Effect of Leaf Extract of *Millettia aboensis* on the Reproductive Hormones and Organs of Female Wistar Albino Rats

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Citation

Abstract
This study assessed the effects of aqueous leaf extract of *Millettia aboensis* on the female reproductive hormones and organs (ovary and uterus) of experimental rats for fourteen days. A total of 24 matured female rats were completely randomized into six groups of 4 each. The six groups received normal food and water. Additionally, Groups 1-5 received 1000, 2000, 3000, 4000 and 5000 mg kg$^{-1}$ body weight of the extract respectively twice daily. The female rats were sacrificed after two weeks of the experiment. Samples were collected from each rat by cardiac puncture using sterile syringes. Administration of the extract produced significant increase (p<0.05) in the serum prolactin, follicle-stimulating hormone and luteinizing hormone concentration whereas those of estradiol, progesterone were significantly reduced. Photomicrographs of rats from the Control Group showed normal architecture of ovarian cells. While rats in Group 1 showed partially destroyed ovarian cells, rats from Groups 2-5 showed completely destroyed ovarian cells. The result showed that the aqueous extract had a significant effect on both the reproductive hormones and the ovary but not on the uterus.

1. Introduction

Medicinal plants are plants or herbs that have healing properties. They have been identified and used throughout human history. Plants have the ability to synthesize a wide variety of chemical compounds that are used to perform important biological functions, and to defend against attack.

Plant-derived chemicals that influence endocrine activities in both humans and animals have received a great deal of attention due to their likely or potential benefit as well as adverse effects (Baker *et al.* 1995). Some of these plants are known to possess anti-fertility effect through their action on hypotlamo-pituitary-gonadal axis or direct hormonal effects on reproductive organs resulting in inhibition of ovarian steroidogenesis (Raja *et al.* 2010). *Millettia aboensis* are small trees of 30–40feet high and up to 2feet in girth but usually 12feet high with reddish-brown pubescence on the petioles, branches, inflorescence and fruits. They are found commonly in low land rain forest. The flowers are purple in erect woody racemes up to 18inches long. It has conspicuously rusty-hairy leaves and handsome purple flowers in erect terminal racemes at branch. Almost all the parts of *M. aboensis* have medicinal properties. The leaf is used
by traditional herbalist for general healing including ulcer and laxatives while the root is used in treating gastro intestinal disturbances and liver diseases. Also a decoction of the leaf, stem and root with other plant materials (herbs) is used to cure venereal diseases such as gonorrhoea, syphilis, etc. (Burkhill, 2005; Adonu et al. 2013). The female reproductive system is primarily regulated by five hormones which include estrogen, progesterone, prolactin, follicle-stimulating hormone and luteinizing hormone. These hormones play a role in one or more stages of development and function of the female reproductive system. Several studies (Telefo et al. 1998; Emmanuele et al. 2002; Benie et al., 2003) have shown that chemical compounds including plant extract could alter the concentrations and functions of female reproductive hormones. This study was therefore designed to provide information on the effect of aqueous leaf extract of *M. aboensis* on female reproductive hormones and the reproductive organs (ovary and uterus).

2. Materials and Methods

2.1. Collection Identification and Extraction of Plant Material

Fresh leaves of *M. aboensis* were collected from the medicinal garden of the Department of Pharmacy, University of Port Harcourt Rivers State, Nigeria. Identification and authentication of plant was as previously reported by Onyegeme-Okerenta et al. (2013). The leaves were thoroughly washed using running water and rinsed with distilled water and air-dried to a constant weight at room temperature (27±1°C) for 2 weeks, after which it was milled to a fine powder with the aid of a Marlex Exceller grinder. The aqueous extract was prepared by dissolving 60g of the powdered plant materials in 150ml of distilled water at room temperature with intermittent shaking. The extract was filtered after 90 mins using Whatmann filter paper (125mm).

2.2. Experimental Design Animal Grouping and Extract Administration

Twenty-four female albino rats (Rattus norvegicus) of Wistar strain weighing 104 – 131kg were obtained from the Animal House of the Department of Animal and Environmental Biology, University of Port Harcourt, Rivers State, Nigeria. They were housed in separate plastic cages and acclimatized for fourteen days and feed on conventional rat feed and water. The rats were completely randomized into six groups of 4 rats each. Rats in Groups 1-5 were orally fed with *M. aboensis* leaf extract of concentrations 1000, 2000, 3000, 4000 and 5000mg kg\(^{-1}\) body weight respectively (09:00-09:45h) and (16:00-16:30h) using plastic syringe. The twenty-four rats were sacrificed after 14 days. This study was carried out following approval from the Departmental Ethical Committee on the Care and Use of Experimental Animals for Research.

