International Journal of Biological Sciences and Applications
2015; 2(5): 42-47
Published online October 20, 2015 (http://www.aascit.org/journal/ijbsa)
ISSN: 2375-3811

AASCIT American Association for Science and Technology

Keywords
Millettia aboensis,
Female Reproductive Hormones,
Ovary,
Uterus,
Photomicrograph

Received: June 28, 2015
Revised: July 8, 2015
Accepted: July 9, 2015

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

Onyegeme-Okerenta B. M.*, Essien E. B.

Department of Biochemistry, Faculty of Biological and Chemical Sciences, College of Natural and Applied Sciences, University of Port Harcourt, Choba, Rivers State, Nigeria

Email address

blessing.onyegeme-okerenta@uniport.edu.ng (Onyegeme-Okerenta B. M.),
minaemy@yahoo.co.uk (Onyegeme-Okerenta B. M.)

Citation

Onyegeme-Okerenta B. M., Essien E. B.. Effect of Leaf Extract of *Millettia aboensis* on the Reproductive Hormones and Organs of Female Wistar Albino Rats. *International Journal of Biological Sciences and Applications.* Vol. 2, No. 5, 2015, pp. 42-47.

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 hypothlamo-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. (Burkill, 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 2weeks, 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 90mins using Whatmann filter paper (125mm).

2.2. Experimental Design Animal Grouping and Extract Administration

Twenty-four female albino rats (Rattusnorvegicus) 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 4rats 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 for fourteen (14) days. Each rat group was also adapted to the commercial feed and water for the fourteen-day study period. Rats in Control Group were given commercial feed and water without the *M. aboensis* leaf extract.

The various groups (1-5) were orally administered with 0.5ml each of distilled water and the extract (1000, 2000, 3000, 4000 and 5000mg kg^{-1} body weight respectively) twice daily (09:00–09:45h) and (16:00-16:30h) using plastic syringe. The twenty-four rats were sacrificed after 14days. 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 anaesthetized 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 120mins at room temperature to clot, after which they were centrifuged at 3000rpm for 10mins 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.

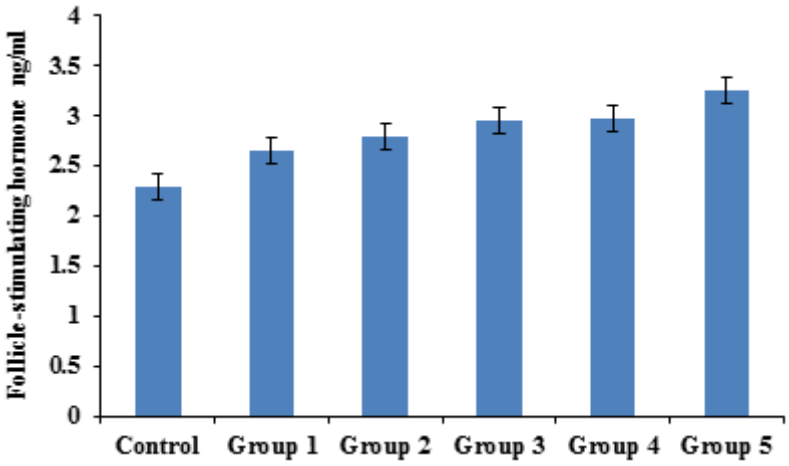

Figure 1. *Effect of aqueous leaf extract of M. aboensis on female rats' follicle-stimulating hormone levels.*

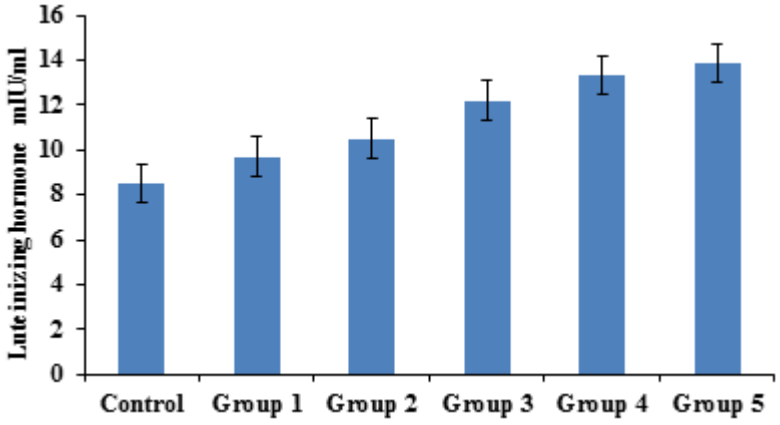

Figure 2. *Effect of aqueous leaf extract of M. aboensis on female rats' luteinizing hormone levels.*

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 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.

