

INTRODUCTION

Portulaca oleracea belongs to the family of Portulacaceae. It is commonly called purslane in English language, babbajibi in Hausa language and esan omode or papas an in Yoruba language. It is a fleshy annual herb, much-branched and attaining 30 cm long (Burkill, 1997).

It is used medicinally in Ghana for heart-palpitations (Johnson, 1997). The plant is used as a diuretic in Nigeria (Ainslie, 1937). A tisane of the plant is drunk in Trinidad as a vermifuge (Wong, 1976).

It has been reported that aqueous and methanolic extracts of *Portulaca oleracea* have contractile effects on isolated intestinal smooth muscle in *in vitro* preparations (Oyedeji *et al.*, 2007).

The extracts of *Portulaca oleracea* have been reported to have protective effects on hypoxic nerve tissue (Wang *et al.*, 2007), anti-inflammatory effects (Xiang *et al.*, 2005) and wound-healing activity (Rashed *et al.*, 2003).

This study aims to investigate the effects of aqueous and methanolic extracts of *Portulaca oleracea* on female reproductive functions in Albino rats.

MATERIALS AND METHODS

Experimental animals: Adult female Albino rats weighing between 180 and 200 g bred in the pre-Clinical Animal House of the College of Medicine, University of Ibadan, were used. They were housed under standard laboratory conditions with a 12 h daylight cycle and had free access to feed and water; they were acclimatized to laboratory condition for 2 weeks before the commencement of the experiments.

Plant materials: Fresh specimens of *Portulaca oleracea* were collected from the Botanical Garden of the Forestry Research Institute of Nigeria, Jericho, Ibadan and was authenticated in the taxonomy unit of the above named institute where a voucher specimen (No. FHI 108334) was deposited.

Preparation of the extracts: Large quantity (2 kg) of the fresh specimens of *Portulaca oleracea* were washed free of soil and debris and the roots were separated from the leaves and stems. The leaves and stems were air-dried for 6 weeks and the dried specimens were pulverished using laboratory mortar and pestle and the divided into two samples A and B.

Aqueous Extract of *Portulaca Oleracea* (AEPO):

Weighted portions (431.33 g) of sample A were macerated and extracted with distilled water (1:2 weight/vol.) for 72 h at room temperature (26-28°C). The resulting solution was then filtered using a wire-gauze and a sieve with tiny pores (0.25 mm). The distilled water was later evaporated using steam bath to give a percentage yield of 11.8% of the starting material.

Methanolic Extracts of *Portulaca Oleracea* (MEPO):

Weighted portion (420.52 g) of sample B were macerated and extracted with 70% methanol (1:2 weight/vol.) for 72 h at room temperature (26-28°C). The resulting solution was then filtered using a wire-gauze and a sieve with tiny pores (0.25 mm). The 70% methanol was later evaporated using steam bath to give a percentage yield of 10.2% of the starting material.

A total of 10 g of AEPO and MEPO were dissolved in 100 mL of distilled water to give a concentration of 0.1 g/mL.

The dosages of AEPO and MEPO administered in these studies were in accordance with those reported by Miladi-Gorgi *et al.* (2004).

Studies on the reproductive functions of female Albino rats

Study of estrous cycle: A total of 10 matured female rats weighing between 180-200 g were randomly divided into two groups (I and II) with each group consisting of 5 rats. Vaginal smear was examined microscopically everyday at a constant interval of 7.00-8.00 a.m. for 21 days. The smears were classified into one of the phases of estrous cycle using the Papanicolaou's staining technique modified by Oyediji and Bolarinwa. The proportion among the types of cells recognized were used to determine the phases of the estrous cycle according to Long and Evans (1922). The duration of the estrous cycle were determined. Then, Groups I and II rats received 75 mg kg⁻¹ BW of AEPO and 75 mg kg⁻¹ BW of MEPO, respectively for 21 days. Vaginal smears were evaluated similarly both during the administration of the extracts and for 21 days after cessation of dosing with the extracts. In this study, the experimental animal also served as the control.

Determination of the phases of estrous cycle

Collection of vaginal smear: Vaginal swab stick was used for smear collection from the vaginal lumen by introducing the swab stick gently into the vaginal and gently rotating it along the floor of the lateral walls of the vaginal. The swab stick was then rotated or smeared in duplicate on a microscope slide and the slide was stained using the Papanicolaou's staining technique modified by Oyediji and Bolarinwa.

