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Original Research Article

OVULATION INDUCING AND FOLLICULOGENETIC ACTIVITY OF *UPPU PARPA* IN FEMALE WISTAR ALBINO RATS

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ABSTRACT

This study has been undertaken to investigate the effect of *Uppu parpa* on folliculogenesis, relative ovary and uterus weight and number of ovarian surface follicles in female Wistar albino rats. The study consisted of 6 female wistar rats in a group and totally 24 rats were taken. Group-I was given 2ml/kg of CMC solution, Group II and III were received 50mg and 100mg of *Uppu parpa* respectively and group IV received Clomiphene citrate 10mg/kg body weight and it is considered as standard. 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 studies. Significant increase in FSH, LH and estradiol levels, ovarian and uterine weight was noticed along with increased folliculogenesis in the experimental groups. Thus the results suggested the significant ovulatory response in female rats, and can be used clinically in reproductive hormonal disorders and in infertility condition of female.

Key words: Clomiphene citrate, Folliculogenesis, FSH, LH, Ovulation inducing activity, Siddha Medicine, *Uppu Parpa*

INTRODUCTION

Siddha system of medicine is enriched with unique and peculiar aspects with treasure house of secret science. They classified various female diseases and had amazing solutions for its curative measures and also handled with its preventive aspects. The system of medicine revitalizes and rejuvenates the internal organs and provides guidelines for healthy lifestyle for healthy living.

But as on date in the modern world the most valued female gender is subjected to many gynecological complaints and ill health, in accordance in the production of healthy future generations such as infertility, PCOS, and other menstrual complaints that interferes with reproduction. Although it was first discovered in 1935 [1], PCOS was then considered an obscure reproductive disorder. However, today this is most common female endocrine disorder, often resulting in infertility and genetic disorder in which each child has 50% of inheriting from the parent who carries the gene [2]. Approximately about 5% to 10% of women of reproductive age (12-45 years old) produce symptoms of PCOS. It is considered to be one of the leading causes of female subfertility [3 - 5]. It is the most common factor of anovulation with normal serum FSH and estradiol levels. Even though the heterogeneity in symptoms associated with PCOS, the chief feature is arrested follicular development at the stage when dominant follicle occurs normally [6].

It is seen that due to insulin resistance, female teenagers are subjected to overgrowth of facial hair, acne, irregular menses, weight gain or increase in body fat. Women during reproductive years may experience not only infertility, miscarriages, but also higher incidence of gestational diabetes during pregnancy. In addition to these later in life, women are at higher risk of developing type 2 diabetes, cardio vascular disease, sleep apnea and endometrial cancer is also noticed in some cases [7, 8]. Further it is visualized that depression is uncommon in women with PCOS. So it is an essential factor to have an eye towards the distressing syndrome and to save the valuable women's health.

As in modern medicine it is treated as in reducing insulin resistance by correcting insulin sensitivity through medications such as metformin, and the newer thiazolidinedione, have been an evident approach and initial studies shows efficacy [9 - 11].

In the present investigation the influence of *Uppu parpa*- a traditionally used Siddha drug was evaluated for its ovulation stimulatory response in female wistar albino rat models and the potency was compared with reference drug clomiphene citrate.

MATERIALS AND METHODS

Preparation of the trial drug

Selection of drug:

The trial drug ***Uppu parpa*** was selected from the Siddha classical text "*Anubava Siddha Vaithiya Muraigal*" [12].

Collection of drug:

The ingredients of *Uppu parpa* as per Siddha literature are *Vediuppu* (Potassium nitrate), *Indhuppu* (Sodium bicarbonate), *Gendhiuppu* (Bitloban), *Valaiyal Uppu* (Sandevere), *Vengaram* (Borax) and *Pooneeru* (Fuller's earth). All the raw materials except Fuller

earth were purchased from raw drug shop at Chennai and the raw material *Pooneeru* (Fuller's earth) was collected from Siddhamalli village near Uthiramerur. They were identified and confirmed by *Gunapadam* experts, Post graduate department of *Gunapadam* (Pharmacology), Government Siddha Medical College, Chennai. The samples of these raw materials have been kept in the department for future reference.

