Original research paper

Evaluation Of The Effect Of Ethanol Bark Extract Of *Moringa oleifera* On Reproductive Biology Of Non-Pregnant Wistar Albino Rats

ABSTRACT

Aim: This study was carried out to evaluate the effect of ethanol bark extract of *Moringa oleifera* (EBMO) on reproductive hormone levels and organ histology in non-pregnant Wistar rats.

Place and Duration Study: This study was conducted at the Department of Pharmacology, Faculty of Basic Medical Sciences, College of Health Sciences, University of Port Harcourt, Nigeria; between March and May, 2017.

Methodology: The study was conducted using 40 non-pregnant adult female rats which were divided into four groups (n=10 per group), labelled as groups I, II, III and IV. Group I was the control (given only distilled water), whereas rats in groups II, III and IV were orally administered EBMO (100, 200 and 400mg/kg, respectively) daily for 30 days. At the end of treatment, blood was collected for estimation of serum concentrations of progesterone, estradiol, prolactin, Luteinizing Hormone (LH) and Follicle Stimulating Hormone (FSH). The ovaries, uteri and fallopian tubes of the rats were examined for histologic changes.

Results: There was a non-significant decline in the concentrations of progesterone and estradiol in EBMO treated rats compared to control. No changes occurred in the serum levels of prolactin, LH and FSH after EBMO treatment. Also, there were no alterations in the histology of all three organs in EBMO treated rats when compared to control.

Conclusion: Results indicate that subacute administration of EBMO does not cause alterations of serum concentrations of reproductive hormones or histology of reproductive organs in non-pregnant Wistar rats.

Keywords*: Gonadotropic hormones, moringa, ovary, progesterone, reproductive biology,*

1. INTRODUCTION

Moringa oleifera Lam. is a perennial tree which has been recognized to be of medicinal value many years ago. It originated from the provinces of Agra and Oudh situated in the Himalayan Mountains but has been domesticated in several tropical and sub-tropical countries (Dhakar *et al*., 2011). *M.oleifera* tree grows very rapidly and can grow up to 7–12 m high and 0.2–0.4m wide in diverse climatic environments (Egbuna, 2015). The plant is cultivated for its medicinal and dietary values (Dalei *et al*., 2016). Each part of *Moringa oleifera* has medicinal value and they are used as remedies for several diseases (Koul and Chase, 2015). *Moringa oleifera* is very rich in nutritional and phytochemical constituents including minerals, vitamins and several antioxidant components (Mahmood *et al.*, 2010; Fakankun *et al*., 2013). Carbohydrates, glycosides, flavonoids and tannins are among photoconstituents that have been identified in the ethanol bark extract of moringa bark (Hassan and Basanagouda, 2013).

The plant has been studied extensively to demonstrate its potent biological activities. The reproductive effects of *M. oleifera* have been studied in male and female animal models fairly well and reports indicate that different parts of the plant influence reproduction differently. The leaf extract had a positive influence on sperm parameters and elevated hormone levels in male rats (Novodita and Varma, 2014). The leaf extract also protected and remediated radiation and chemical induced testicular damage (Nithya and Elango, 2014). The seed extract increased sexual activities in male rats (Zade *et al.,* 2013). The root extract possesses contraceptive potentials and has been shown to be teratogenic and abortifacient in rats (Seth *et al.,* 1992; Das *et al.,* 2014). The root has equally induced post-coital antifertility effect in rats and has been demonstrated to induce fetal resorptions in pregnant rats (Prakash *et al*., 1987). However, there is a dearth of data concerning the bark. In many unorthodox settings, it is claimed that the various parts of the plant have valuable effects on all aspects of reproduction and so it is consumed by many women of reproductive age.

The aim of this study was to evaluate the effect of ethanol bark extract of *M.oleifera* on serum levels of reproductive hormones (progesterone, estradiol, prolactin, FSH and LH) and histology of reproductive organs (uterus, ovary and fallopian tube) in non-pregnant female Wistar rats.

2. MATERIALS AND METHODS

Fresh bark of *Moringa oleifera* was obtained from an horticulture (11 Nkakini Road, Port Harcourt, Nigeria). It was identified and authenticated by a botanist of the Department of Plant Science and Biotechnology, University of Port Harcourt, Nigeria and voucher number of UPH003/1017 was assigned to the plant specimen and deposited at the herbarium of the University. The bark was shade–dried, powdered, and macerated in 95% ethanol in a ratio of 1: 4 (moringa: ethanol) for 2 weeks and filtered with size 15 mm Watman filter paper. The filtrate was concentrated under reduced pressure using a rotary evaporator (RV0 400 SD, BOECO, Germany). Residual ethanol was evaporated in water bath at 40^{o}C (Techmel & Techmel, USA) at 40^{o}C to obtain dry extract. Extract was preserved in refrigerator until used for experiments.

