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Research Article

EVALUATION OF OVULATION-INDUCING ACTIVITY OF *INJI RASAYANAM* IN FEMALE WISTAR ALBINO RATS

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ABSTRACT

Polycystic ovarian disease (PCOD) is a common endocrine disorder characterized by anovulation, hyperandrogenism, and hormonal imbalance. *Inji Rasayanam*, a classical Siddha polyherbal formulation, is traditionally used for reproductive disorders. This study aimed to evaluate the ovulation-inducing activity of *Inji Rasayanam* in female Wistar albino rats. Twenty-four female Wistar albino rats were divided into four groups (n = 6): normal control (2% CMC), low dose (36mg/kg), high dose (360mg/kg), and standard (clomiphene citrate 10mg/kg). Estrous cycles were synchronized using estradiol and progesterone. The drug was administered orally for 10 days. Serum levels of LH, FSH, estradiol, progesterone, and testosterone were estimated using ELISA. Uterine and ovarian weights were recorded, and histopathological examination of ovarian tissues was performed. Statistical analysis was done using one-way ANOVA followed by Dunnett's test. *Inji Rasayanam* significantly increased FSH levels and reduced estrogen and testosterone, with minimal changes in LH and progesterone. Histology showed improved follicular development comparable to the standard drug. *Inji Rasayanam* may possess ovulation-inducing activity through hormonal modulation and restoration of ovarian architecture.

INTRODUCTION

Polycystic Ovarian Syndrome (PCOS) is a multifactorial and polygenic condition, manifested by amenorrhea, hirsutism and obesity associated with enlarged polycystic ovaries. [1] This complex endocrine disorder occurs at a frequency of 11.2% among women of reproductive age group. [2] It was first described by Stein and Leventhal in 1935 and is linked with anovulation[1]. Ovulation is regulated by HPO axis through coordinated secretion of gonadotropins and ovarian hormones. However, in PCOS, hormonal imbalance particularly elevated androgen levels and altered gonadotropin secretion-interferes with follicular development and ovulation [3].

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Conventional management of PCOD-related infertility mainly focuses on ovulation induction using drugs such as clomiphene citrate, letrozole, and gonadotropins[4]. Although these treatments are effective in many patients, they are often associated with adverse effects, including ovarian hyperstimulation syndrome, multiple pregnancies, and other metabolic complications. Furthermore, some patients exhibit resistance to these therapies, which highlights the need for alternative therapeutic approaches that are safe, effective, and affordable.

Siddha Medicine, one of the oldest traditional medical systems practiced in South India, describes conditions similar to PCOD under *Soothaga Vaayu*, involving disturbances in the reproductive and hormonal balance. Several polyherbal formulations used in Siddha medicine are believed to possess properties that regulate menstrual cycles, improve reproductive health, and enhance fertility. Among these, *Inji Rasayanam*, a classical Siddha polyherbal formulation, is traditionally used for reproductive health and digestive disorders. This formulation is rich in bioactive phytoconstituents that may exert

beneficial effects by modulating hormonal balance, improving ovarian function, and promoting follicular maturation. However, scientific validation of this formulation through experimental studies remains limited.

Therefore, the present study aims to evaluate the ovulation-inducing activity of *Inji Rasayanam* using an experimental animal model. The study also seeks to provide scientific evidence supporting its traditional therapeutic claims and to explore its potential role as a safer alternative in the management of ovulatory disorders associated with PCOD.

MATERIALS AND METHODS

Inji Rasayanam was prepared as per siddha literature "Anuboga Vaithya Navanitham Part-8"[5]. The formulation consists of fifteen ingredients.