Preparation of Uppu parpa:

The raw drugs were subjected to *Suddhi* (Purification process) as per classical Siddha text [13].*Vengaram* is grounded to fine powder in *Kalvam* (Stone mortar with pestle) and it was divided into two parts and kept separately. Then other raw materials were ground in *Kalvam* until the fine powder obtained. Mud vessel was taken and one of the part of *Vengaram* was spread evenly and pressed inside the mud vessel. Then other powdered drugs from one to five placed in the vessel. Then the other half of *Vengaram* powder was spread and pressed uniformly over the other raw materials. Then another suitable mud lid was kept which was covered with seven layers of *Seelaiman* (seven layers of moist mud cloth) was made and dried. It was subjected to *Pudam* process (Calcination) with 20 -25 cow dung cakes. After 24 hours of burning, the vessel allowed to cool and the resultant was collected and kept it in an clean and air tight container.

Animal Selection

Mice weighing 28-32 gms of either sex of wistar strain 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

XIII/ VELS/ PCOL/ 04/ 2000/ CPCSEA/ IAEC/ 08.08.2012.

Acute toxicity study:

Acute oral toxicity test of *Uppu parpa* was carried out as per OECD Guidelines 425 up and down method [14]. 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 *Uppu parpa* was given. Body weight and behavioral changes were noted. Animals are observed individually and were systematically recorded. The acute toxicity was occurred at 500mg/kg after 48 hours of oral drug treatment. Hence, one-tenth and one fifth dose was selected as therapeutic dose from maximum tolerable dose for further pharmacological study.

Drug and stock solution

The *Uppu parpa* was accurately weighed using electronic balance and suspended in 2% carboxy methyl cellulose (CMC) solution to so as to get 200mg/kg of main stock solution and this was used in this study. All the chemicals and standard drugs

were procured from authorized suppliers.

Ovulation stimulation activity:

In the present study, twenty four Virgin female wistar rats weighing of around (88- 130 gm) of 2 month old were obtained from the animal house at Vel's University, Chennai. Before starting drug treatment, the reproductive cycles of the rats were synchronized by using 100µg of estradiol dissolved in 2 ml olive oil was injected subcutaneously. After 24 hours period all rats received 50µg progesterone dissolved in olive oil and administered intramuscularly. Vaginal smears were obtained by vaginal lavage after few hours to monitor ovulation and estrous cycle. 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 [15]. All the animals were weighed daily after drug administration for 10 days. The suitable sensitive rats were divided into four groups of six each as follows:

Group I Normal Control animals given only 2ml/kg of CMC solution.

Group II animals were administered 50 mg/kg of *Uppu parpa* for 10days,

Group III rats were received 100mg/kg of *Uppu parpa* for 10 days

Group IV received Clomiphene citrate 10mg/kg and served as standard. All the drugs were given orally for ten days.

On 11th day, 2ml of blood was collected by retro orbital 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, Progesterone, Testosterone and estradiol by ELISA method. At the end of experiment, the animals were sacrificed using ether anesthesia and the uteri were removed and weight was recorded. The oviduct was dissected out from the rats, suspended in normal saline and placed on a microscopic slide with a cover slip to count the number of ova in the oviduct [16].

Statistical analysis

Statistical significance of data was assessed by analysis of variance (one-way ANOVA) followed by a comparison between different groups using Dunnet test. $P<0.05$ considered as statistically significant.

RESULTS

From the acute toxicity study, it was confirmed that the *Uppu parpa* had no toxic effect at 500mg/kg on mice after 48 hours of oral drug treatment.

Effect of *Uppu parpa* on mean uterus and ovary weight

Uppu parpa and the standard drug Clomiphene citrate had no significant effect on the uterus and ovary weight of the rats which was summarized in Table No.1 and graph No.1.