2.3. Collection of Tissue and Blood Samples

The procedure used was described by Yakubu et al. (2005). Each of the adult rats was anesthetized in chloroform vapor in desiccators and dissected using surgical forceps and scissors. Blood samples were collected by cardiac puncture using sterile syringe and needle into plain sample tubes and were allowed to stand for 120 mins at room temperature to clot, after which they were centrifuged at 3000rpm for 10 mins using a bench top centrifuge Uniscope Laboratory Centrifuge (Model 802, Surgifriend Medicals and Essex, England), to obtain the serum. The sera obtained from the respective samples were carefully removed using Pasteur pipettes, into respective labeled plastic specimen bottles and stored frozen in a bio-freezer until ready for analysis. Ovarian and uterine tissues were fixed in 10% formal saline in labeled plain bottles for histological studies. The tissues were subjected to standard routine histological procedures as described by Brown, (2002). The slides were viewed using the light microscope and histopathological changes were observed and recorded at X40 magnification identifying both the normal and the degenerated hepatocytes.

2.4. Hormonal Assay

The assay kits for prolactin, progesterone, estradiol, follicle-stimulating and luteinizing hormones were supplied by Diagnostic Automation Inc., Calabasa, CA, USA. All other reagents used were of analytical grade and were prepared in volumetric flask using glass-distilled water. The procedure described in the hormone assay kits was used according to the principle highlighted by Uotilia et al. (1981) was used for all the hormonal analysis that is, prolactin, estradiol, progesterone luteinizing and follicle-stimulating hormones.

2.5. Statistical Analysis

The experimental data were analyzed for statistical significance by one-way analysis of variance (ANOVA) using the Excel computer-based program. Data were expressed as mean ± SEM and the probability tested at 95% level of significance (P<0.05).

3. Result

3.1. Effects of Aqueous Extract of *M. Aboensis* on the Reproductive Hormones of Female Rats

Fig.1 shows the result of the effect of *M. aboensis* in the serum level of follicle-stimulating hormone (FSH), a
reproductive hormone of female Wistar rats. When compared with the control, administration of the extract produced significant increase (p<0.05) in the serum follicle-stimulating hormone.

Figure 1. Effect of aqueous leaf extract of M. aboensis on female rats’ follicle-stimulating hormone levels.

Figure 2 shows the result of the effect of M. aboensis on luteinizing hormone (LH), the reproductive hormones in the serum level of female Wistar rats. Results obtained show that administration of the extract produced a significant increase (p<0.05) in the level of serum luteinizing hormone when compared to the Control Group.

Figure 2. Effect of aqueous leaf extract of M. aboensis on female rats’ luteinizing hormone levels.

Figure 3 shows the result of the effect of M. aboensis on prolactin (PRL), the reproductive hormones in the serum of female Wistar rats. Results obtained show that administration of M. aboensis extract caused a significant increase (p<0.05) in the level of serum prolactin when compared with the control.

Figure 3. Effect of aqueous leaf extract of M. aboensis on female rats’ prolactin levels.
Figure 4 shows the result of the effect of *M. aboensis* on the reproductive hormones of female Wistar rats for progesterone in the serum. Results obtained show that administration of *M. aboensis* extract caused a significant decrease (p<0.05) in the level of serum progesterone when compared to the Control Group.

![Figure 4](image)

**Figure 4.** Effect of aqueous leaf extract of *M. aboensis* on female rats’ progesterone levels.

Figure 5 shows the result of the effect of *M. aboensis* on serum level of estradiol, a reproductive hormone, of female Wistar rats. Results obtained show that administration of *M. aboensis* extract caused a significant decrease (p<0.05) in the level of serum estrogen when compared to the Control Group.

![Figure 5](image)

**Figure 5.** Effect of aqueous leaf extract of *M. aboensis* on female rats’ estrogen levels.

3.2. Effect of Aqueous Leaf Extract of *M. Aboensis* on the Ovaries of Female Rats

Plate 1 is the histopathological changes in ovaries of control and experimental rats. Photomicrograph showed a normal architecture of ovarian cells of the control group (a); partially destroyed ovaries were observed in rats from Group 1 (b) while completely destroyed ovarian cells were observed in rats from Groups 2-5 (c-f).

![Plate 1](image)

**Plate 1.** Histopathological changes in ovaries of control and experimental rats. a. Control Group - shows normal ovarian cells; b. Group 1 - shows partially destroyed ovary; c– f. Groups 2-5 show completely destroyed ovarian cells.
3.3. Effect of Aqueous Extract of M. Aboensis on the Uterus of Female Rats

Figure 6 shows the photomicrograph of the uterus of Group 5 rats. The aqueous extract of M. aboensis had no effect on the cells and lining of the uterus of Group 5 which showed normal uterine wall architecture. Normal uterine wall architecture was observed in all the groups.