1. *Inji- Zingiber officinale*
2. *Sukku -Zingiber officinale*
3. *Arisi* thippili- *Piper longum*
4. *Seeragam -Cuminum cyminum*
5. *Elam -Eletteria cardamomum*
6. *Sirunagapoo -Mesua ferrea*
7. *Athimathuram- Glycyrrhiza glabra*
8. *Lavangathiri- Cinnamomum tamala*
9. *Thalisapathiri- Abies spectabilis*
10. *Moongiluppu- Bamboo salt*
11. *Thiratchaipalam- Vitis vinifera*
12. *Milagu- Piper nigrum*
13. *Nei*
14. *Sarkkarai- Saccharum officinarum*
15. *Thaen*

Animal selection

Female Wistar albino rats (100-155 g) were used for toxicity study. Female Wistar rats or mice are generally preferred animal models for evaluating ovulation inducing activity. The female Wistar albino rats (95-135gm) were used for ovulation inducing activity. All the experimental rats were housed in metal cages with corncob bedding, maintained under standard conditions of temperature (22-26°C), relative humidity 40-70% and a 12-hour light/12-hour dark cycle. Proper lighting was maintained to preserve the normal reproductive cycle. All animals were fed on standard pellet diet and clean drinking water continuously (ad libitum). The experimental animals were acclimatized under lab conditions for one week and monitored regularly for signs of illness such as hair loss, weight loss or abnormal behavior. This study was conducted at Arulmigu Kalasalingam College of Pharmacy, Krishnankovil after obtaining Institutional Animal Ethical Committee (IAEC) clearance AKCP/IAEC/14/2025-2026 and followed CPCSEA guidelines.

Toxicity study

Acute toxicity study of *Inji Rasayanam* was performed according to OECD guideline 425[6] and sub-acute toxicity according to OECD guideline 407[7]. This was performed at Madras Veterinary College, Vepery. For acute toxicity study (single dose toxicity), all rats were fasted overnight (12hrs) and administered a single oral dose of *Inji Rasayanam* via oral gavage. Dose levels were calculated as per OECD acute toxicity guidelines. Animals were observed continuously for first 4 hours, then periodically for 24 hours and daily for 14 days. Based on mortality data, LD 50 (median lethal dose) was estimated. For subacute toxicity study, the test drug was administered daily for 28 days to evaluate toxic effects of the test drug.

Drug and stock solution

The test drug *(Inji Rasayanam)* was weighed using electronic balance and stock suspension was prepared in 2% Carboxymethylcellulose (CMC) solution to obtain 360mg/kg of main stock solution and was used as a vehicle for oral administration in experimental animals.

Ovulation inducing activity

For ovulation inducing activity, 24 female Wistar albino rats weighing about 95 - 135g were used for this experiment. Before starting this experiment, the reproductive cycles of all rats were synchronized by the following method[8]. 100µg of estradiol dissolved in 2ml of olive oil was injected subcutaneously. After 24 hours, all rats received intramuscular injection of 50µg progesterone dissolved in olive oil. Progesterone inhibits the release of GnRH, preventing the rats from moving into proestrus stage. Vaginal smear analysis was used to monitor the estrous cycle. The smears were collected at the same time each morning because they were nocturnal and hormonal changes occur overnight. The smear was prepared by washing the vaginal opening using a small plastic pipette with a tiny amount of saline (0.9% NaCl) with a glass dropper and placed in a clean glass slide and viewed under a light microscope at 40 X magnification. In the four stages of estrous cycle, the proestrus stage shows predominance of round nucleated epithelial cell, estrus stage with anucleated cornified cell, metestrus stage consists of the same proportion of leukocytes, cornified, and nucleated epithelial cells while a diestrus smear shows predominance of leukocytes. A rat in persistent estrus stage indicates hormonal synchronization. Assessment of vaginal smear ensured that all animals in a study group were at the same estrus stage for testing the trial drug (I.R).

Experimental design

Group I- (Normal Control): Received 2ml/kg of 2% CMC solution orally for 10 days.

Group II- (IR-36): Received 36mg/kg of IR orally for 10 days,

Group III- (IR- 360): Received 360mg/kg of IR orally for 10 days

Group IV-(Standard): Received 10mg/kg of Clomiphene citrate orally for 10 days.

