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Research article

Effects of Extracts of *Portulaca oleracea* on Reproductive Functions in Female Albino Rats

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ABSTRACT: The effects of aqueous (AEPO) and methanolic (MEPO) extracts of *Portulaca oleracea* were investigated on estrous cycle and histopathology of the ovaries and uteri in female albino rats. Treatments of rats for 21 days with 75mg/kg 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 75mg/kg BW MEPO produced no significant change in the duration of all the phases of estrous cycle. Treatment of rats for 25 days with 75mg/kg BW AEPO and MEOP produced no significant (P>0.05) changes in the ovarian and uterine weights of the treated rats relative to the control. Also both extracts did not induce noticeable 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.

Keywords: *Portulaca oleracea*, estrus cycle, ovaries, uteri.

INTRODUCTION

Portulaca oleracea belongs to the family of Portulacaceae. It is commonly called Purslane in English language, *babbajibji* in Hausa language and *esan omode* or *papasan* in Yoruba language. It is a fleshy annual herb, much-branched and attaining 30 cm long (Bukill, 1997).

It is used medicinally in Ghana for heart-palpitations (Johnson, 1997). The plant is used as a diuretic in Nigeria (Ainslie, 1973). A tisane of the plant is drunk in Trinidad as a vermifuge (Wong, 1976).

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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). Parry et al (1987) also reported the skeletal muscle relaxant effect of the plant. A number of compounds have been isolated from the Australian varieties of *Portulaca oleracea.* These include alpha-linolenic acids and beta-carotene (Liu et al, 2000), omega-3 fatty acids and melatonin (Simopoulos *et al*, 2005).

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). Imal et al (2007) showed that P. oleracea has the ability to efficiently remove from water bisphenol A (BPA), which is well known as an endocrine disrupting compound (EDC) having estrogenic properties. However, there is a paucity of information from literature on the effects of *Portulaca oleracea* on reproductive functions.

This study aims at investigating the effects of aqueous and methanolic extracts of *Portulaca oleracea* on female reproductive functions in albino rats.

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MATERIALS AND METHODS

Experimental Animals

Adult female albino rats weighing between 180g and 200g 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 hours daylight cycle and had free access to feed and water; they were acclimatized to laboratory condition for two 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 quantities (2kg) of the fresh specimens of *Portulaca olerace* 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 six 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 (431:33g) of sample A were macerated and extracted with distilled water (1:2 wt/vol) for 72 hours at room temperature (26-28^{0}C). The resulting solution was then filtered using a wire-gauze and a sieve with tiny pores (0.25mm). 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 (420.52g) of sample B were macerated and extracted with 70% methanol (1:2 wt/vol.) for 72 hours at room temperature (26-28^{0}C). The resulting solution was then filtered using a wire-gauze and a sieve with tiny pores (0.25mm). The 70% methanol was later evaporated using steam bath to give a percentage yield of 10.2% of the starting material.

Ten grammes of AEPO and MEPO were dissolved in 100mlof distilled water to give a concentration of 0.1gml. 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

Study of Estrous cycle

Ten matured female rats weighing between 180g-200g were randomly divided into two groups (I and II) with each group consisting of five rats. Vaginal smear was examines 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. The relative proportions 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 was determined. Groups I and II rats received 75mg/kg BW of AEPO and 75mg/kg 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.

Virginal 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

Histopathological study

Fifteen matured albino rats (180-200g) 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 75mg/kg of AEPO
Group II received 75mg/kg of MEPO
Group III received 0.5ml of distilled water (control).

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 below.

