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Original Research Article

Effects of Aqueous Leaf Extract of *Mormodica Balsamina* on the Histology of the Ovary and Female Reproductive Hormones

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Abstract

***Mormodica balsamina* is use traditionally for abortions, birth control, increasing milk flow, menstrual disorders, vaginal discharge, constipation, diabetes, hyperglycemia, and fever (malaria). This study was aimed at evaluating the effect of aqueous leaf extract of *Momordica balsamina* on the histology of the ovary and female reproductive hormones. A total of twenty five (25) Wistar albino rats weighing between 96-120g were used for the study. The rats were divided into five groups of 5 rats each. Group I served as the control group and was given normal feed and water. While groups II, III and IV were administered with 100, 200, and 500mg/kg of the extract respectively for 28 days. Group V was administered with 500mg/kg and was allowed for 14 days recovering period. At the end of the experiment, blood samples were collected by cardiac puncture and serum were used to determine the levels of estrogen, progesterone, follicle stimulating hormone, and leutinizing hormones. Tissue sample were collected for routine histological analysis. Plasma serum concentration of estrogen, progesterone, FSH, LH, significantly reduced in high dose group ($p<0.05$). Histological section in high dose group showed mild reduction in graffian follicles. The result of the present study suggest that *Mormodica balsamina* poses anti-estrogenic properties which was attributed to the mild reduction in granulose cells.**

Keywords: *Mormodica balsamina,* Ovary, FSH, LH, Progesterone, Estrogen

INTRODUCTION

Historically, plants have provided sources of inspiration for drug compounds, as plant- derived medicines have made large contributions to human health and well- being (Ebonyi and Iyawe, 2000). Nowadays, given the side effects due to manmade drugs, plants and traditional medicine are being increasingly used and the benefits of different plants are attracting more attention day to day.

Various substances including herbal mixture or plant extract have been reported to affect fertility in females as infertility is a major problem for 15-20% of couples and about 50% of infertility is related to problems associated with female disorders (Vander and Wyns, 2018). Female infertility may be caused by an underlying variety of disorders, and hormonal imbalances. These hormonal conditions include polycystic ovary syndrome (PCOS), endometriosis, premature ovarian failure (POF) and pelvic inflammatory diseases (Mustafa *et al.,* 2019).

Traditional medicines were used for fertility regulation in ancient times (Ogbuewu *et al.,* 2011). Since then a large number of plant species have been screened for their antifertility efficacy and female contraception (Yama *et al.,* 2011). *Mormodica balsamina linn* belongs to the family Cucurbitaceae. It is native to Africa, Asia, Australia and Europe. It is commonly called Balsam apple (English) Garahuni (hausa) Akbon-ndewe (Igbo) and Ejirin (Yoruba) (Burkill, 2005). Their leaves and fruits are

eaten as vegetables in Nigeria, Cameroon, Sudan, and South Africa (Grover and Yadav, 2004). Traditionally, *Mormodica balsamina* has been evaluated for its benefits in the treatment of high fever, excessive uterine bleeding, syphilis, rheumatism, hepatitis, diabetics, skin diseases, and microbial infections (Gills, 1992). Its antifertility effect on the female reproductive system has been evaluated by other researchers (Osonuga *et al.,* 2014 and Sheeja *et al.,* 2012). However, most people in the developing countries including Nigeria consume herbal medication without the knowledge of the side effects of such medications (Osemene *et al.,* 2011). Therefore, we intend to explore the effects of aqueous leaf extract of *Mormodica balsamina* being one of the commonly consumed herbs in northern Nigeria in order to advise patients especially when infertility is the problem.

MATERIALS AND METHODS

Collection and Identification of the Plant Materials

The leaves of *Momordica balsamina linn* were purchased from a local market of herbalists located within the Monday market in Maiduguri Metropolis. The plant was identified and authenticated by a plant taxonomist, Department of Biological Sciences, University of Maiduguri, Borno state.

Extraction Procedures

The extraction of the plant material was conducted as described by the World Health Organization. The leaves of *Mormodica balsamina* obtained were air dried and powdered using a surface sterilized pestle and mortar. A total of 500g of pulverized material was subjected to exhaustive soxhlet extraction in distilled water (1000ml) for 72h at 60^0c. A yield of 18.4g w/w was obtained from the extraction process. The dried extract was stored in a refrigerator at 4^0c.