2.1 Animals handling

Forty (40) female rats weighing 140–200 g were used for the experiment. The animals were fed with rodent pellets and allowed free access to water throughout the period of the experiment. They were maintained under natural lighting condition. The experiments were commenced after obtaining Ethical Clearance from the Research and Ethics Committee of our institution (UPH/CEREMAD/REC/04).

2.2 Design of the study

The animals were divided into 4 groups (I, II, III and IV) each containing 10 animals. Group I received distilled water (Control), while groups II, III and IV received 100, 200 and 400 mg/kg body weight of ethanol bark extract of *M. oleifera* (EBMO), respectively. Extract was administered by oral gavage daily for 30 days. The choice of these doses was based on the median lethal dose (LD_{50}) of the leaf extract of *M*. *oleifera* which is >2000 mg/kg body weight when administered orally to rats (Adedapo *et al*., 2009). The doses selected were approximately equivalent to 5, 10 and 20% of the LD_{50} of the plant and was similar to doses used in other studies (Zade and Dabhadkar, 2015). At the end of the treatment, the rats were anesthetized with ether and then sacrificed. Blood was collected by cardiac puncture and serum levels of reproductive hormones (FSH, LH, progesterone, estradiol and prolactin) were measured using ELISA technique. Briefly, blood samples were allowed to clot and centrifuged at

3000 rpm for 15 min to separate serum. Serum was analyzed with auto-analyzer machine- microplate reader (RT-2100C, China) using AccuBind assay kits (FSH: EIA-4K4A7, LH: EIA-6K2C7, prolactin: EIA-7K1C7, progesterone: EIA-48K1H6, and estradiol: EIA-49K637; Monobind Inc., USA). Reproductive organs (ovaries, fallopian tubes and uteri) were harvested, fixed in Bouin's solution and processed for histological analysis.

2.3 Histological analysis

Tissues (ovary, oviduct and uterus) were sliced, dehydrated with ascending grades of alcohol and embedded with paraffin wax. Sections were cut (5 µm thick) with a microtome and tissues were fixed to slides and allowed to dry. The slides were stained with hematoxylin–eosin (H & E) solution and examined under the light microscope (Optitech model RC 1321, California, USA). Photomicrographs of the tissues were taken.

2.4 Statistical analysis

All data were expressed as means ± standard deviation of mean (mean±SD). Data obtained were analyzed by one–way analysis of variance (ANOVA) using SPSS version 21. Values were considered to be significant at $p < 0.05$.

3. RESULTS AND DISCUSSION

The serum levels of progesterone, estrogen, prolactin, FSH and LH in moringa extract treated rats were not different ($p>0.05$) compared to control (Table 1). The hormone concentrations obtained in the control rats were: progesterone (4.18±1.06 ng/ml), estrogen (300.00±69.63 ng/ml), prolactin (2.60±0.82 ng/ml), FSH (13.20±1.30 mIU/ml) and LH (8.00±1.41 mIU/ml). Although, the concentrations of progesterone and estrogens in rats that received moringa extract were lower than control levels, the values were not significant (Table 1).

Table 1. Effect of ethanol bark extract of *Moringa oleifera* on serum reproductive hormone levels in female Wistar rats

Group	Progesterone (ng/ml)	Estradiol (ng/ml)	LH (mIU/ml)	FSH (mIU/ml)	Prolactin (ng/ml)

Control	4.18±1.06	300.00±69.63	8.00±1.41	13.20±1.30	2.60±0.82
100 mg/kg	3.64±1.20	292.00±75.19	8.00±1.41	13.60±1.67	2.80±0.57
200 mg/kg	3.14±1.71	252.00±95.50	8.20±2.05	13.00±1.00	2.50±0.61
400 mg/kg	3.06±0.68	236.00±43.36	8.80±1.10	14.20±1.79	2.90±0.55
F	0.901	1.509	0.305	0.644	0.398
p-value	0.462	0.250	0.821	0.598	0.756

Values are expressed as mean±SD, n=10 per group

The histologic analyses of the uteri, ovaries and fallopian tubes of rats revealed normal histoarchitecture for the control rats (Figures 1A, 2A and 3A). Mild inflammatory cell infiltrations were observed in the uteri, ovaries and fallopian tubes of extract administered groups of rats. However, the structural outline and cellular composition of the structures were preserved in all the organs (Figures 1, 2 and 3).

