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EFFECT OF EHRETIA MICROPHYLLA LAMK ON STIMULATION OF REPRODUCTIVE FUNCTION AND OVARIAN FOLLICULOGENESIS IN RATS

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ABSTRACT

This study has been undertaken to investigate the effect of *Ehretia microphylla L.*_ on folliculogenesis, relative ovary and uterus weight and the number of ovarian surface follicles in female Wistar albino rats. The study consisted of 24 female wistar rats that were divided into four groups. Group-I 0.5 ml distilled water, Group II, III and IV were orally administered with powdered form of *E. microphylla L* and adjuvant at 500 mg/kg and 1000 mg/kg and only adjuvant (Palm jaggery)respectively. After 10 days, blood samples were taken from all groups in order to measure the serum levels of luteinizing hormone (LH), follicle stimulating hormone (FSH) and estradiol hormones. Ovaries and uterus were removed for histopathology. Significant increase in FSH, LH and estradiol levels, ovarian and uterine weight was observed along with enhanced folliculogenesis in the experimental groups. Thus results suggest significant stimulatory effect on female reproductive activity which can enhance fertility in female adult rats.

KEYWORDS: ***Ehretia microphylla*, Palm Jaggery, FSH, LH, Estradiol**

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INTRODUCTION

Siddha system is said to be divine and holistic system of medicine as it offers excellent medicines and lifestyle guidelines for healthy living. Siddha heritage is invaluable as it helps to acquire health for physique, peace for mind and purity for the soul. Women experience menstrual cycle every month reflecting the greater natural cycle of earth, hence women is given time to nurture their creative potential. Infertility is a complex disorder with significant medical, psychosocial and economic aspects. Medications can be administered to induce follicular development and ovulation.[1]Siddha system has a long history of utilization of plants in enhancing fertility and curing reproductive problems for women. Siddha drugs have incredible remedial supremacy for regulating hormones, stimulating healthy ovulation, and maintaining regular menses in women. Herbs have been the highly valued source of medicine throughout human history since knowledge and usage of plants are of divine origin. Herbs are superior due to their well documented, non toxic and inexpensive healing properties. According to the World Health Organization about 80% of the world's population uses plant products in handling primary medical problems due to their accessibility, availability and affordability.[2]The hormonal therapy currently in use is “accused” to impair some physiological activity and while mitigating another disorder. This has resulted in the greater acceptability of herbal drugs as alternative therapy.[3] In recent decades, due to the undesirable side effects of chemical drugs, more emphasis has been placed on the use of traditional medicine, particularly plant therapy. *Ehretia microphylla* is an erect, much branched shrub growing up to 1.5-4 m high. It contains flavonoids, phytosterols, and alkaloids. It has anti-inflammatory, antibacterial, analgesic, anti-allergic, anti-mutagen, anti-diarrheal, antimicrobial and anti-tumor activity. Thus far, there have been no research studies concerning the effects of *E. microphylla* on the female reproductive system and ovarian function. Hence the present study was undertaken to validate the possible effects of *E. microphylla* on LH, FSH, estradiol levels and development of number of ovarian surface follicles in female rats and its acute toxicity. The results of this study can be useful to stimulate ovulation and regulate women’s menstrual cycles and reduces the incidence of anovulatory infertility.

MATERIALS AND METHODS

(i) Collection and authentication of plant material:

The plant material was collected from Kollimallai Hills, Salem district, Tamilnadu, India in the month of May 2011. The plant materials were authenticated by Botanist, Central Research Institute for Siddha, Chennai. The drug “*kuruvichipoondu Chooranam*” (powdered form of *Ehretia microphylla*) was selected from the classical Siddha literature *Pathartha Guna Villakam* written by Kannusamiyam Pillai.

(ii) Preparation of drug:

The fresh whole plant of Kuruvichipoondu was thoroughly cleaned to remove soil particles and impurities. Then the plant was cut into small pieces and dried in shade. Later they were finely powdered.