Effect of *Uppu parpa* on Serum Concentration of reproductive hormones of female rats

The effect of the administration of trial drug *Uppu parpa* and standard drug Clomiphene citrate on serum concentration of reproductive hormones were presented in Table No.2 and Graph No.2, Graph No.3. LH, FSH, Estrogen, Progesterone and Testosterone were analyzed. The result of

Table No.2 showed that the administration of *Uppu parpa* in the dose of 50 mg and 100 mg (UP – 50 and UP – 100) caused no significant effect on LH which was approximately similar to the normal and standard group. There was significant increase in FSH level ($p<0.01$) in standard drug Clomiphene citrate. Animals are pre treated with *Uppu parpa* 100 mg and 50 mg was also an increase in the level of FSH ($p<0.01$).

In accordance with the results related to estrogen and testosterone also showed a significant decrease after the administration of *Uppu parpa* when compared with standard and normal control group. Both trial drug treated groups and standard group produce little decreasing effect on progesterone level which was insignificant.

Histopathological study of ovary tissue

Histological studies of ovarian tissues of normal group, standard group, UP – 50 and UP -100 trial drug treated groups were presented in Fig No.1 A, B, C and D respectively. The ovarian tissue of the normal group showed the normal histological features with presence of few primordial follicles, matured graffian follicle. (Fig.1). Standard group and both doses of *Uppu parpa* (UP – 50 mg and UP-100 mg) showed some well defined histological features with increased number of primary follicles, matured graffian follicles and also corpus luteum when compared with normal rat. These were more pronounced in rat ovary that received UP – 100 mg and Clomiphene citrate. (Fig No: 1 -B and C)

Discussion:

Uppu parpa significantly increases the serum FSH and increases LH in animal model ($p< 0.05$) which is primary step for inducing ovulation. Folliculogenesis is the process whereby the primordial follicle is recruited for further development. Majority of the developing follicles will undergo atresia, some will mature into preovulatory follicles. During the development of ova until the preovulatory stage, the gonadotropin FSH is the primary stimulus for the growth and differentiation. The time span for the development of primordial follicle into a preovulatory follicle will be stimulated by LH [17].

The features of atresia include degeneration and shrinkage of the ovum, apoptosis of the granulosa cell or their transformation of the connective tissue [18].Uppu parpa increases FSH, LH and estradiol may be due to ovarian steroidogenesis [19].

The administration of *Uppu parpa* in the dosage of 50 mg and 100 mg decrease the level if testosterone and this helps in the management of PCOS which is the leading cause for hyperandrogenism (hirsutism, hair loss, acne, obesity, depression and deepening of voice).

The test drug *Uppu parpa* 50mg and 100 mg increased the level of FSH and LH. The results of *Uppu parpa* suggested the ovulatory response in female rats. The body weights of the rats treated with *Uppu parpa* 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 [20].

Table.1. Effect of *Uppu parpa* on Weight of Uterus and Ovary after 10 days Treatment

S.No	Group	Treatment and dose	Weight of uterus (g%)	Weight of ovary (g %)
1.	Normal	2ml/kg 2% CMC	0.401±0.02	0.125±0.02
2.	Test-I	Uppu parpa 50mg/kg	0.430±0.01	0.131±0.02
3.	Test-II	Uppu parpa 100mg/kg	0.455±0.01	0.140±0.02
4.	Standard	Clomiphene 10mg/kg	0.490±+0.02	0.150±0.03

N = 6. Values are expressed as Mean±SEM. $^{ns}P>0.05$ compared to normal control.

