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EFFECT OF MESUA FERREA FLOWER AND CARICA PAPAYA SEED EXTRACTS ON REPRODUCTIVE HORMONES IN FEMALE ALBINO RATS

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ABSTRACT
Flowers of Mesua ferrea (M.ferrea) and seeds of Carica papaya (C.papaya) medicinal plants used as having fertility and anti fertility potentials. Present investigation study the effect of both the plants on reproductive hormones of female rat. The Distilled Water and ethanolic extract of flowers of M.ferrea and seeds of C.papaya used for testing fertility and anti fertility activity in female rat. D.W and ethanolic extract of plants were administered orally to female rat for 21 consecutive days. Reproductive hormones (LH, FSH, Estrogen, Progesterone and Prolactine) were studied in both control and extract-administered groups by using standard methods. The present study which was undertaken to estimate the reproductive hormones of female albino rats after oral administration of different doses of D.W and ethanolic extracts of M.ferrea and C.papaya showed a significant fertility and anti fertility activity.

KEYWORDS: Fertility, Anti fertility, Reproductive hormones, Mesua ferrea, Carica papaya.

INTRODUCTION

Fertility hormones regulate the reproductive cycle and are used to early or delayed puberty as well as non reproductive test for various associated conditions including infertility and impotence in men and women disorder. The most common causes of female infertility are hormones. These are commonly associated with ovulation, ovarian syndrome, premature ovarian failure, damage to the Fallopian tube or uterus and problem with cervix. Endocrine disorders results from excessive production of hormones or insufficient production of one or more hormones or the lack of the tissues responses to normal circulating hormones.[1]

The female reproductive cycle function primarily by the interplay between the luteinizing hormone (LH), follicle stimulating hormone (FSH), progesterone, estradiol and prolactin. The integrity of the female reproductive organs can be assayed by the serum level of these hormones.[2]

According to our ethano medicinal survey, the plant is used in managing myriad of ailments and the leaves are widely consumed within many localities in Nigeria. This is however, without information on its effect on reproductive hormones of the females. This study was therefore designed to provide information on the effect of aqueous extract of C. aconitifolius leaves on female reproductive hormones. Several studies[3] have shown that chemical compounds including plant extracts could alter the concentrations and functions of female reproductive hormones.

Many plant species, half of them from tropical forest have been found to contain anti-fertility compounds. About 370 have been shown to provide assurance for safer and effective contraceptives suitable for both males and females.[4] Also more than 600 medicinal plant species are believed to have potential abortifacient property a good number of them from tropical forests[5]

Reproduction is an important phenomenon of all living organisms and essential for the perpetuation of species. It is controlled by the function of hypothalamo-hypophyseal gonadal axis. The mechanism regulating the growth and differentiation of an antral follicle and estrous cycle involves the hypothalamo-hypophyseal-ovarian axis and their hormones GnRH, gonadotrophins and steroids.[6] The hypothalamus and pituitary forms a functional unit that secretes the gonadotrophins in pulses. There is a tonic (low) pulsatile secretion and cyclic (high) pulsatile secretion. The hypothalamus regulates the rhythmic release of gonadotrophins i.e., FSH and LH through neural stimulus to gonadotrophins releasing hormone-GnRH.[7] The process of reproduction in mammals includes several stages like ovarian growth, follicular development, fertilization, implantation, gestation and lactation in females. Testicular growth and spermatogenesis maintenance of accessory reproductive organs in males. In females, the different stages of

ovarian development are reflected through the different phases of reproductive cycle.

Although, there were any reports on the effects of M.ferrea flowers and C.papaya seeds on the female reproductive functions have not been reported. The present study was designed to investigate the impact of M.ferrea and C.papaya on the female reproductive hormones.

MATERIALS AND METHODS

Fertility plant (Mesua ferrea**)**

(Fig: 1) (Plant)

(Fig: 2) (Flowers)

Anti-Fertility plant (Carica papaya)

(Fig: 3) (Plant & fruit)

(Fig: 4) (Seeds)

Collection and identification of plant material

The Medicinal plants of Mesua ferrea flowers collected from Anantapuramu super market and Carica papaya seeds collected from Anantapuramu surrounding gardens of Rayalaseema in A.P.The identification of plants material have been done by plant Taxonomist, Department of Botany,S.K.University,ATP.