Table 1: Papanicolaou's staining procedure modified by Oyediji and Bolarinwa

No. of coplin jar	Reagents	Duration (min)
1	95% Alchol	15
2	Distilled water	2
3	Harris Hematoxylin	3
4	Distilled water	2
5	95% Alchol	3
6	95% Alchol	3
7	EA ₅₀	3
8	95% Alchol	3
9	95% Alchol	3
10	OG ₆	3
11	95% Alchol	3
12	95% Alchol	3
13	Absolute alchol	3
14	Xyline (after drying properly)	3-10

Papanicolaou's staining technique: Each slide was placed in the reagent contained in each of the Coplin jars for the specific length of time in the order shown in the Table 1.

Histopathological study: A total of 15 matured Albino rats (180-200 g) showing at least three regular 4-5 day cycles were divided into three groups (n = 5). The different groups received the following doses of the extracts and vehicle (control) orally per day for 25 days as follows:

- Group I received 75 mg kg⁻¹ of AEPO
- Group II received 75 mg kg⁻¹ of MEPO
- Group III received 0.5 mL of distilled water was the control group

On the 26th day, all the rats were sacrificed by an overdose of diethyl ether vapour. The ovaries and uteri were dissected out, cleaned of fat, blotted with filter papers, weighed quickly on a sensitive balance and then fixed in Bouin's fluid. The tissues were then processed histologically as described in this study.

Ovarian and uterine histology: After weighing the ovaries and uteri, they were immediately fixed in Bouin's fluid for 12 h and the Bouin's fixative was washed from the samples with 70% alcohol. The tissues were then cut in slabs of about 0.5 cm transversely and the tissues were dehydrated by passing through different grades of alcohol: 70% alcohol for 2 h, 95% alcohol for 2 h, 100% alcohol for 2 h and finally 100% alcohol for 2 h. The tissues were then cleared to remove the alcohol, the clearing was done for 6 h using xylene. The tissues were then infiltrated in molten paraffin wax for 2 h in an oven at 57°C, thereafter the tissues were embedded. Serial sections were cut using rotary microtome at (5 µm). The satisfactory ribbons were picked up from a water bath (50-55°C) with microscope slides that had been coated on one slide with egg albumin as an adhesive and the slides were dried in an oven. Each

section was deparaffinised in xylene for 1 min before immersed in absolute alcohol for 1 min and later in descending grades of alcohol for about 30 sec each to hydrate it. The slides were then rinsed in water and immersed in alcoholic solution of hematoxylin for about 18 min. The slides were rinsed in water, then differentiated in 1% acid alcohol and then put inside a running tap water to blue and then counterstained in alcoholic eosin for 30 sec and rinsed in water for a few seconds, before being immersed in 70, 90% and twice in absolute alcohol for 30 sec each to dehydrate the preparations. The preparations were cleared of alcohol by dipping them in xylene for 1 min. Each slide was then cleaned, blotted and mounted with DPX and cover slip and examined under the microscope. Photomicrographs were taken at x40, x100 and x400 magnifications.

Statistical analysis: The mean and Standard Error of Mean (SEM) were calculated for all values. Comparison between the control and experimental groups was done using one-way Analysis of Variance (ANOVA) with Least Significant Difference (LSD). Differences were considered statistically significant at $p < 0.05$.

RESULTS

Treatment of rats for 21 days with 75 mg kg^{-1} BW AEPO produced no significant ($p > 0.05$) change in the duration of all the phases of estrous cycle relative to the pre-treatment (before treatment) as shown in Table 2. However, withdrawal of the treatment (post-treatment) for 21 days produced a significant ($p < 0.05$) decrease in the proestrous phase and a significant ($p < 0.05$) increase in the estrous phase relative to the pretreatment period.

Table 3 shows that treatment of rats for 21 days with 75 mg kg^{-1} BW MEPO produced no significant ($p > 0.05$) change in the duration of all the phases of estrous cycle relative to the pre-treatment. However, withdrawal of treatment (post-treatment) for 21 days produced a significant ($p < 0.05$) decrease in the proestrous phase and a significant ($p < 0.05$) increase in the metestrous phase relative to the pre-treatment period.

Table 4 and 5 show that treatment of rats for 25 days with 75 mg kg^{-1} BW AEPO and MEPO produced no significant ($p > 0.05$) change in the uterine and ovarian weights of the treated rats relative to the control.

Figure 1 shows the transverse section through the ovary of control rats given 0.5 mL of distilled water for 25 days with normal ovarian size and fibrohemorrhagic stroma. About 3 follicles were at different stages of maturation with one having a prominent oocyte.