After that 2ml of blood was collected from all animals by retroorbital puncture. These samples were centrifuged at 4000 rpm for 15 mins, and the serum was separated. These samples were frozen at -20°C to estimate LH, FSH, estradiol, progesterone by using ELISA method. All the animals were euthanized using an approved protocol under ether anesthesia. A mid-ventral incision was made to expose the ovaries and uterus. The uteri and oviducts were weighed. Oviducts were examined under a microscope to count the number of ova released.

Histological analysis

Following sacrifice, ovaries were carefully dissected from uterine horns and fixed in 10% neutral buffered formalin for 24 hours. Fixed tissue samples were dehydrated in a graded series of ethanol, embedded in paraffin wax and sliced into thin sections (5 to 7µm). Sections were stained with haematoxylin and Eosin (H&E) to identify and count follicles based on the morphology of surrounding epithelial cells. For example: Primordial follicles show squamous cells whereas primary follicles are surrounded by cuboidal cells. Folliculogenesis was quantified by counting follicles in every 10th section to avoid double- counting.

Statistical Analysis

Data were expressed as mean±SEM. Statistical analysis was performed using one-way ANOVA followed by Dunnet's multiple comparison test. $p<0.05$ was considered statistically significant.

RESULTS

In the acute and subacute toxicity study, *Inji Rasayanam* administered orally at 500mg/kg was well tolerated. At this dose, no mortality, significant changes in body weight, or alterations in physiological and behavioral parameters were observed, indicating the formulation was safe at this dose.

Effect *of Inji Rasayanam* on mean uterus and ovary weight

The effect of *Inji Rasayanam* (IR) on the mean uterus and ovary weight of female rats after 10 days of treatment is presented in Table 1, Graph 1 and Graph 2. The results indicated that treatment with *Inji Rasayanam* at doses of 36mg/kg and 360mg/kg did not produce any statistically significant change in the weight of the uterus and ovary when compared with the normal control group. Similarly, the standard drug Clomiphene citrate (10mg/kg) also showed no significant effect on uterine and ovarian weight.

These findings indicate that *Inji Rasayanam* does not significantly influence reproductive organ weight, suggesting that its ovulation-inducing activity may occur through hormonal modulation rather than structural changes in reproductive organs.

Effect of *Inji Rasayanam* on serum concentration of reproductive hormones of female Wistar rats

The ovulation-inducing activity of *Inji Rasayanam* was evaluated by assessing the serum levels of reproductive hormones including LH, FSH, estrogen, progesterone, and testosterone in female rats are presented in Table 2, Graph 3, Graph 4, Graph No.5 and Graph 6 respectively.

Administration of *Inji Rasayanam* at doses of 36mg/kg and 360mg/kg produced a dose-dependent increase in serum FSH levels, with the higher dose significantly elevating FSH levels ($p<0.05$). LH levels showed a mild, non-significant increase in treated groups, comparable to the standard drug. Significant reduction in estrogen levels was observed, particularly at 360mg/kg ($p<0.01$), suggesting normalization of ovarian steroidogenesis and hormonal balance. There was a significant decrease in serum testosterone levels, indicating a potential anti-androgenic effect beneficial in ovulatory disorders. Progesterone levels showed a slight, non-significant decrease, suggesting luteal phase activity was not adversely affected

The hormonal modulation produced by *Inji Rasayanam* was comparable to that of the standard ovulation-inducing drug Clomiphene citrate, which significantly increased both LH and FSH levels. Although the effect of *Inji Rasayanam* on LH was less pronounced than that of the standard drug, the formulation demonstrated a significant stimulatory effect on FSH secretion along with a reduction in androgen levels.

Overall, the findings of this study indicate that *Inji Rasayanam* possesses ovulation-inducing activity, possibly mediated through modulation of gonadotropin secretion and regulation of ovarian steroid hormones. The formulation may promote follicular maturation and restore hormonal balance, thereby facilitating ovulation. These results support the potential therapeutic use of *Inji Rasayanam* in the management of ovulatory disorders such as PCOD.