![Figure 6. Section of the uterus of rat of Group 5 showing normal uterine architecture.](image)

4. Discussion and Conclusion

The focus of this study was to determine the effects of various doses of aqueous leaf extract of M. aboensis on plasma follicle-stimulating hormone, luteinizing hormone, prolactin, estrogen (B-estradiol) and progesterone, five important fertility hormones in females. Maturation of pre-ovulatory follicles and ovulation are under the combined and balanced influences of ovarian and extra ovarian hormones. Imbalances or alterations in these hormones lead to irregularity in the ovarian functions and duration of estrous cycle (Shivatingappa, et al. 2002). Administration of the extract produced significant increases (p<0.05) in the serum follicle-stimulating hormone, prolactin and luteinizing hormone concentration. Follicle-stimulating hormone is the central hormone of mammalian reproduction, essential for gonadal development and maturation at puberty as well as gamete production during the fertile phase of life (Simoni & Nieszlag, 1995). It stimulates the growth and maturation of ovarian follicles by acting directly on the receptors located on the granulosa cells.

Administration of leaf extract of M. aboensis significantly reduced (p<0.05) estrogen and progesterone concentration. This is in agreement with Osonuga et al. (2014) who reported that oral administration of leaf extract of Momordica charantia caused a reduction in estrogen levels of adult female Wistar rats. The pituitary-gonadal axis is important for the maintenance of the reproductive system hence any distortion to this axis can be deleterious (Amah et al. 2012; Koneri et al. 2006; Yama et al.2011). According to Moore et al. (2008), follicle-stimulating hormone stimulates maturation of the Graafian follicle while luteinizing hormone causes it to synthesize testosterone which is then converted to estrogen by aromatase. High estrogen levels are important for the luteinizing hormone surge that induces ovulation. The subsequently formed corpus luteum secretes progesterone that favors implantation and establishment of pregnancy (Sheeja et al.2012) while a decline in estrogen prevents ovulation hence low progesterone levels. Also a direct toxic effect on the corpus luteum may be a possible mechanism for decline in progesterone levels. The attendant effect of this is spontaneous abortion and failure of implantation reported by other workers (Koneri et al. 2006; Sheeja et al. 2012).

These hormonal imbalances may be caused by numerous chemical agents contained in plant extract. Benie et al. (2003) and Yakubu et al. (2005) reported that phytochemical screening has revealed many bioactive as well as toxic agents of plant extract that can affect the regulation of oestrous cycle, conception and reproduction. Alkaloids and flavonoids have been shown to reduce plasma concentrations of estradiol (Lauritzen et al. 1997; Browning et al. 1998; Bianco et al. 2006). Therefore, the presence of these phytochemicals in M. aboensis (Onyegeme-Okerenta et al. 2013) may account for the alterations in the levels of the circulating hormone observed in this study. Prolactin helps to initiate breast development by inducing lobuloalveolar growth of the mammary gland. It also stimulates lactogenesis. Dopamine serves as the major-inhibiting factor or break on prolactin secretion (Fitzgerald et al. 2008). The enhanced level of prolactin observed in this study may be attributed to the effect of the extract probably acting as a dopamine antagonist. According to Fitzgerald et al. (2008), high prolactin levels tend to suppress the ovulatory cycle by inhibiting the secretion of both follicle-stimulating and gonadotropin-releasing hormones (GnRH) which are necessary for ovulation. Such increase in prolactin may inhibit ovulation and promote the loss of menstrual periods which will hinder conception. The elevated level of prolactin in this study justifies the folkloric use of the plant in stimulating lactation.

Photomicrographs of rats from the control group showed normal architecture of ovarian cells. However, photomicrographs of rats in group one showed partially destroyed ovarian cells while that of groups 2-5 were dose dependant and showed completely destroyed ovarian cells. It is probable that M. aboensis may have adverse effect on the ovary and may be one of the factors causing female infertility by its varied use in the management of other medical conditions by alternative medical practitioners and rural dwellers. This result agrees with the findings of Eweka, (2009). Photomicrograph of a section of the uterus of rats from all the groups showed normal uterine architecture. This implies that the extract did not have any effect on normal uterine architecture. This study showed that the aqueous extract of M. aboensis has a significant effect on the female reproductive hormones when compared with the control rats. Consequently, the extract may impair fertility and conception in female rats. Thus, leaf extract of M. aboensis may be explored as a female contraceptive.

References