The results of the present study indicate that subacute exposure of ethanol bark extract of *M. oleifera* may have no effect on serum concentrations of progesterone and estradiol in non-pregnant rats. An earlier report concluded that the stem/bark of the plant elevated estrogen level in pregnant rats and eventually produced abortifacient effect (Zade and Dabhadkar, 2014). In another study, low progesterone has equally been described to induce abortion at certain stages of gestation, which may explain the abortifacient activity of antiprogesterone agents like mifepristone (Ganong, 2015). The maintenance of normal serum levels of these hormones suggests thus that the extract may not affect ovarian activity and not be harmful to reproductive function in the non-pregnant rats. Interestingly, limited data on the effect of moringa bark on non-pregnant rats existed prior to this study. Additionally the lack of effect on prolactin level by the extract in this study disagrees with some results that were obtained with the leaf extract in pregnant animals. Oral administration of the leaf extract increased number of mammary glands and serum prolactin level in rats and goats (Titi and Nunung, 2014). This effect was ascribed to the phytosterol content of moringa which indirectly stimulates ductular proliferation by increasing estrogen formation. Although, the major physiologic regulator of prolactin secretion is a negative feedback inhibition by dopamine, it has been suggested that progesterone may as well be a putative positive regulator of prolactin secretion (Ganong, 2015). The effect of the extract on progesterone thus correlated with the non-alteration of prolactin level.

In previous studies, ethanol extract of *M. oleifera* leaf increased FSH and LH levels in male albino rats (Manhal *et al*., 2016). From our results, there was no change in the serum levels of FSH and LH following treatment with the extract in female rats. This zero effect on gonadotropic hormones is explained by the normal estrogen and progesterone levels that were seen after extract treatment, being that they are feedback regulators of gonadotropins release, particularly LH (Ganong, 2015). Similarly, the histology results revealed no histological changes of concern supporting the insignificant hormonal influence of the extract and indicating it may not be harmful to the reproductive biology of non-pregnant rats. Although animals' menstrual cycles can influence the outcome of this study, the consistent results obtained provides some reliability. However, future studies are necessary to establish the influence of the plant on female sex hormones in specific terms.

4. CONCLUSION

This study shows that 30 days treatment with crude ethanol moringa bark extract may cause no change in serum reproductive hormone concentrations as well as reproductive organs in the non-pregnant female rats.

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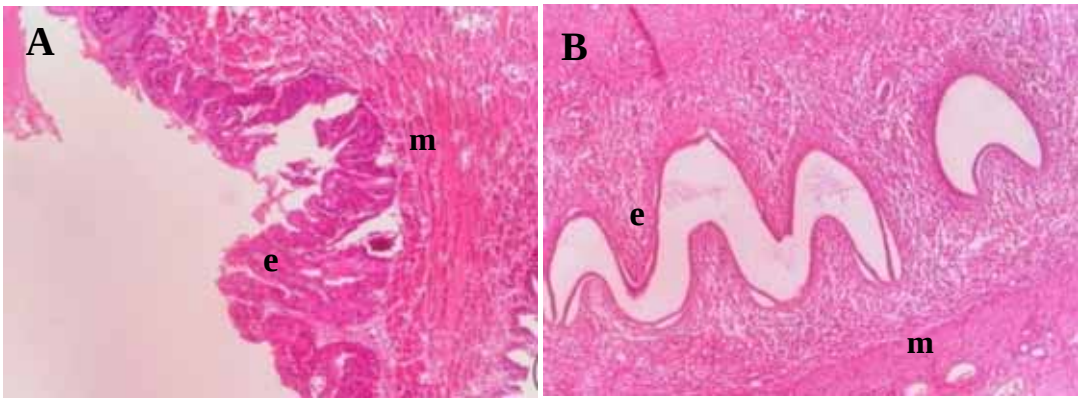