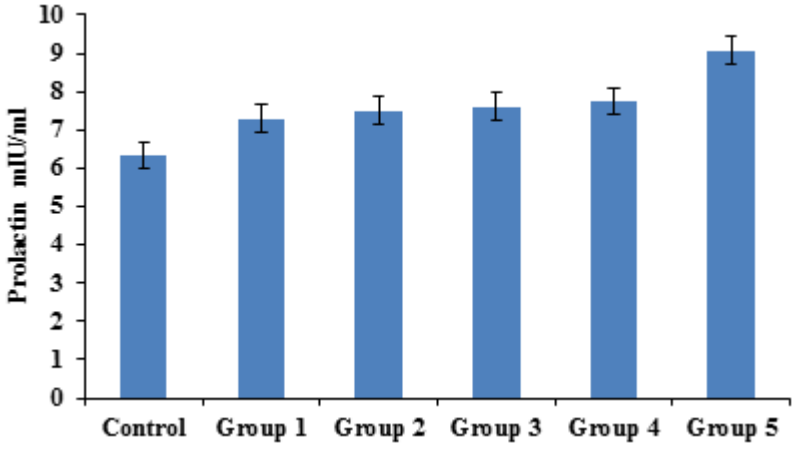

Figure 3. *Effect of aqueous leaf extract of M. aboensis on female rats' prolactin levels.*

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.

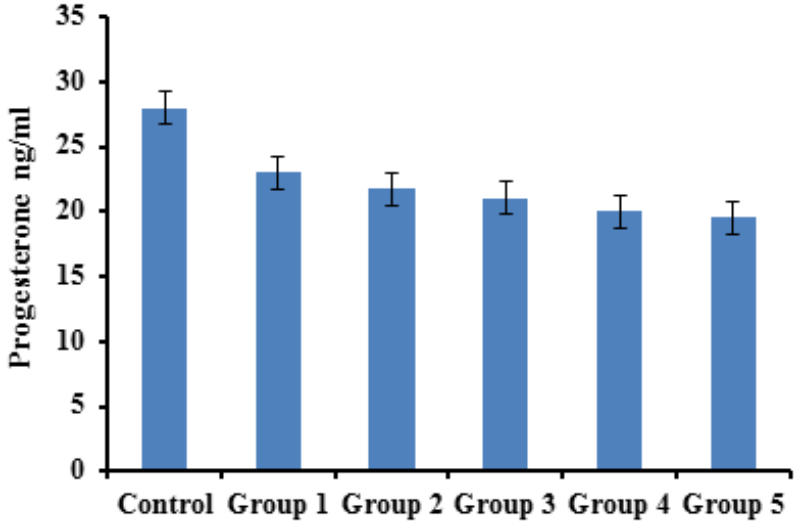

Figure 4. *Effect of aqueous leaf extract of M. aboensis on female rats' progesterone levels.*