Histopathological Examination

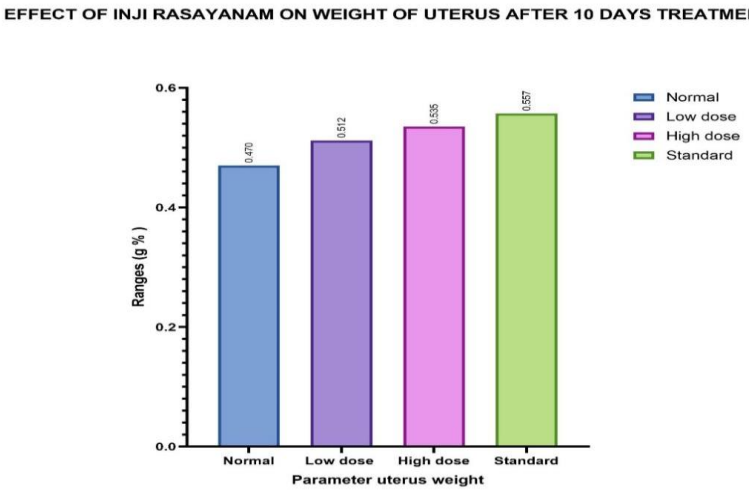
Histological examination of ovarian sections from normal control, test drug (IR-36mg, IR-360mg) and Standard drug were presented in Fig 1, Fig 2, Fig 3, Fig 4 respectively. It Indicates that administration of IR

particularly at high dose preserves normal ovarian histology and promotes follicular development comparable to standard drug.

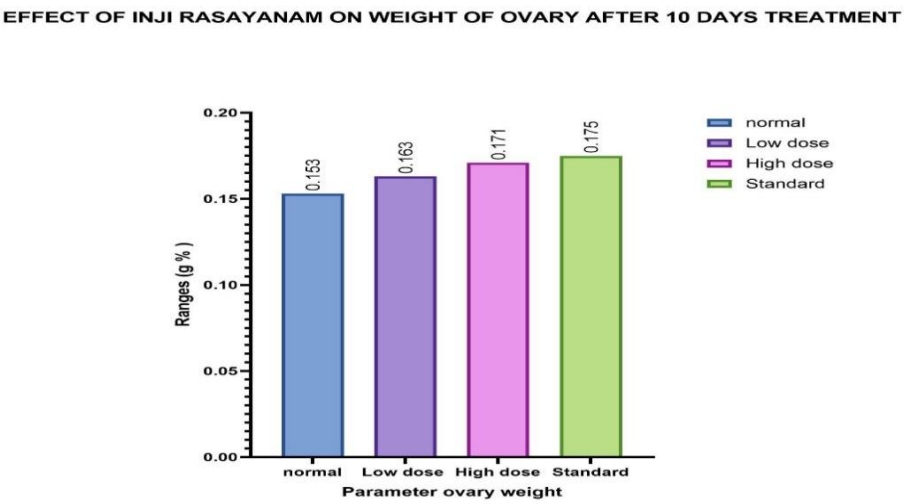
Table 1: Effect of *Inji Rasayanam* on weight of uterus and ovary after 10 days treatment

Group	Treatment and dose	Weight of uterus (g%)	Weight of ovary (g%)
Normal	2ml/kg 2% CMC	0.475±0.05	0.153±0.03
Low dose	IR 36mg/kg	0.512±0.01	0.163±0.01
High dose	IR 360mg/kg	0.535±0.02	0.171±0.01
Standard	Clomiphene citrate10mg/kg	0.557±0.03	0.175±0.01

Values are expressed as Mean ±SEM (n= 6) one way ANOVA followed by Dunnet's multiple comparison test. Where the values are ns P>0.05 compared to normal control