(iii) Selection of animals:

Mice of either sex of wistar strain weighing 25-30 gm and Female albino rats of wistar strain weighing about 95–135 gm were used. Pregnant animals were excluded. Animals were fed on conventional diets and water *ad libitum* and they were maintained under standard conditions of humidity, temperature (20- 24°C) and light (12 h light: 12 h dark cycle).Animals were kept in polycarbonate cages with laced steel roofs. The animals were acclimatized for one week under laboratory conditions. The study was conducted at the Vel’s University, Chennai after obtaining

Institutional Animals Ethical Committee clearance bearing the number (XII/VELS/COL/16/CPCSEA/IAEC/23.09.11).

(v) Acute toxicity study:

Acute oral toxicity test was carried out as per OECD Guidelines 425 up and down method. Animals are observed individually after dosing at least once during the first 30 minutes, periodically during the first 24 hours, with special attention given during the first 4 h, and daily thereafter, for a total of 14 days. Initially starting at a dose of 2000 mg/kg of *Ehretia microphylla L* was given. Body weight and behavioral changes were noted. [4]Animals are observed individually and were systematically recorded. The acute toxicity was not occurred at 5000mg/kg (as per the OECD-425) after 48 hours of oral drug treatment at the dose level of 5000mg/kg and total duration of study was 14 days. Hence, one-tenth and one fifth dose was selected as therapeutic dose from maximum tolerable dose for further pharmacological study.

(vi) Ovulation stimulation activity:

In the present study, twenty four Virgin female wistar rats weighing of around (95- 135 gm) of 2 month old were obtained from the animal house at Vel's University, Chennai. Rats were divided into four groups of six as follows: control group I (no intervention, other than dry food and water).Three experimental groups which received adequate food and water in addition to *Kuruvichi poondu chooranam* (KPC) in the following doses: experimental group II rats were administered lower dose of trial drug KPC of 500 mg/kg with adjuvant palm jaggery orally for 10days,experimental group III received a higher dose of 1000mg/kg KPC with palm jaggery of for 10 days and experimental group IV were given only adjuvant palm jaggery of 500 mg/kg. Following groups are designed.

***(vii) Synchronization of animals reproductive cycles*:**

Prior to beginning of experiment, the reproductive cycles of the rats were synchronized by the following method. 100µg estradiol dissolved in 2 ml olive oil was injected subcutaneously. All rats after a 24 hr period, received intramuscular injections of 50 µg progesterone dissolved in olive oil. After few hours, vaginal smears were prepared by washing vaginal opening with 0.9% w/v of sodium chloride with a glass dropper and placed in a clean glass slide and viewed under light microscope at 40X magnification. Examination of vaginal smears showed that all the animals were in the estrous stage.[5] All the animals are weighed daily after drug administration for 10 days.

(viii) Experimental procedure:

After 10 days, the animals in each group were weighed. Groups of animals were killed by decapitation (n=3) at the end of the experiment. 2 ml of blood was collected by cardiac puncture. Blood samples were centrifuged for 15 minutes at 4000 rpm and the separated serum samples were frozen at -20°C and kept for later estimation of LH, FSH and estradiol by ELISA method. At autopsy, the oviducts and uterus were dissected and weighed.[6] Uterus and ovaries were carefully removed and fixed in a formalin solution at a pH of 7.0. The organs were fixed in Bouin's fluid, embedded in paraffin wax, sectioned serially at 10 mm, and stained with haematoxyline-eosin. Uterus and ovarian sections were studied with a light microscope.

(ix) Statistics:

Statistical significance of data was assessed by analysis of variance (one-way ANOVA) followed by a comparison between different groups using Dunnet test.

RESULTS

(i) Acute toxicity study:

The acute toxicity of *Kuruvichi poondu chooranam* had not occurred at 5000mg/kg (as per the OECD-425) on mice after 48 hours of oral drug treatment at the dose level of 5000mg/kg and total duration of study was 14 days and behavioral changes are normal. The data present in table no.1.