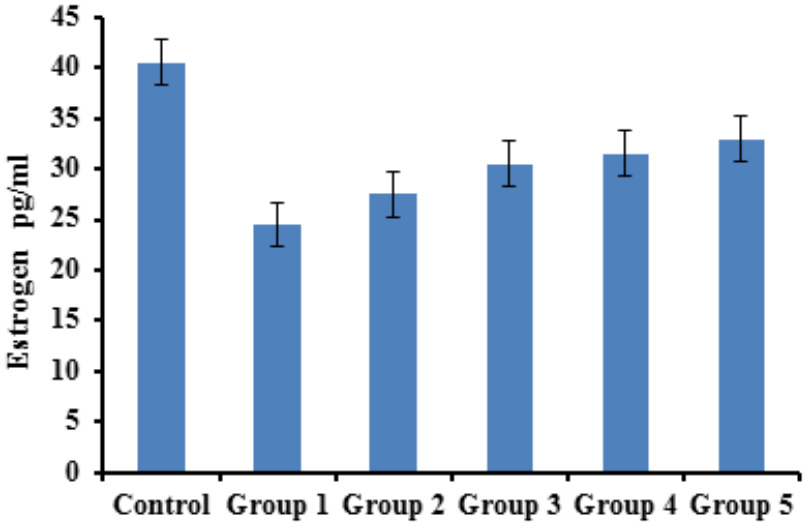

Figure 5. *Effect of aqueous leaf extract of M. aboensis on female rats' estrogen levels.*

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.

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).

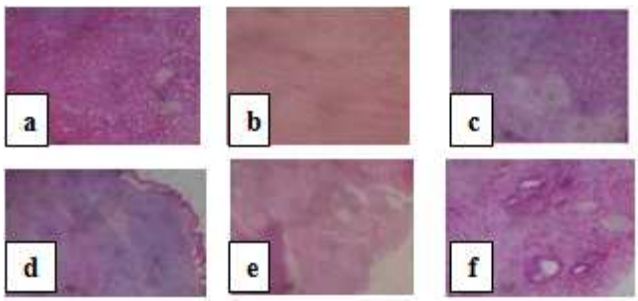

Plate 1. *Histopathological changes in ovaries of control and experimental rats. a. Control Group - shows normal ovarian cells; b. Group 1 - shows partially destroyed ovary; c– f. Groups 2–5 show completely destroyed ovarian cells.*

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.

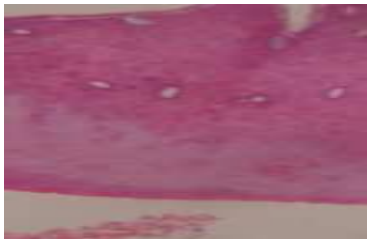

Figure 6. *Section of the uterus of rat of Group 5 showing normal uterine architecture.*

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 (Shivalingappa, *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 & Nieschlag, 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 gonadotropic-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

[1] Adonu, C. C., Ugwueze, M. E. & Ozioko, C. A. (2013). Phytochemical analyses of ethanol and water extracts of millettia aboensis, cuscuta reflexa, Daniella oliveri and synclisia scabrida International Journal of Pharmacy and Biological Sciences, 3(2): pp 64-69.

[2] Amah, C. I., Yama, O.E. & Noronha, C. C. (2012). Infecund evaluation of cycling female Sprague-Dawley rats: an aftermath treatment with Momordica charantia seed extract. Middle East Fertility Society Journal, 17(1): pp 37–41.

[3] Baker, J. T., Borris, R. & Carte, P. B. (1995). "Natural product drug discovery and development: new perspectives on international collaboration," Journal of Natural Products, 58 (9): pp 1325–1357.

[4] Benie, T., Duval, J. & Thieulant, M. L. (2003). Effects of some traditional plant extract on rat oestrous cycle compared with clomid. Phytotherapy Research, 17(7): pp 748-755.

[5] Bianco, F., Basini, G. & Grasselli, F. (2006). The plant alkaloid Sanguinarine affects swine granulose cell activity. Reproductive Toxicology, 21(3): pp 335-340.

[6] Brown, H. S. (2002). Hematoxylin and eosin (the routine stain). H&H informational primer. Sigma-Aldrich Corporation, pp 1–3.

[7] Browning, R., Schrick, F. N., Thompson, F. W. & Wakefield, T. (1998). Reproductive hormonal responses to ergotamine and ergonovine in cows during the luteal phase of the estrous cycle. Journal of Animal Science, 76(5): pp 1448-1454.

[8] Burkill, H.M. (2005). The useful plants of West Tropical Africa. Vol. 3, 1st Edition, Royal Botanical Garden, Kew, ISBN:10-0947643648.