Graph 1: Effect of IR on weight of uterus

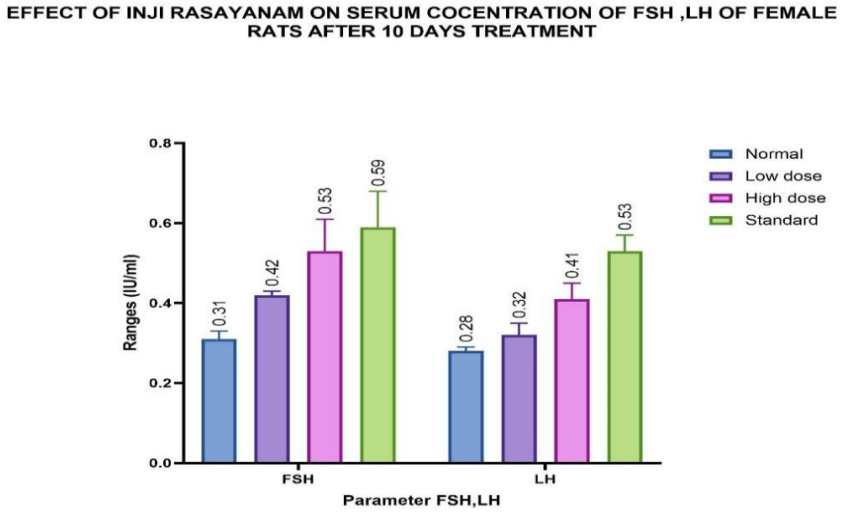


Graph 2: Effect of IR on weight of ovary

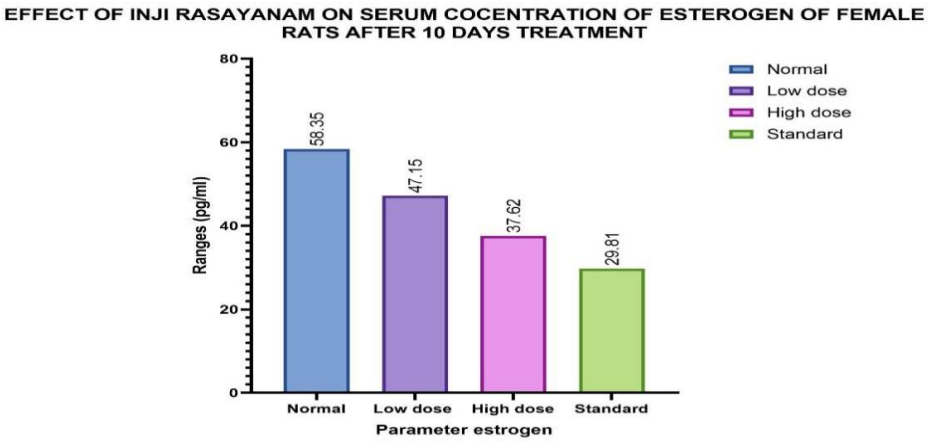
Table 2: Effect of *Inji Rasayanam* on serum concentration of reproductive hormones of female rats after 10 days treatment

Group	Treatment and dose	LH (IU/ml)	FSH (IU/ml)	Estrogen (pg/ml)	Progesterone (ng/ml)	Testosterone (ng/ml)
Normal	2ml/kg-2% CMC	0.28±0.01	0.31±0.02	58.35±2.1	7.9±0.85	0.9±0.001
Low dose	36mg/kg	0.32±0.03	0.42±0.01	47.15±1.7*	6.7±0.02	0.5±0.07**
High dose	360mg/kg	0.41±0.04	0.53±0.08*	37.62±2.7**	6.2±0.41	0.3±0.04
Standard	Clomiphene 10mg/kg	0.53±0.04*	0.59±0.09**	29.81±0.01	5.8±0.29	0.2±0.09*