Ovarian and Uterine Histology

After weighing the ovaries and uteri, they were immediately fixed in Bouin's fluid for 12 hours and the Bouin's fixative was washed from the samples with 70% alcohol. The tissues were then cut in slabs of about 0.5cm transversely and the tissues were dehydrated by passing through different grades of alcohol: 70% alcohol for 2 hours, 95% alcohol for 2 hours, 100% alcohol for 2 hours and finally 100% alcohol for 2 hours. The tissues were then cleared to remove the alcohol; the clearing was done for 6 hours using xylene. The tissues were then infiltrated in

molten paraffin wax for 2 hours in an oven at 57^0C, thereafter the tissues were embedded. Serial sections were cut using rotary microtome at 5 microns (5μm). The satisfactory ribbons were picked up from a water bath (50-55^0C) 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 minute before immersed in absolute alcohol for 1 minute and later in descending grades of alcohol for about 30 seconds each to hydrate it. The slides were then rinsed in water and immersed in alcoholic solution of hematoxylin for about 18 minutes. 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 seconds and rinsed in water for a few seconds, before being immersed in 70%, 90% and twice in absolute alcohol for 30 seconds each to dehydrate the preparations. The preparations were cleared of alchol by dipping them in xylene for 1 minute. 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 75mg/kg 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 1. 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 2 shows that treatment of rats for 21 days with 75mg/kg 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.

Figures 1 and 2 show that treatment of rats for 25 days with 75mg/kg 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.

Table 1:
Effect of 75mg/kg 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

** indicates significant difference from "Before Treatment"*

Table 2:
Effects of 75mg/kg 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 ± 0.4

** indicates significant difference from "Before Treatment"*

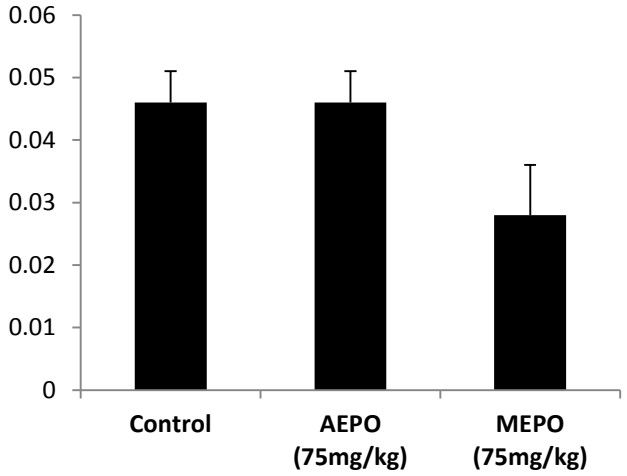

Fig. 1:
Effects of aqueous (AEPO) and methanol (MEPO) extracts of *Portulaca oleracea* on Ovarian Weights

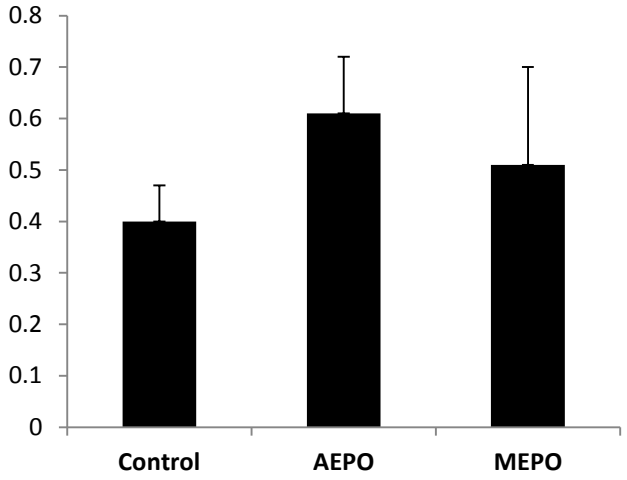

Fig. 2:
Effects of aqueous (AEPO) and methanol (MEPO) extracts of *Portulaca oleracea* on uterine Weights

DISCUSSION

Tables 1 and 2 shows that treatment of rats for 25 days with 75mg/kg 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).