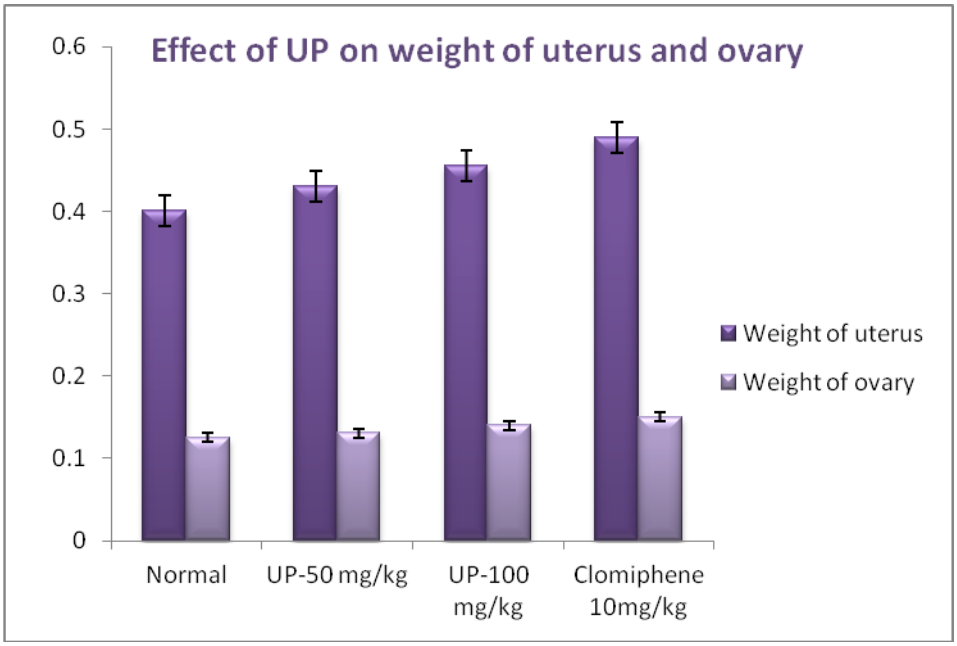


Fig.1.Effect of UP on Weight of Uterus and Ovary

Table.2. Effect of *Uppu parpa* on Serum Concentration of Reproductive Hormones of Female rats after 10 days Treatment

Group	Treatment and dose	LH (IU/ml)	FSH (IU/ml)	Estrogen (pg/ml)	Progesterone (pg/ml)	Testosterone (ng/ml)
Normal	2ml/kg 2% CMC	0.28±0.04	0.34±0.02	63±3.2	8.6±1.22	1.3±0.11
Test-I	Uppu parpa 50mg/kg	0.34±0.06	0.46±0.05	55±2.8[a]	7.6±1.03	0.9±0.04[**,a]
Test-II	Uppu parpa 50mg/kg	0.46±0.09	0.52±0.06[**]	50±1.7[**,a]	7.2±0.88	0.6±0.02[**]
Standard	Clomiphene 10mg/kg	0.51±0.12	0.64±0.08[**]	32±1.2[**]	6.5±0.69	0.4±0.02[**]

*N = 6. Values are expressed as Mean±SEM. **p<0.01 Vs Normal control; [a]p<0.01 Vs Standard.*