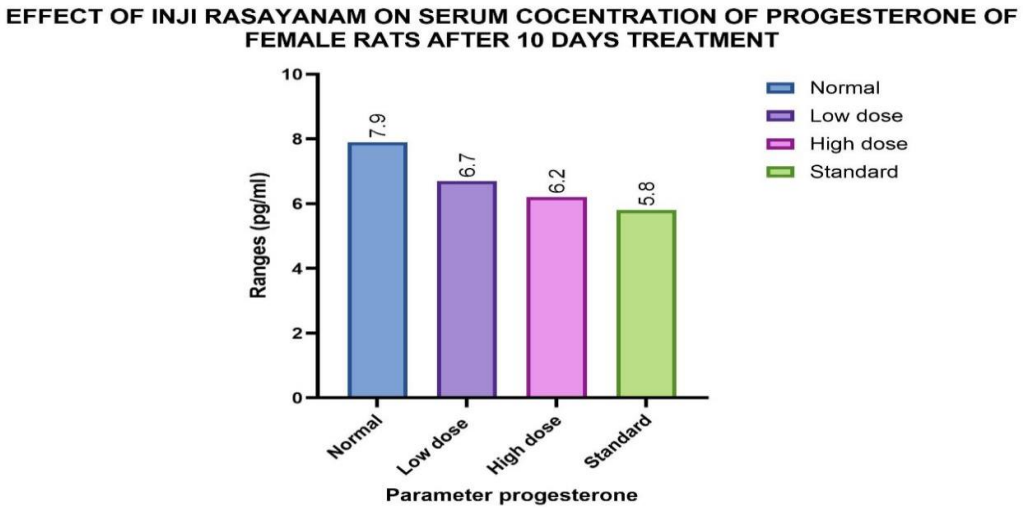
All values were expressed as mean ± SEM, n = 6, * P<0.05, **P<0.01, ***P < 0.001 as compared to the control group. Results were done by one-way ANOVA followed by Dunnett's test



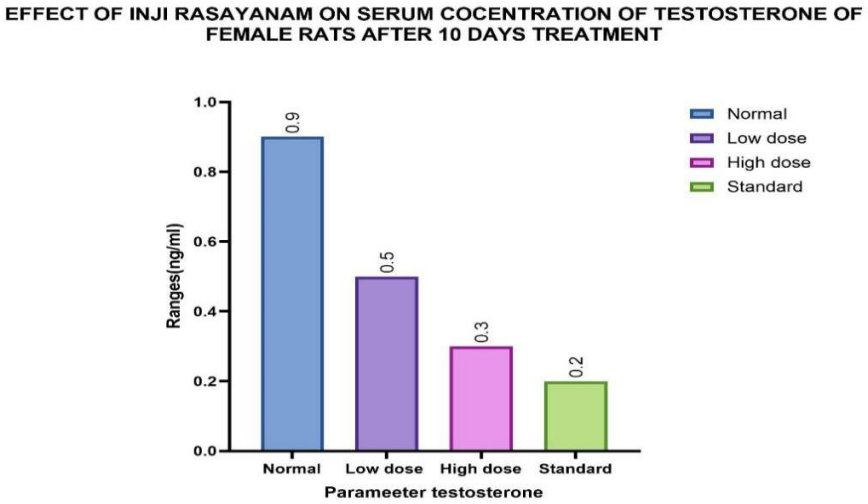
Graph 3: Effect of IR on Serum LH and FSH in rats