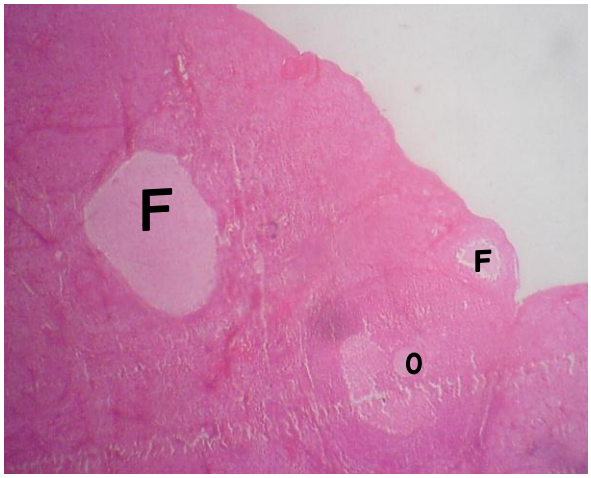

Plate 1:
Photomicrograph of rat's ovary treated with 0.5ml of distilled water for 25 days showing a normal sized ovary with follicles (F) and oocyte (O) at different stages of maturation (x100).

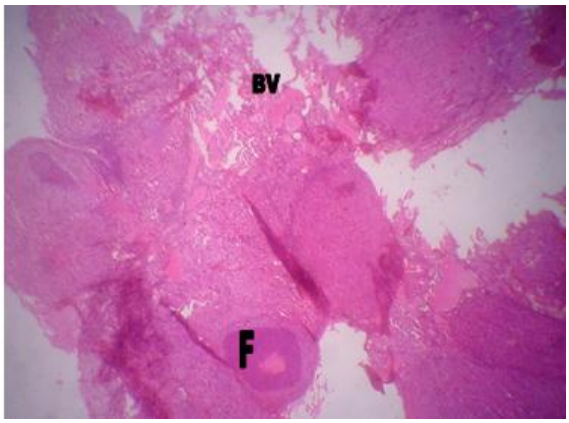

Plate 2
Photomicrograph of rat's ovary treated with 75mg/kg BW AEPO for 25 days showing a normal sized ovary with blood vessel (BV) and a maturing follicle (F) (x100)

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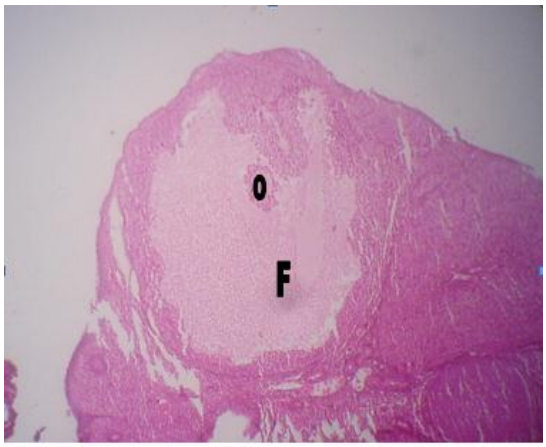

Plate 3
Transverse section through the ovary of rat treated with 75mg/kg BW MEPO for 25 days with normal ovarian size and fibrohemorrhagic stroma with a cystically dilated follicle preparatory to ovulation.

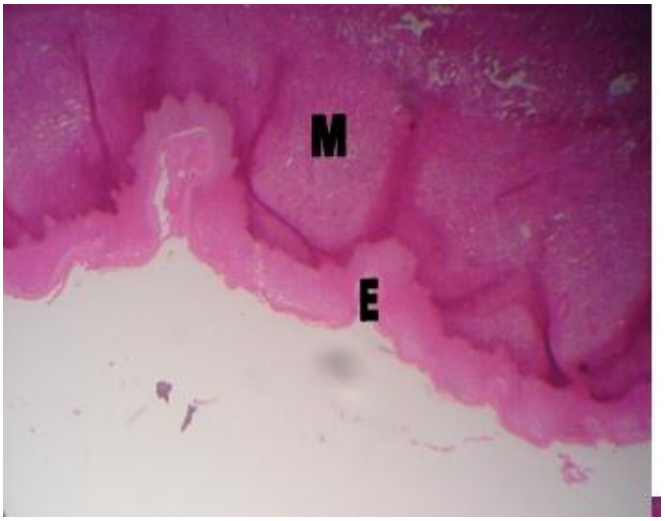

Plate 4
The transverse section through the uterus of control rats given 0.5ml of distilled water for 25 days with normal uterine size with a normal thickness (10 cell layer) of the endometrium and myometrium.