Table No. 1
Dose finding experiment and its behavioral Signs of Toxicity

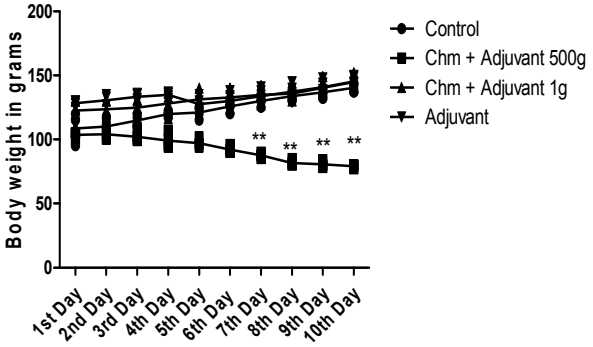
S. No	Dose mg/kg	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1.	500	+	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	+	-
2	1000	+	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	+	-
3	2000	+	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	+	-
4	5000	+	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	+	-

1. Alertness, 2. Aggressiveness, 3. Pile erection, 4. Grooming, 5.Gripping, 6. Touch Response, 7.Decreased Motor Activity, 8.Tremors, 9.Convulsions, 10. Muscle Spasm, 11. Catatonia, 12.Muscle relaxant, 13. Hypnosis, 14.Analgesia, 15.Lacrimation, 16.Exophthalmos, 17.Diarrhoea, 18.Writhing, 19.Respiration, 20. Mortality,

(ii) Ovulation stimulation activity:

KPC administration shows gradual increase in body weight in control, 1gm/kg and adjuvant alone. However, significant decrease ($P<0.001$) in was observed in low dose treated group from 7-10thday as compared with control group. The result present in graph 1.

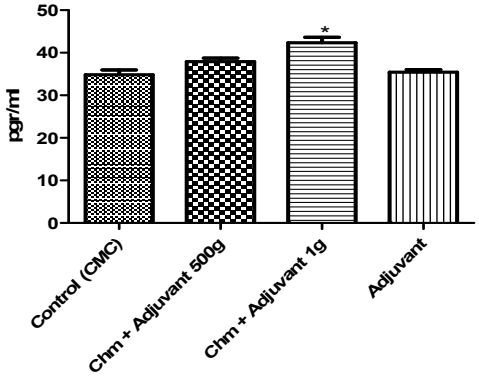
Graph 1
Effect of KPC + adjuvant, Adjuvant alone in body weight changes



The relative weight of uterus and ovary were significantly increased ($P<0.05$) in experimental groups that received 1gm/kg at the end of the tenth day the result present in graph 2 and 3 as compared with CMC treated normal female rats. There is no significant changes were observed in low dose and adjuvant treated female rats.

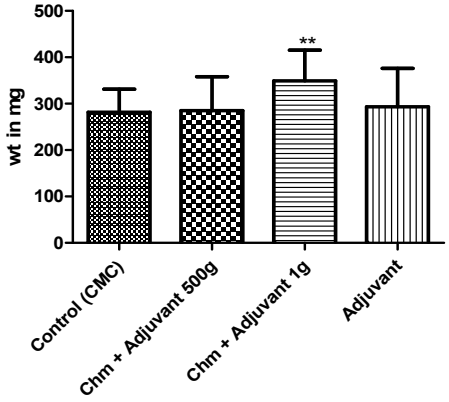
Graph 2

Effect of KPC + adjuvant, Adjuvant alone in ovary weight in female rats



Graph 3

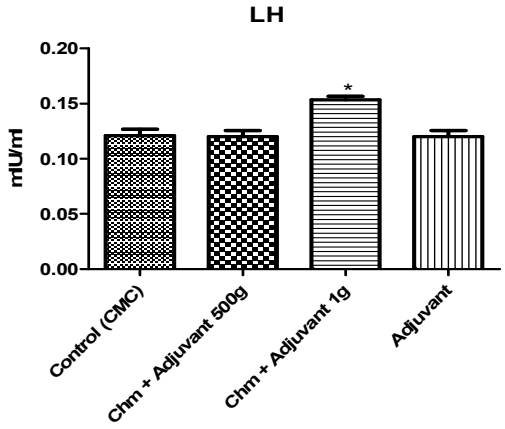
Effect of KPC+ adjuvant, Adjuvant alone in Uterus weight in female rats



In addition, the concentration of LH, FSH, estradiol hormones showed a significantly ($P<0.05$) increase in rats significant rise in experimental group III, which received the high dose the result present in graph 4, 5, 6.