Graph 4: Effect of IR on Serum estrogen in rats



Graph 5: Effect of IR on Serum progesterone in rats



Graph 6: Effect of IR on Serum testosterone in rats

Histopathological Study on Ovary Tissue

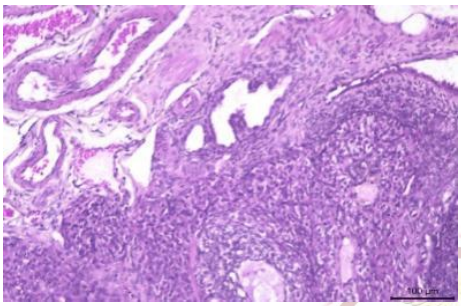


Fig 1: Ovary of normal control rat with IR-36mg

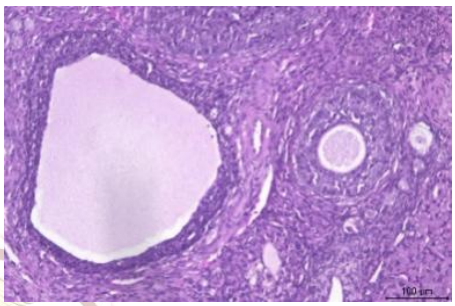


Fig No:2 Ovary of rat treated

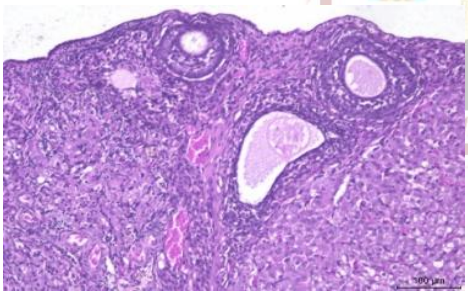


Fig 3: Ovary of rat treated with

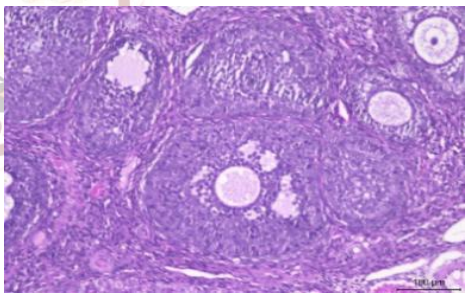


Fig 4: Ovary of rat treated with IR-360mg Clomiphene citrate (Standard drug)

DISCUSSION

The present study evaluated the ovulation-inducing potential of *Inji Rasayanam* (IR) in an experimental rat model. The findings demonstrate that administration of IR produced a dose-dependent increase in serum FSH levels, with the higher dose showing statistical significance. Elevated FSH levels play a critical role in stimulating follicular growth and maturation, thereby facilitating ovulation. This suggests that IR may enhance folliculogenesis through modulation of pituitary gonadotropin secretion.

Although LH levels showed a mild increase, the change was not statistically significant. However, the levels were comparable to those observed with the standard drug, clomiphene citrate. This indicates that IR may exert a balanced regulatory effect on LH secretion, avoiding excessive stimulation, which is often associated with adverse effects such as ovarian hyperstimulation.

A significant reduction in serum estrogen and testosterone levels was observed, particularly at higher doses. Hyperandrogenism is a hallmark feature of PCOD; therefore, the reduction in testosterone levels suggests that IR possesses anti-androgenic properties, which may help restore normal ovarian function. The decrease in estrogen levels may reflect normalization of ovarian steroidogenesis and improved endocrine balance.

Progesterone levels did not show significant variation across treatment groups, indicating that IR does not adversely affect luteal phase function. Preservation of progesterone levels suggests that ovulation, if induced, is likely followed by normal luteal activity.

Histopathological examination further supported the biochemical findings. Ovarian sections from IR-treated groups showed restoration of normal ovarian morphology, with increased numbers of developing follicles and reduced cystic changes. The absence of inflammatory infiltration or degenerative alterations indicates the safety of the formulation. These changes were comparable to those observed in the standard drug-treated group.

Therefore, IR did not produce significant changes in uterine and ovarian weights, suggesting that its mechanism of action is primarily through hormonal modulation rather than structural alteration of reproductive organs. These results support the potential therapeutic use of *Inji Rasayanam* in the management of ovulatory disorders such as PCOD.

CONCLUSION

This study demonstrates that *Inji Rasayanam* exhibits significant ovulation- inducing activity. The observed effects of the drug stimulate the HPO axis and promotes folliculogenesis. Histopathological findings further support the restoration of normal ovarian architecture. These results suggest that IR may be beneficial in the management of ovulatory disorders such as PCOD. However, further clinical trials are required to confirm its safety and efficacy in humans.

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