The plant material was dried under room temperature without exposure to sunlight. The dried plant material was powdered in the grinder.5gm of Mesua ferrea flower powdered was soacked in 50 ml of D.W and Ethanol for 48 h, and filtered through whatmann filter paper No: 1 and using Soxhlet apparatus. Thus the filtrate was used as test solution in female albino rats.

Processing of plant extraction

(Fig: 5)

(Fig: 6)

(Fig: 7)

Figure 5, 6, and 7: Steps in Extract Preparation which includes grinding to form powder-like material and soxhlet extraction.

(Fig: 8)

(Fig: 9)

Figure 8, 9: Steps in Extract Preparation which includes grinding to form powder-like material and filter paper extraction.

Preparation of Test Drug /Dose
For fertility and ant fertility studies, concentrated ethanolic and D.W extracts of M. ferrea and C. papaya were freshly prepared at different concentrations and administered orally.

Animal model
Sexually matured, healthy female albino rats weighing 120- 160g were used for the experiments. The rats were housed in polypropylene cages, uder well ventilated animal house conditions (minimum temperature: 27-30^{0}c, photoperiod=12h natural light and 12h dark).The rats were given pelleted feed and tap water ad libitum. The experimental protocol was approved by the Institutional Animal ethics committee.

Experimental Design
The animals were divided into 4 groups, consisting of 6 animals in each group. Group-I was maintained as Control and the remaining groups II, III and IV were administered orally 100 mg/kg, 200 mg/kg and 300 mg/kg body weight of M.ferrea and C.papaya extract by orally using intragastric tube for 21 days daily.

Experimentation I
The animals were divided into 4 groups, consisting of 6 animals in each group.
Group- I: - Control (Received D.w).
Group- II: - Treated with 100 mg D.w Extract /kg b.w.
Group-III: -Treated with 200 mg D.w. extract /kg b.w.
Group-IV: - Treated with 300 mg D.W. extract/kg b.w.

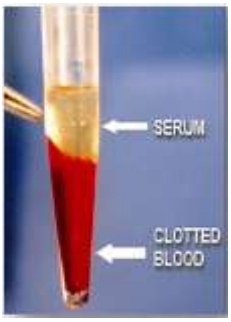
Experimentation II
The animals were divided into 4 groups, consisting of 6 animals in each group.
Group- I: - Control (Received D.w).
Group- II: - Treated with 100 mg Ethanol Extract /kg b.w.
Group-III: -Treated with 200 mg Ethanol extract /kg b.w.
Group-IV: - Treated with 300 mg Ethanol extract/kg b.w.

All the above treatments were given orally by using intragastric catheter for 21 days to studies the hormonal parameters.

Quantitative estimation of hormones in control and treated animals:-Sample Collection
At the end of 21days experiment, the animals were anaethesized in a chloroform chamber and the blood samples were collected by Cardiac puncture. The serum was separated out and used to estimate the levels of Estrogen, progesterone, luteinizing hormone (LH), follicle stimulating hormone (FSH) and prolactin concentrations by Enzyme Linked Immunosorbent Assay (ELISA).


(Fig: 10)


(Fig: 11)



(Fig: 12)


(Fig: 13)

Terminal blood collection by Vacutainer tubes without Centrifuge for 15min Serum containing Cardiac puncture in a deeply containing anticoagulant at 2500 Rpm Cryovials Anesthetized rat. 5 ml Lavender top

Hormonal Assay
Estrogen, progesterone, luteinizing hormone (LH), follicle stimulating hormone (FSH) and prolactin were measured using Enzyme Linked Immune sorbent Assay (ELISA). This assay is based on the high specificity of antibodies to bind molecules which in this case hormones. The antibody is tagged with an enzyme. Since the enzyme labelled with antibody reacts/binds with the hormone, the amount of the hormone present can be obtained by introducing a substrate for the enzyme that forms a coloured product and the optical density (OD) of the sample is measured. The intensity of colour is proportional to the concentration of the bind hormone.

Statistical Analysis
The results obtained from this study were analyzed using the Statistical Package for Social Sciences version 2007 for windows and expressed as Mean ± SD. Analysis of variance with Tukey test for multiple comparisons was used to analyze LH, FSH, prolactin, estrogen and progesterone data. Statistical significance was defined as $P < 0.05$.