Animal and Husbandry

Mature female Wistar albino rats were obtained from the Department of Biochemistry animal units, University of Maiduguri. The study was conducted on twenty five (25) adult female albino Wister rats weighing between 96 - 120g. The animals were maintained on standard growers' pelletised diet (from Grand Cereals and Oil Limited, Bukur, Plateau State) and potable drinking water. The rats were housed ten per cage and allowed to acclimatize to existing conditions in the animal house for a period of two weeks before the commencement of administration of the extract.

Statistical

The data obtained were analyzed using statistical software (SPSS) version 16.0 by one way analysis of variance (ANOVA) and were expressed as Mean ± Standard error of the mean (SEM).*P* values ($P< 0.05$) was considered statistically significant

Experimental Design

A total of fifty adult Female Albino Wister rats were used for this research. The rats were divided into five groups with each group consisting of five (5) rats. Group I served as the control group and was given normal feed and water *ad libitum* throughout the research. Groups II, III and IV were administered with 100, 200 and 500 mg/kg respectively. Group V was designated as the post recovery group and was administered 500 mg/kg. The extract was administered to groups II, III, IV and V for the period of 28 days.

This study was carried out following approval from the Departmental Ethical Committee on the Care and Use of Experimental Animals for Research.

Hormonal Assay

Hormonal assay was carried out at the Department OF Chemical Pathology University of Maiduguri Teaching Hospital. Blood samples were collected by cardiac puncture method in a plain bottle, it was centrifuged at 3000 rpm x g for 5mins and the serum was used to determine the levels of estrogen, LH, FSH and progesterone using enzyme linked immunosorbant Assay (ELISA) as was previously described by (Lilaram and Nazeer 2012).

Tissue Processing

After the 28^{th} day of administration of extract, the ovaries were carefully dissected out, trimmed off fat and connective tissues. The tissues were fixed with 10% formalin, dehydrated using different grade series of alcohol, embedded in paraffin wax, and sectioned at 5, cleared in xylene and then stained with heamatoxylin and eosin and observed under light microscope (Muhammad *et al*., 2016).

RESULTS

Effects of the Extract on Female Reproductive Hormones

The effect of aqueous extract of *Mormodica balsamina* on

Table 1. Levels of Progesterone, Estrogen, FSH and LH on Rats Treated with Aqueous Leaf Extract of *Mormodica balsamina*

Dose (mg/kg)	Progesterone (ng/ml)	Estrogen (pg/ml)	FSH (miu/ml)	LH (miu/ml)
00	1.94 ± 0.05	20.10 ± 0.46	2.99 ± 0.06	2.83 ± 0.83
100	1.83 ± 0.05	19.70 ± 0.40	2.84 ± 0.06	2.81 ± 0.81
200	1.84 ± 0.04	18.60 ± 0.83*	2.87 ± 0.04	2.62 ± 0.06
500	1.79 ± 0.02*	18.10 ± 0.28*	2.78 ± 0.28*	2.51 ± 0.08*
500PRG	1.88 ± 0.03	19.00 ± 0.26	2.96 ± 0.02	2.77 ± 0.08

Result are presented as Mean ± SEM.
PRG= Post Recovery Group: Sacrificed 14 days after the last administration of the extract

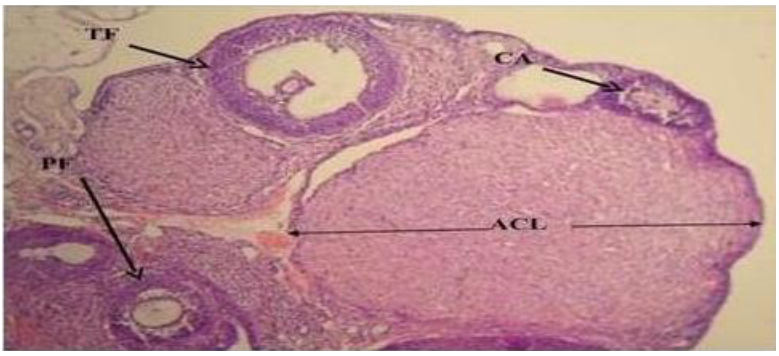


Figure 1. Photomicrograph of rats showing normal histological features, with tertiary follicles (TF) primary follicle (PF), active corpuse luteum (ACL), and corpus atritecum (CA) H&E ×200.