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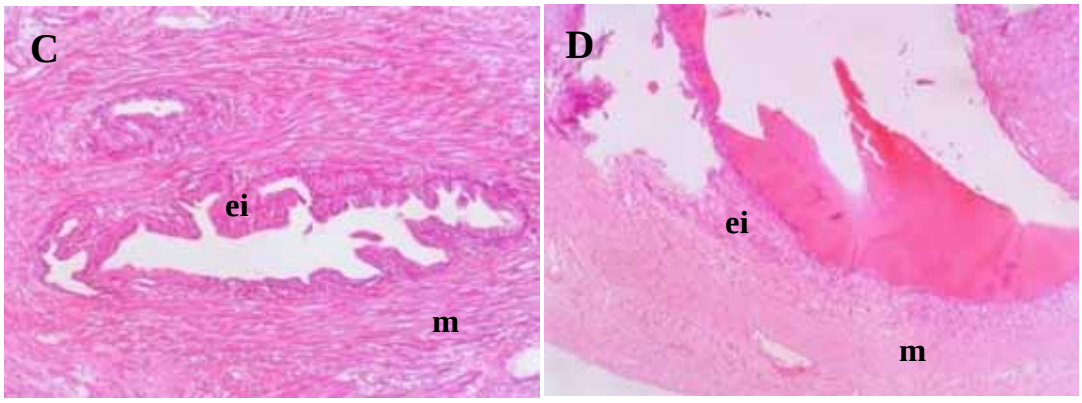

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227 **Fig 1. Photomicrographs showing uteri of non-pregnant female Wistar rats**
228 **treated with ethanol bark *M. oleifera* extract for 30 days. H & E stain, 400x.**

229

230 *A- (Control): Section shows normal uterine tissue with straight and tubular endometrial glands and normal sized*

231 *myometrium.*

232 *B - (100 mg/kg): Section shows uterine tissue with normal histology.*

233 *C - (200 mg/kg): Section has normal histology with few endometrial leukocytic infiltrations.*

234 *D - (400 mg/kg): Section shows uterine tissue with convoluted endometrial glands with few sub-epithelial*

235 *leukocytic infiltrations*

236 *Key: e = endometrium, m = myometrium, ei = sub-endometrial inflammation.*

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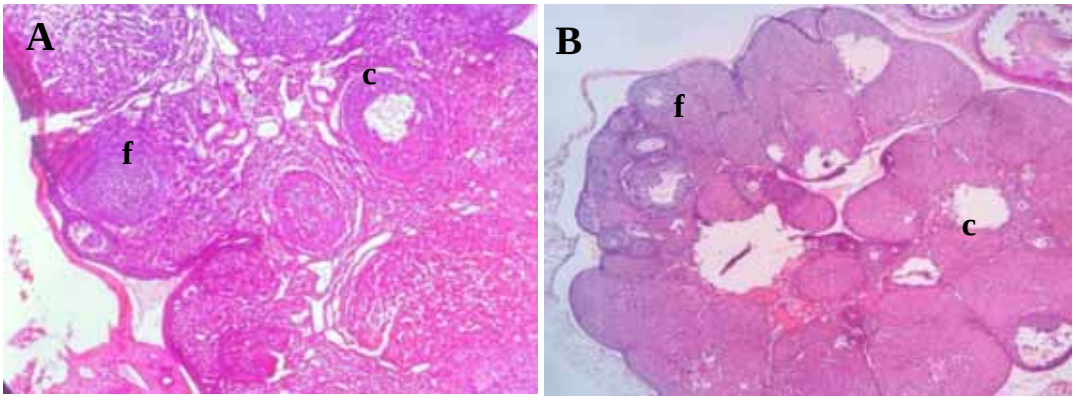

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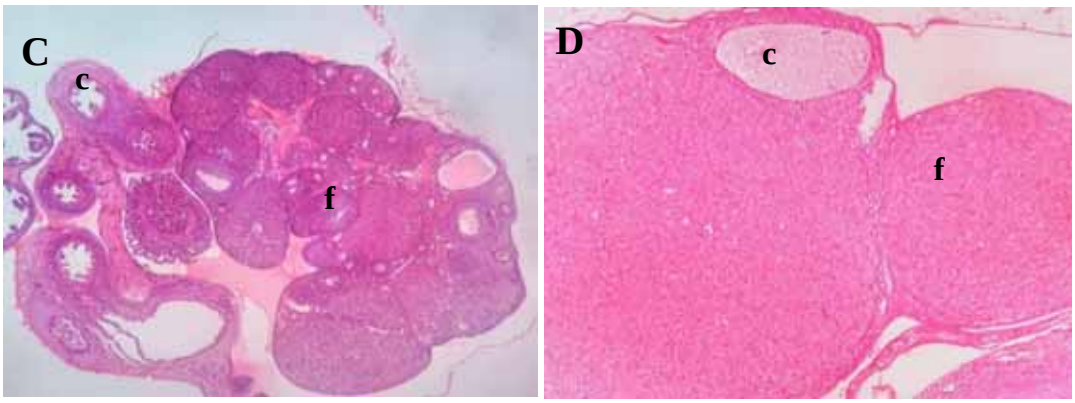


Fig 2. Photomicrographs showing ovaries of non-pregnant female Wistar rats treated with ethanol bark *M. oleifera* extract for 30 days. H & E stain, 400x.