RESULTS

Table 1: Effect of 100mg/kg, 200mg/kg and 300mg/kg D.W Extract of Mesua ferrea flowers on Estrogen, progesterone LH, FSH and Prolactin Level in Female Albino Rats:

Treatment of the group	Dose mg/kg body weight	No. of days	Name of the hormones				
			Estrogens Pg/ml	Progesterone ng/ml	FSH miU/ml	LH miU/ml	Prolactin ng/ml
Group-I	Control	____	6.22 ± 0.32	0.85 ± 0.10	1.99± 0.25	3.2 ± 0.5	1.97 ± 0.48
Group-II	100	21	6.15 ± 0.29	1.74 ± 0.16	1.92± 0.21	2.8 ± 0.4	1.91 ± 0.42
Group-III	200	21	6.01 ± 0.24	1.98 ± 0.29	1.81± 0.18	2.5 ± 0.3	1.82 ± 0.38
Group-IV	300	21	5.85 ± 0.15	2.45 ± 0.38	1.62± 0.14	2.2 ± 0.1	1.62 ± 0.30

*Values are means and standard errors for 6 rats per treatment. Means along the row with different superscript are significantly different (P > 0.05).

Table 2: Effect of 100mg/kg, 200mg/kg and 300mg/kg Ethanol Extract of C.papaya seeds on Estrogen, progesterone LH, FSH and Prolactin Level in Female Albino Rats.

Treatment of the group	Dose mg/kg body weight	No. of days	Name of the hormones				
			Estrogens Pg/ml	Progesterone ng/ml	FSH miU/ml	LH miU/ml	Prolactin ng/ml
Group-I	Control	____	6.22 ± 0.32	0.85 ± 0.10	1.99± 0.25	3.2± 0.5	1.97 ± 0.48
Group-II	100	21	6.18 ± 0.28	0.70 ± 0.4	1.89± 0.19	2.6±0.3	1.89 ± 0.42
Group-III	200	21	6.14 ± 0.18	0.54 ± 0.2	1.81± 0.17	2.4± 0.2	1.82 ± 0.28
Group-IV	300	21	5.90 ± 0.15	0.25 ± 0.1	1.72± 0.12	2.1± 0.1	1.71 ± 0.13

*Values are means and standard errors for 6 rats per treatment. Means along the row with different superscript are significantly different (P < 0.05).

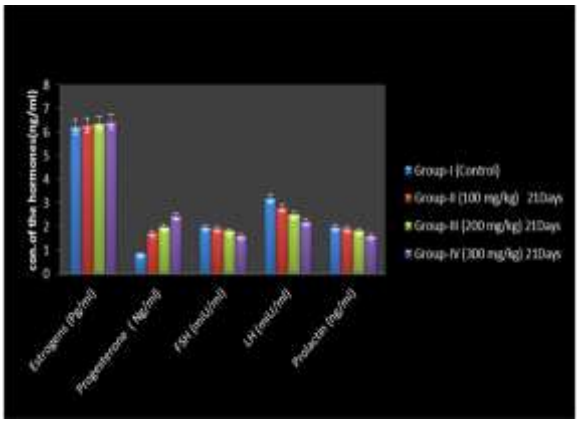


Figure 14: Effect of 100mg/kg, 200mg/kg and 300mg/kg D.W Extract of Mesua ferrea Flowers on Estrogen, progesterone LH, FSH and Prolactin Level in Female Albino Rats.

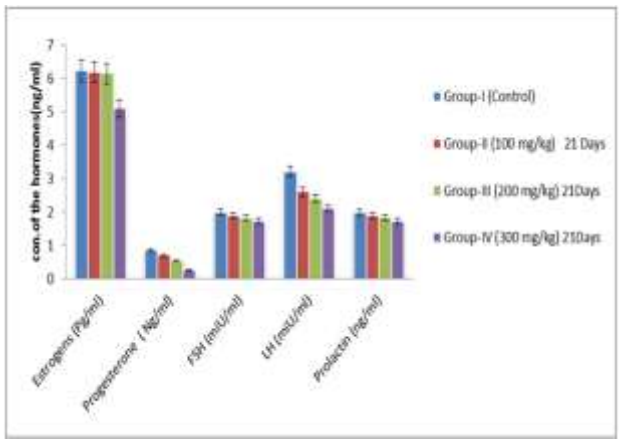


Figure 15: Effect of 100mg/kg, 200mg/kg and 300mg/kg Ethanol Extract of C.papaya seeds on Estrogen, progesterone LH, FSH and Prolactin Level in Female Albino Rats.