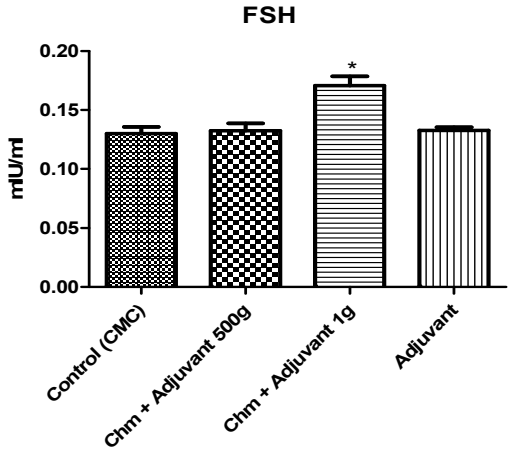
Graph 4

Effect of KPC + adjuvant, Adjuvant alone in luteinizing Hormone (LH) level in female rats



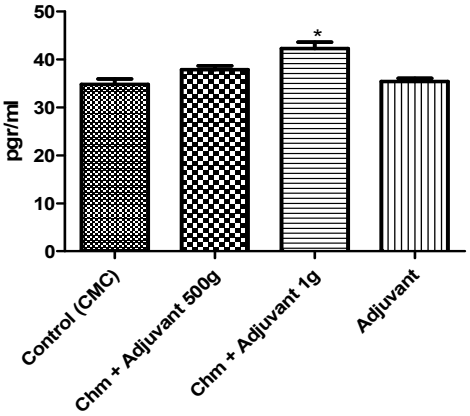
Graph 5

Effect of KPC + adjuvant, Adjuvant alone in Follicle Stimulating Hormone (FSH) level in female rats