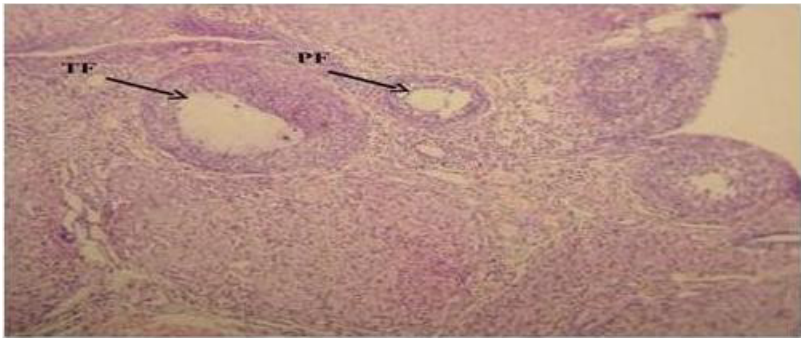


Figure 2. Photomicrograph of rats ovary treated with 100mg/kg showing ovarian tissues and mild reduction in graffian folicle (GF) and primary follicle (PF) (H&E)×200.

the female reproductive hormones is presented on Table 1. The results showed a significant decrease in estrogen, progesterone, FSH and LH levels in all the groups administered with 500 $mgkg^{-1}$ of the extract ($P < 0.05$) when compared to the control group as seen in Table 1.

HISTOPATHOLOGICAL FINDINGS

Photomicrographs of rat ovary from the control group showed normal architecture of ovarian cells (Figure 1). However, photomicrographs of rats in groups administered 100mg/kg of extract showed ovarian tissues with mild reduction in Graffian follicles (Figure 2). The group administered 200mg/kg (Figure 3) also showed mild reduction in Graffian follicles. The group that was administered with 500mg/kg (Figure 4) showed Graffian follicles that are composed of mainly granulose cells which fail to mature. The slide from the recovery group (Figure 5) did not show any significant change.

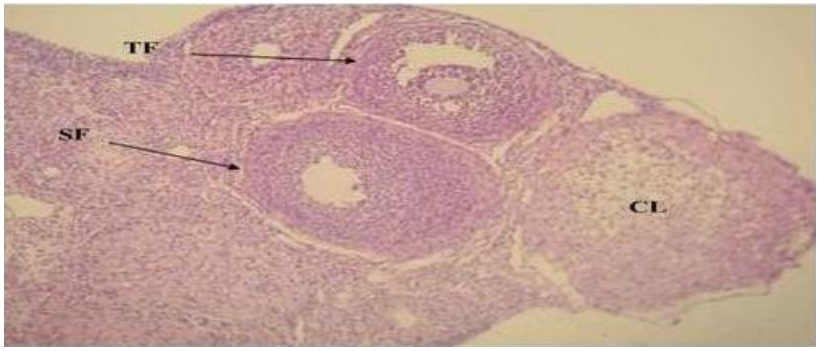


Figure 3. Photomicrograph of rat treated with 200mg/kg of extract showing corpus luteum (CL), secondary follicle (SF) and mild reduction in tertiary follicle (TF) (H&E)×20