Table 2: Effect of 75 mg kg^{-1} BW AEPO on estrous cycle

Phases	Before treatment	During treatment	Post-treatment
Proestrous	9.25±2.84	6.75±0.75	3.50±1.04*
Estrous	5.25±1.55	8.50±1.26	10.80±2.75*
Metestrous	3.00±0.58	2.75±0.25	3.25±0.95
Diestrous	2.50±0.65	2.50±0.65	3.25±1.11

Table 3: Effects of 75 mg kg^{-1} BW MEPO on estrous cycle

Phases	Before treatment	During treatment	Post-treatment
Proestrous	7.75±1.89	6.25±0.25	3.00±0.41*
Estrous	5.25±1.38	7.75±0.85	8.50±0.50
Metestrous	1.50±0.65	2.75±0.48	4.25±0.48*
Diestrous	5.00±2.42	2.00±0.91	5.00±0.40

*Significant difference from before treatment

Table 4: Effects of AEPO and MEPO on ovarian weights

Parameter	Control	BW AEPO	BW MEPO
		-----75 mg kg ⁻¹ -----	
Weights (g)	0.046±0.005	0.046±0.006	0.028±0.009

Table 5: Effects of AEPO and MEPO on uterine weights

Parameter	Control	BW AEPO	BW MEPO
		-----75 mg kg ⁻¹ -----	
Weights (g)	0.40±0.07	0.61±0.15	0.51±0.17

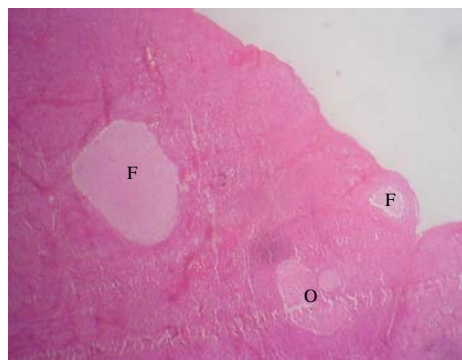


Fig. 1: Photomicrograph of rat's ovary treated with 0.5 mL of distilled water for 25 days showing a normal sized ovary with Follicles (F) and Oocyte (O) at different stages of maturation (x100)

Figure 2 shows the transverse section through the ovary of rat treated with 75 mg kg^{-1} BW AEPO for 25 days with normal ovarian size and fibrohemorrhagic stroma with dilated blood filled vascular channels. A follicle lacking oocyte was seen undergoing maturation.

Figure 3 shows that transverse section through the ovary of rat treated with 75 mg kg^{-1} BW MEPO for 25 days with normal ovarian size and fibrohemorrhagic stroma with a cystically dilated follicle preparatory to ovulation.

Figure 4 shows the transverse section through the uterus of control rats given 0.5 mL of distilled water for 25 days with normal uterine size with a normal thickness (10 cell layer) of the endometrium and myometrium.

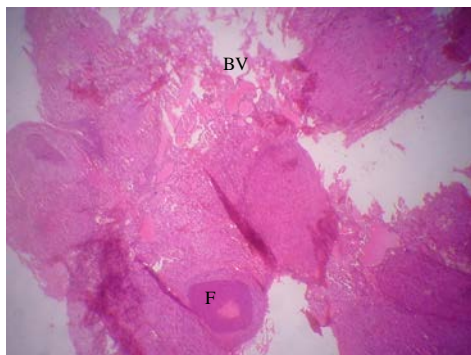


Fig. 2: Photomicrograph of rat's ovary treated with 75 mg kg⁻¹ BW AEPO for 25 days showing a normal sized ovary with Blood Vessel (BV) and a maturing Follicle (F) (x100)

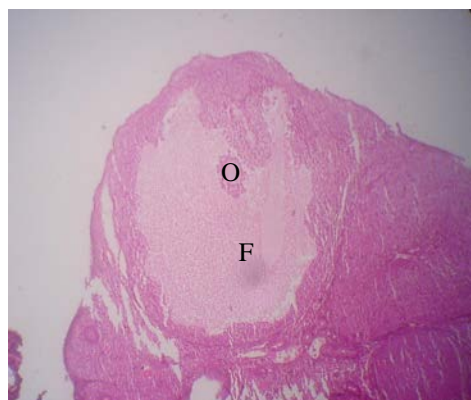


Fig. 3: Photomicrograph of rat's ovary treated with 75 mg kg⁻¹ BW MEPO for 25 days showing an ovarian Follicle (F) with the Oocyte (O) (x100)



Fig. 4: Photomicrograph of rat's uterus treated with 0.5 mL distilled water for 25 days showing normal sized Endometrium (E) and Myometrium (M) (x100)

Figure 5 shows the transverse section through the uterus of rat treated with 75 mg kg⁻¹ BW AEPO for



Fig. 5: Photomicrograph of rat's uterus treated with 75 mg kg⁻¹ BW AEPO for 25 days showing atrophied Endometrial (E) and Myometrial (M) layers (x100)



Fig. 6: Photomicrograph of rat's uterus treated with 75 mg kg⁻¹ BW MEPO for 25 days showing slightly hypertrophied Endometrium (E) and Myometrium (M) (x100)

25 days presenting with smaller uterine size with atrophied endometrial and myometrial tissues. Figure 6 shows the transverse section through the uterus of rat treated with 75 mg kg⁻¹ BW MEPO for 25 days presenting with normal uterine size with slightly hypertrophied endometrium and myometrium.