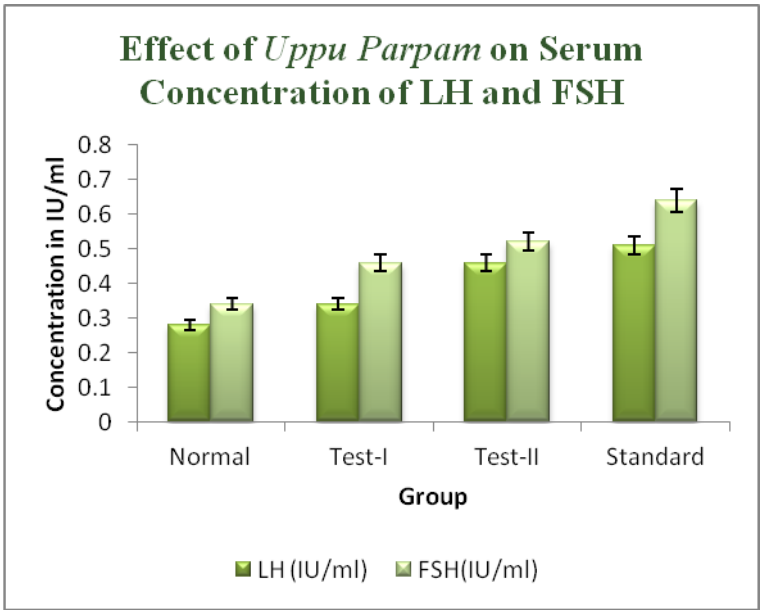


Fig.2.Effect of UP on Serum Concentration of LH and FSH

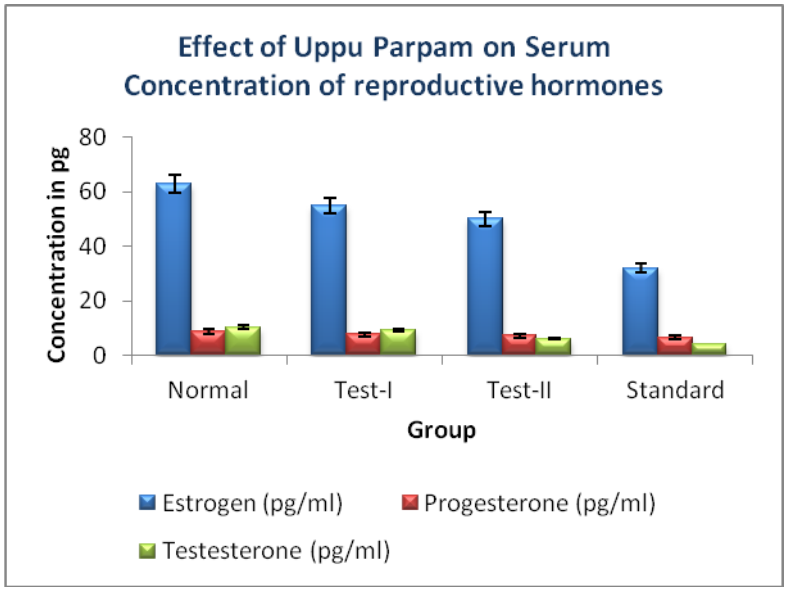
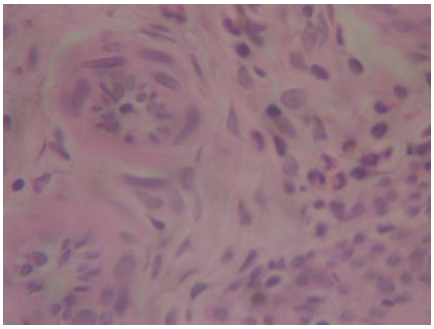
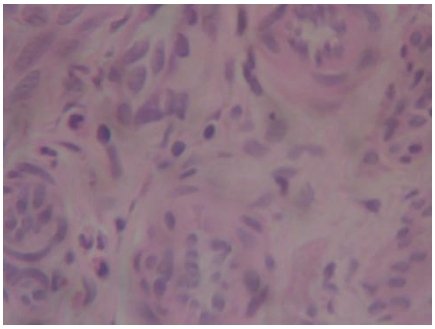


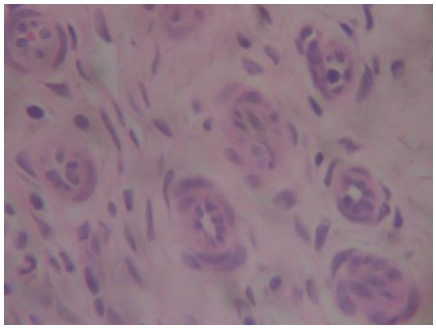
Fig.3.Effect of UP on serum concentration of reproductive hormones



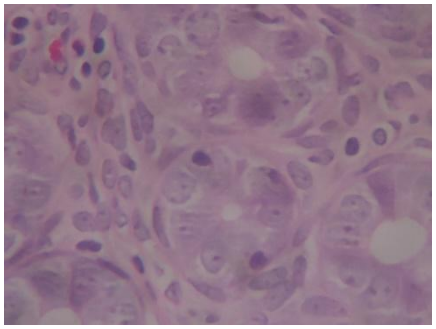
(A) Control group



(B) Standard group



(C) *Uppu parpa* 50mg



(D) *Uppu parpa* 100mg

Fig.4.Histopathological pictures

CONCLUSION

The results of this study ensures that *Uppu parpa* was significantly increases the number of ova in the oviduct of treated rats ($p<0.01$) when compared with the control indicates enhancement of ovulation and also elevates the serum concentrations of LH, FSH and estradiol hormones, and increased ovarian weight. Hence, It can be concluded that Uppu parpa promotes folliculogenesis and further clinical study can be conducted to evaluate its potential in reproductive hormonal disorders and in infertility condition in female.

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