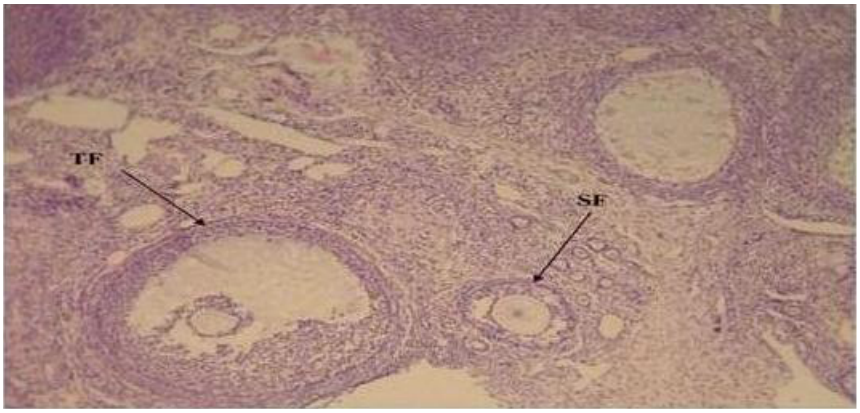


Figure 4. Photomicrograph of rat treated with 500mg/kg of extract showing secondary follicle (SF), and tertiary follicle (TF) that is compose mainly of granulose cells which fails to mature (H&E)×200.

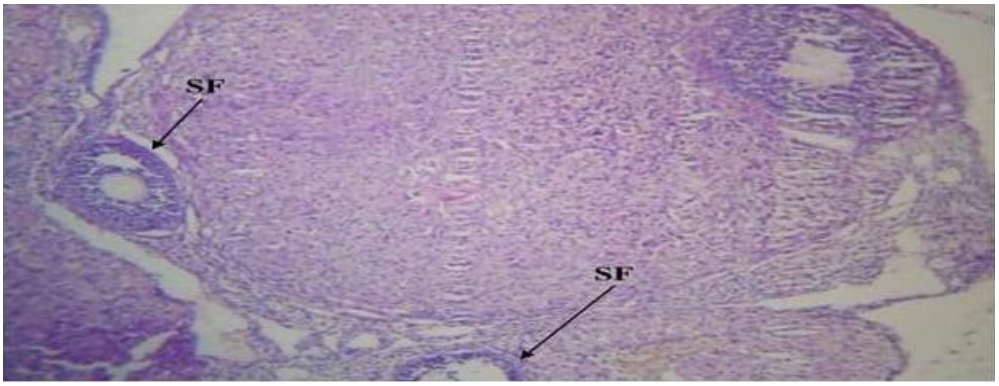


Figure 5. Photomicrograph of rat treated with 500mg/kg (recovery) showing secondary follicle that fails to mature (H&E)×200.

DISCUSSION

At the end of this present study, administration of *Mormodica balsamina* leaf extract significantly reduces plasma concentration of estrogen, progesterone, LH, and FSH levels of female Wistar rats in the group administered with 500mg/kg body weight of extract. The decrease in estrogen level observed in this study may be due to the inhibitory effect of *Mormodica balsamina* on concentration pituitary gonadotropins, direct toxic effects on follicular and theca cells as seen with the seminiferous tubules in males rats (Yama *et al*., 2011).

This is also in accordance with the assertion of (Koneri *et al.,* 2001 and Amah *et al.,* 2012) that the pituitary gonadal axis is important for the maintenance of the reproductive system , thus any distortion to this axis can be deleterious. High estrogen levels are important for luteinizing hormones that induce ovulation. A decline in estrogen prevents ovulation hence low progesterone levels (Sheeja *et al.,* 2012). Follicle stimulating hormones are the central hormones of mammalian reproduction essential for gonad development and maturation at puberty as well as gamete production during the fertile phase of life (Simoni and Nieschlag, 1995). It stimulates the growth and maturation of ovarian follicles by acting directly on the receptors located on the granulosa cell. In females, ovulation of mature follicles in the ovary is reduced by a large surge of LH secretion during the pre-ovulatory period. Any substance capable of inhibiting this release will provoke disruption of ovulation by decreasing the number of mature follicles or induce an estrous cycle disruption at rest (Benie *et al.,* 2003). Therefore, the reduction in serum LH levels observed in this study may be explained by an inhibitory effect of the extract on the release of LH. It is possible that *Mormodica balsamina* contains anti gonadotropin substances which may affect the estrous cycle and hamper reproduction in females.

The histological changes observed might be due to inhibitory effects of the extract. The reduction in the granulosa cell observed in this study may be attributed to the decrease availability of ovarian estrogens as these follicles are the major source of estrogens in the ovary (Mc Natty *et al.,* 1998).

CONCLUSION

The results of the present study suggest that *Mormodica balsamina* poses antiestrogenic properties which was attributed to the reduction in granulosa cells, and a consequence of reduced serum levels of ovarian hormones.

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