DISCUSSION

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.[8] These hormonal imbalances may be used by numerous chemical agents contained in plant extracts. Phytochemical screening has revealed many bioactive as well as toxic agents of plant extracts that can affect the regulation of estrous cycle conception and reproduction.[9,10] Alkaloids and flavonoids have been shown to reduce serum concentrations of LH, FSH, estradiol and Progesterone.[11,12] Therefore, the presence of these phytochemicals 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 lacto genesis. Dopamine serves as the major-inhibiting factor or break on prolactin secretion.[13] 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.[14] It stimulates the growth and maturation of ovarian follicles by acting directly on the receptors located on the granulose cells. The reduction in the levels of FSH by the extract may hamper folliculogenesis and delay maturation of the follicle in the pre-ovulatory phase.[15] It is possible that the extract might have exerted its effect on the anterior pituitary or the hypothalamus since the secretion of FSH is regulated by the gonadotropic releasing hormone

secreted by the hypothalamus. The reduction in the levels of the hormone may adversely affect conception in the female animals. This study agreed with the work of Benie et al.[16] where administration of Afrormosia laxiflora, Pterocarpus erinaceus and Cola nitida stem bark decreased the release of the gonadotropins (LH and FSH). Luteinizing hormone stimulates secretion of sex steroids from the gonads, in females.

This present study was aimed at investigation the effect of D.W extract of Mesua ferrea on gonadotropins showed that Mesua ferrea L. contains phytochemical contents such as glycosides, flavonoids terpenoids and alkaloids. Alkaloids and flavonoids have been shown to reduction serum concentration of LH, estradiol and FSH (11, 12). Udoh et al.,[17] also reported that increased dose of alkaloid extract significantly increased serum levels of FSH, LH and testosterone.

The effect of administration of aqueous extract of M.ferra flowers extract at 100, 200 and 300 mg kg- body weight for 21days on the concentration of serum reproductive hormone (Estrogen and Progesterone) was increased in all treated groups when compared with the control, but LH, FSH and prolactin concentrations were decreased after the first, second and third dose when compared with the control. At 21st day low dose (100mg/kg body weight) administration produced high significant effect on the serum hormone (Table: 1). The concentration of estrogen and progesterone in the serum was increased by the extract, and the concentrations of follicle stimulating hormone (FSH), luteinizing hormone (LH) and prolactine in the serum were decreased by the extract when compared with control. The least dose (100 mg /kg body weight) on the 21st day produced high significant change ($p>0.05$) in the concentration of the hormones (table: 1). However, by the end of the treatment period, the concentration of estrogen and progesterone had increased by 20%, 24% and 31% respectively in all treated groups when compared with control.

The effect of administration of aqueous extract of C.papaya seeds at 100, 200 and 300 mg/ kg body weight for 21days on the concentration of serum reproductive hormones in the female rats were decreased in all treated groups when compared with the control, administration of the extract produced reduces ($p<0.5$) in serum of Estrogen, progesterone, LH, FSH and prolactin concentration after the first, second and third dose. The 21st day low dose(100mg/kg) administration produced low significant effect on the serum hormone (table: 2). The concentrations of follicle stimulating hormone (FSH), luteinizing hormone (LH), progesterone and estradiol levels in the serum were decreased by the extract. While the concentrations of FSH, LH and progesterone were decreased in all the groups, the least dose (100 mg /kg body weight) on the 21st day produced low significant change ($p<0.05$) in the concentration of the hormones (table: 2). However, by the end of the treatment period, the concentrations of Estrogen, progesterone, FSH, LH and prolactine had decreased by 15%, 22% and 30% respectively in all treated group when compared with control.

Reported that several plant alkaloids inhibit aromatase activity, thus altering the potential for steroid production and reproductive performance. Therefore, the alkaloid in the extract may be responsible for the reduced level of estradiol probably by inhibiting aromatase activity. Thus, it is possible that the aqueous extract of Carica papaya seeds contain biologically active phytochemicals which may be endocrine disrupting. Such substances in the plant extract may induce hormonal imbalance or disorders such as infertility and contraception in hormones dependent organs like the ovary and mammary glands. Our findings in this study have important implications for female contraceptive development. Plant products as contraceptive will be more acceptable for economic reasons and side effects that are less than chemical agents.