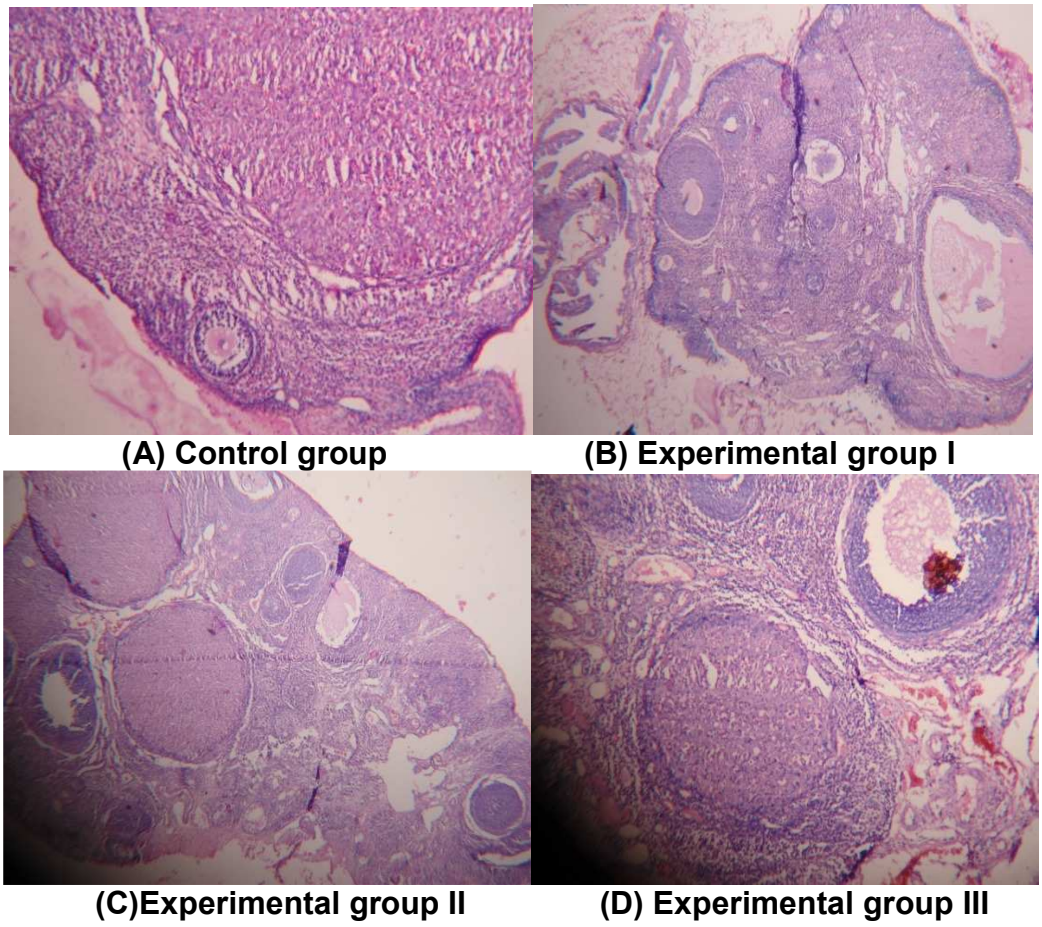


Graph 6

Effect of KPC + adjuvant, Adjuvant alone in Estradiol level in female rat



Histological studies revealed that KPC treated at high dose showed important changes in ovarian tissues with increased number of Primordial follicles, matured graffian follicles and corpus luteum. The result present in figure 1(A-D).



(A) Control group **(B) Experimental group I**

(C)Experimental group II **(D) Experimental group III**

Figure 1 (A-D)
Histopathology study of ovary tissue

DISCUSSION

Summation of all results shows that there is a marked increase in body, uterine and ovary weight, LH, FSH, estradiol is noted in group III (1000mg/kg.+ adjuvant) p.o treated rats compared to group I, group II, and group IV. Moreover number of ovarian follicles with matured graffian follicle and corpus luteum are increased in group III when compared to other groups. Follicle development is a complex and dynamic process requiring the coordinate interactions of multiple intra gonadal and extra gonadal factors.[7]The results shows that (KPC) at high dose stimulates ovulation in female rats. The body weights of the rats treated with high dose KPC increased significantly. It may stimulate hypothalamus-pituitary-ovarian axis which is responsible for the synthesis and storage of gonadotropins LH and FSH which play a major role as regulators of folliculogenesis.[8]Administration of KPC increases FSH, LH and estradiol due to ovarian steroidogenesis.[9]The increase in ovarian weight is regulated by plasma gonadotropins (FSH and LH)and uterine weight by ovarian steroids(estrogen and progesterone).[10]Our results suggest that ovarian steroidogenic function is increased after treatment with high dose of KPC along with increased pituitary gonadotropins release when compared to other group.[11] Histological examinations of follicles of ovaries treated with high dose of KPC showed that there was an increase in the number of primary and secondary follicles, graffian follicle and corpus luteum with less atretic follicles. Hence, this study shows *Ehretia microphylla* enhances

folliculogenesis by acting through hypothalamic- pituitary-ovarian axis.[12]

CONCLUSION

The findings of this study show that 1000mg/kg of powdered form of *Ehretia microphylla* promote the pituitary-ovary axis activities at all levels, cause an elevation in the serum concentrations of LH, FSH and estradiol hormones, as well as increase the mean numbers of follicles and eventually ovarian weight. Hence *Ehretia microphylla* KPC helps in treating irregular ovulation disorders and promotes fertility in female.

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