[9] Emmanuele, M. A, Wezeman, F, & Emmanuele, N. V. (2002).Alcohol's effect on female reproductive function. Alcohol Research and Health, 26(4): pp 274-281.

[10] Eweka, A. O. (2009). Histological studies of the effects of oral administration of Aspilia africana (Asteraceae) leaf extract on the ovaries of female wistar rats. African Journal of Traditional, Complementary and Alternative Medicines, 6(1): pp 57-61.

[11] Fitzgerald, P. & Dinan T. G. (2008). Prolactin and dopamine: What is the connection? A review article. Journal of Psychopharmacology, 22(2): pp 12-19.

[12] Koneri, R., Balaraman, R. & Saraswati, C. D. (2006). Antiovulatory and abortifacient potential of the ethanolic extract of roots of Momordica cymbalaria Fenzl in rats. Indian Journal of Pharmacology, 38(2): pp 111–114.

[13] Lauritzen, C., Reuter H. D., Repges R, Bohnert, K. J., Schmidt, U. (1997). Treatment of premenstrual tension syndrome with Vitexagnus castus controlled double-blind study versus pyridoxine. Phytomedicine, 4(3): pp 183–189.

[14] Moore, K. L. & Persaud, T. V. N. (2008). The developing human. 8th ed. Netherlands: Saunders Elsevier Science. The Beginning of human development: first week; pp. 14–41.

[15] Onyegeme-Okerenta, B. M. Onyeike, E. N. & Esialekpe, F. O. (2013). Effect of ethanol leaf extract of millettia aboensis on selected haematological indices of Wistar albino rats. Global Advanced Research Journal of Medicinal Plants, 2(1): pp 004-011.

[16] Osonuga, O. A., Osonuga, I. O. & Osonuga, A. (2014). Oral administration of leaf extract of Momordica charantia affect reproductive hormones of adult female Wistar rats. Asian Pacific Journal of Troical Biomedicine, 4(Suppl 1): S521–S524.

[17] Raja, A., Gajalakshmi, P. & Raja, M. M. M. (2010). Drugs from the natural bio sources for human disease. International Journal of Pharmacology, 6(4): pp 360– 363.

[18] Sheeja, E. J., Ajeet, P., Papiya, B. & Shilpi, S. (2012). Antifertility activity of Momordica charantia descourt pulp and seed hydroalcohol extract. Journal of Applied Pharmaceutical Science, 3(4): pp 682–696.

[19] Shivalingappa, H., Satyanaranyan, N., Purohit, M. G., Sahranabasappa, A., & Patil, S.B. (2002). Effect of ethanol extract of Riveahypocraterifomis on the estrous cycle of the rat. Journal of Ethnopharmacology, 82(1): pp 11-17.

[20] Simoni, M. & Nieschlag, E. (1995). FSH in therapy: physiological basis, new preparations and clinical use. Reproductive Medicine Review, 4(3): pp 163–177.

[21] Telefo, P. B., Moundipa, P. F., Tchana, A. N., Tchouanguep Dzickotze, C. & Mbiapo, F. T. (1998). Effects of an aqueous extract of Aloebuettneri, Justiciainsularis, Hibiscus macranthus, Diclipteraverticillata on some physiological and biochemical parameters of reproduction in immature female rats. Journal of Ethnopharmacology, 63(3): pp 193-200.

[22] Uotila, M., Ruoslathi, E. & Envall, E. (1981). Two-site sandwich enzyme immunoassay with monoclonal antibodies to human alpha fetoprotein. Journal of Immunological Methods, 42(1): pp 11-15.

[23] Yakubu, M. T., Akanji, A. & Oladiji, A.T. (2005). Aphrodisiac potentials of the aqueous extract of Fadogiaagrestis (Schweinf.Ex Hiern) stem in male albino rats. Asian Journal of Andrology, 7(4): pp 399-404.

[24] Yama, O. E., Duru, F. I., Oremosu, A. A., Noronha, C. C. & Okanlawon, A. (2011). Suppressive effects of Momordica charantia on pituitary-testicular axis and sperm production in male Sprague-Dawley rats. International Journal of Medical Sciences, 3(12): pp 354–350.