CONCLUSION

It was concluded that the study of aqueous extract of M. Ferrea flower & C. Papaya seed administered orally to Female albino rats increased and decreased the hormones, suggesting that these two plant extracts might boost up the general health of the animal. As the rat is a mammal the estrous cycle is same what similar with that of the humans, Hence these plant materials can also be used in human beings.

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REFERENCES

1. Baird D.T, V.H.T. James, M. Serio and G. Giusti, "Ovarian steroid secretion and metabolism in women. In: the endocrine function of the human ovary". Academic press, New York, 1976; 125-133.
2. Bowman W.C. and Rand M.J. The endocrine system and drug affecting endocrine function. In: W.C.W. Bowman and M.J. Rand (Eds.), Textbook of Pharmacology, 1980; 2: 19.1-19.64. Oxford: Blackwell Scientific Publications.
3. Telefo PB, Moundipa PF, Tchana AN, Tchouanguep Dzickotze C, Mbiapo FT. Effects of an aqueous extract of Aloe buettneri, Justicia insularis, Hibiscus macranthus, Dicliptera verticillata on some physiological and biochemical parameters of reproduction in immature female rats. J Ethnopharmacol, 1998; 63: 193-200.
4. Maurga R, Scrivstara S, Kulshresh D.K. and Gupta C.M. Traditional remedies for fertility regulation. Current Medical Chemistry, 2004; 11: 1431-1450.

5. World Health Organization. 1981: Report of Task Force on Anti-fertility plants Especially from Tropical Forests. WHO Geneva, Switzerland.
6. Devi GU, Changamma C, Govindappa S. Regulation of estrous cycle in hysterectomized albino rats by activation of follicular development. Ind J Exp Biol, 1992; 30: 778.
7. Silverman AJ, Livne I, Witkin JW. The Gonadotrophin-Releasing Home (GnRH), Neuronal Systems: Immunocytochemistry and In situ Hybridization. In: The physiology of Reproduction, 2nd Edn., Knobil, E. and J.D. Neill (Eds.). Raven Press, New York, ISBN: 9780781700863, 1994; 3312.
8. Shivalingappa H, Satyanaranyan ND, Purohit MG, Sahranabasappa A, Patil SB. Effect of ethanol extract of Rivea hypocraterifomis on the estrous cycle of the rat. J Ethnopharmacol, 2002; 82: 11-17.
9. Benie T, Duval J, Thieulant ML. Effects of some traditional plant extracts on rat oestrous cycle compared with clomid. Phytother Res., 2003; 17: 748-755.
10. 21. Yakubu MT, Akanji MA, Oladiji AT. Aphrodisiac potentials of the aqueous extract of Fadogia agrestis (Schweinf. Ex Hiern) stem in male albino rats. Asian J Androl, 2005; 7: 399-404.
11. Lauritzen C, Reuter HD, Repges R, Bohnert KJ, Schmidt U. Treatment of premenstrual tension syndrome with Vitex agnus castus controlled double-blind study versus pyridoxine. Phytomedicine, 1997; 4: 183–189.
12. Bianco F, Basini G, Grasselli F. The plant alkaloid Sanguinarine affects swine granulose cell activity. Reprod Toxicol, 2006; 21: 335-340.
13. Fitzgerald P, Dinan TG. Prolactin and dopamine: What is the connection? A review article. J Psychopharmacol, 2008; 22: 12-19.
14. Simoni M, Nieschlag E. FSH in therapy: physiological basis, new preparations and clinical use. Reprod Med Rev., 1995; 4: 163–177.
15. Kumar TR, Wang Y, Lu N, Matzuk MM. Follicle stimulating hormone is required for ovarian follicle maturation but not male fertility. Nature Genetics, 1997; 15: 201–204.
16. Benie T, El Izzi A, Tahiri C, Duval J, Thieulant ML. Combretodendron africanum bark extracts as an antifertility agent. 1: estrogenic effects in vivo and LH release by cultured gonadotrope cells. J Ethnopharm, 1990; 29: 13-23.
17. Udoh F.V, Udoh P.B, Umoh E.E, Activity of alkaloid extract of Carica papaya seeds on reproductive functions in male wistar rats. Pharm. Bio., 2005; 43: 563-567.