DISCUSSION

Table 1 and 2 shows that treatment of rats for 25 days with 75 mg kg⁻¹ AEPO and MEPO produced no significant ($p > 0.05$) changes in the duration of all the phases of the estrous cycle compared to the pre-treatment period and this suggests that the extracts (AEPO and

MEPO) did not cause an imbalance of the ovarian and extraovarian hormones, since it has been reported that imbalance in these hormones leads to irregularity in the ovarian functions and duration of the estrous cycle (Circosta *et al.*, 2001).

However, the post-treatment period of the AEPO showed a significant decrease in the duration of the proestrous phase and a significant increase in the duration of the estrous phase relative to the pre-treatment period. Also, the post-treatment period of the MEPO showed significant decrease in the duration of the proestrous phase and a significant increase in the duration of the metestrous phase relative to the pre-treatment period. These post-treatment effects of AEPO and MEPO on the different phases of the estrous cycle might be due to non-total renal clearance of AEPO and MEPO leading to their accumulation in the ECF with a resultant potentiation of their biological activities. The post-treatment significant decrease in the duration of the proestrous phase induced by 75 mg kg⁻¹ BW AEPO indicates that maturation of the follicle in the preovulatory phase was hastened, leading to maturation of the graafian follicle, while the significant increase in the duration of the estrous phase indicates the availability of matured graafian follicle which leads to ovulation. The post-treatment significant ($p < 0.05$) increase in the duration of the metestrous phase induced by 75 mg kg⁻¹ BW MEPO indicates the availability of matured graafian follicle.

The ovary can be considered as an aggregate of three endocrine tissues; the stroma, the follicle and the corpus luteum. The weights of these tissues constitutes the net weight of the ovary. During the estrous cycle the weight of the ovarian tissue increases under the influence of gonadotrophic and steroidal hormones. The non-significant change in the weights of ovaries of rats treated with 75 mg kg⁻¹ BW of AEPO and MEPO relative to the control could indicate normalcy in the activity of the stroma, the follicle and the corpus luteum of the ovary which suggests the availability of gonadotrophic or steroidal hormones or both (Shivalingappa *et al.*, 2002).

The non-significant change in the uterine weights of rats treated with 75 mg kg⁻¹ BW of AEPO and MEPO relative to the control could be due to the absence of estrogenic substance in the extracts (AEPO and MEPO), since it has been reported that estrogenic substance increase the wet weight of uterus (Turner, 1971).

Figure 2 and 3 show the photomicrographs of the ovaries of rats treated with 75 mg kg⁻¹ BW of AEPO and MEPO, respectively. When compared with Fig. 1, they showed a normal ovarian size with fibrohemorrhagic stroma with follicles at different stages of maturation and there were no pathologic lesions of effects which suggests the non-toxic effects of AEPO and MEPO on the

ovaries. Figure 5 and 6 show the photomicrographs of the uteri of rats treated with 75 mg kg⁻¹ BW of AEPO and MEPO, respectively. When compared with Fig. 4, Fig. 5 presents with smaller uterine size and an atrophied endometrial and myometrial tissues, these effects suggest that the rat was in the late diestrous phase of estrous cycle. Also, there were no pathologic lesions on the ovary which suggests the non-toxic effect of AEPO on the ovary. When compared with Fig. 4, Fig. 6 presents with slightly hypertrophied endometrium and myometrium, these effects suggest the rat was in the estrous or early diestrous (luteal phase) phase of the estrus cycle; there were also no pathologic lesions on the uterus which suggests the non-toxic effect of MEPO on the uterus.

CONCLUSION

In this study, the results shows that treatment with AEPO and MEPO caused no significant change in the duration of the different phases of estrous cycle, caused no significant change in the weights of the ovaries and uteri and have no pathologic effects on the ovaries and uterus. These findings indicate that AEPO and MEPO have no deleterious effects on the reproductive functions of female Albino rats and this could be the reason why the plant (*Portulaca oleracea*) is taken along with other ingredients as an aid to the development of foetus by the local people living near Benin City (Nigeria) (Vermeer, 1976).

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