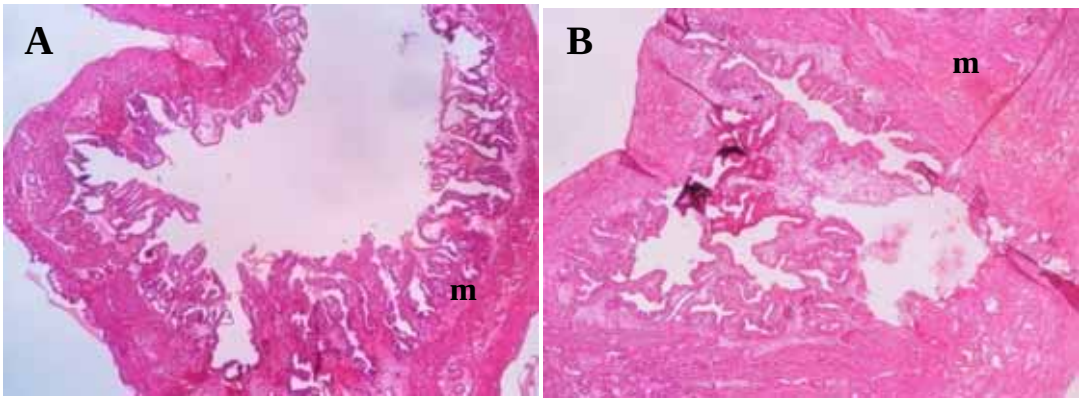
A - (Control): Section shows ovarian tissue composed of maturing follicles of different stages (primary and Graafian). There are several corpus lutei.

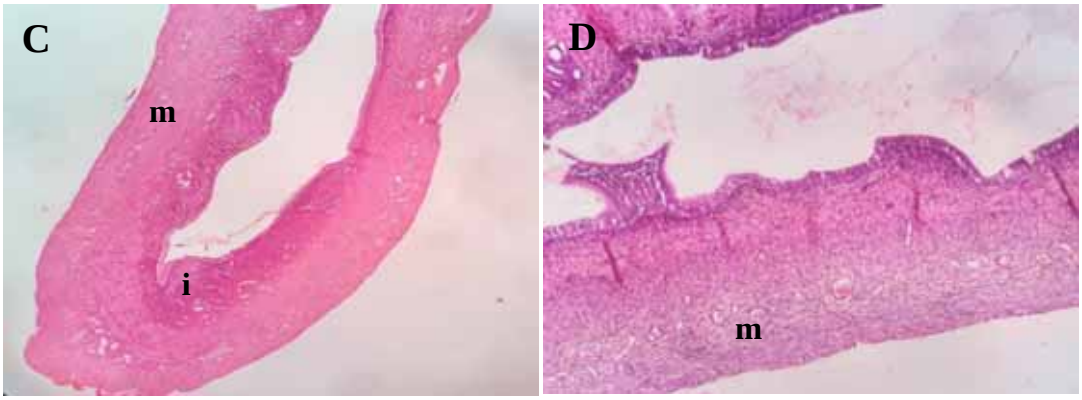
B - (100 mg/kg): Section shows ovarian tissue composed of few maturing follicles of different stages (primary and Graafian). There are few corpus lutei.

C - (200 mg/kg): Section shows ovarian tissue composed of maturing follicles of different stages (primary and Graafian). There are few corpus lutei.

D - (400 mg/kg): Section shows ovarian tissue with maturing follicles and few corpus lutei.

Key: c = corpus luteum, f = follicle at various stages of maturation





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268 **Fig 3. Photomicrographs of fallopian ducts of non-pregnant female Wistar rats**
269 **treated with ethanol bark *M. oleifera* extract for 30 days. H & E stain, 400x.**

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271 *A - (Control): Section shows fibro-muscular tubal tissue lined by tall columnar epithelium with normal histology.*

272 *B – (100 mg/kg): Section shows fibro-muscular tube lined by columnar epithelium with no leukocytic infiltration*

273 *C– (200 mg/kg): Section shows fibro-muscular tube with normal epithelia and mild leukocytic infiltration.*

274 *D – (400 mg/kg): Section shows fibro-muscular tube with mild mucosal and muscularis leucocytic inflammation.*

275 *Key: m = fibro-muscular layer, i = inflammation.*