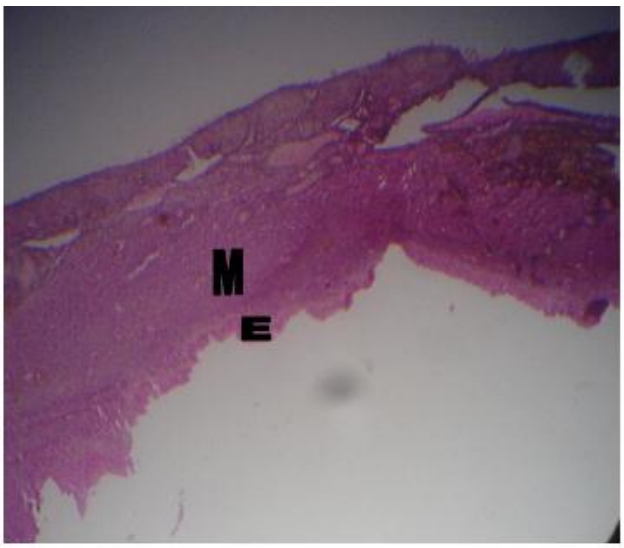

Plate 5
Transverse section through the uterus of rat treated with 75mg/kg BW AEPO for 25 days presenting with smaller uterine size with atrophied endometrial and myometrial tissues.

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 75mg/kg 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 induce by 75mg/kg BW MEPO indicates the availability of matured graafian follicle.

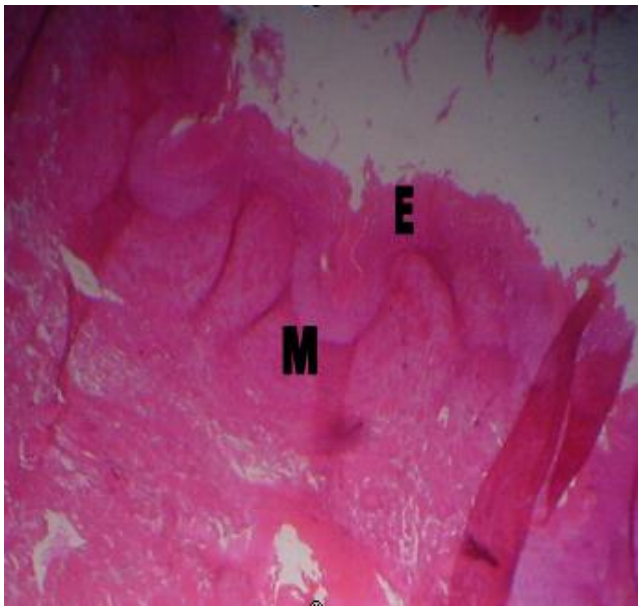

Plate 6:
Photomicrograph of rat's uterus treated with 75mg/kg BW MEPO for 25 days showing slightly hypertrophied endometrium (E) and myometrium (M) (x100).

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 he 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 75mg/lg 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 75mg/kg 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).

Plates 2 and 3 show the photomicrographs of the ovaries of rats treated with 75mg/kg BW of AEPO and MEPO respectively. When compared with plate 1, they showed a normal ovarian size with fibroh emorrhagic 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.

Plates 5 and 6 show the photomicrographs of the uteri of rats treated with 75mg/kg BW of AEPO and MEPO respectively. When compared with plate 4, plate 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 plate 4, plate 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.

In conclusion, 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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