

ANTI FERTILITY ACTIVITY OF *JAPAKUSUMADI YOGA* IN FEMALE WISTAR ALBINO RATS

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ABSTRACT

Background: Ayurvedic literature mentions the use of a number of herbomineral preparations for fertility control. Documented experiments or clinical data are however lacking. **Objective:** The present study was carried out to determine the anti fertility effect of *Japa kusumadi yoga* in the female wistar albino rats. **Methods:** The experimental study was carried out in female wistar albino rats to determine the effect of *Japa kusumadi yoga* on estrous cycle and on post coital antifertility activity. **Results:** Study on the estrous cycle revealed the disruption of the estrous cycle with the prolongation of the diestrous phase and shortening of the estrous, proestrous and the metaestrous phase. Correspondingly significant changes in the hormonal levels, Cholesterol levels, weight of the ovaries and weight and the cytoarchitecture of the uterus were appreciated. Study on the post coital pregnancy interruption activity showed the anti implantation and abortifacient activity with significant decrease in the estrogen and progesterone levels. **Conclusion:** The study on wistar albino rats concluded that *Japa kusumadi yoga* has an anti fertility effect and has no adverse effect.

Keywords: *Japa kusumadi yoga*, Histopathological study, experimental study.

INTRODUCTION

The population of India was estimated to be 1.34 billion [as per Jan 2017]¹. In spite of promotion of family planning methods by government, the population control is not effectively achieved in our country. Today, there is an imperative need for limiting the family size at personal level and to control popu-

lation at national level. One of the effective remedy to check the overgrowth of population is to control the birth rate and Contraception is the only "Tool" which can control the fertility.

Contraception is of two types, permanent and temporary. Steroidal contraception is the most popularly

used temporary method of contraception owing to its less failure rate. Hormone based contraceptive medicines because, adverse effects like weight gain, nausea, headache, cervical cancer, carcinoma of breast, deep vein thrombosis² Now a day, very low dose contraceptives are available but these too have adverse effect on the health of the lady. Hence there is a need of a contraceptive measure which is free from adverse effects, more effective, easily available and cheaper.

Contraception is mentioned in Ayurvedic classics also. Various local and oral anti fertility measures have been explained in the text books of *Bhava Prakasha* and *Yogarathnakara*.³ *Japa* is a locally grown plant with abundant availability and no controversy. The use of *Japa kusuma* with Guda and kanji as a contraceptive is mentioned in classics. An attempt is made here to evaluate the contraceptive efficacy of *Japa kusuma* with Guda and kanji.⁴

Various studies using a systemic scientific approach have demonstrated the anti fertility activity of *Japa kusuma* or flower of *Hibiscus rosa sinensis*. The demonstrated anti fertility could be because of its estrogenic activity⁵, anti estrogenic activity⁶ or could be because of the presence of phytoestrogens⁷. But till today no study has been carried out on the formulation containing *Japakusuma*, guda and kanji. Hence the present study “Anti fertility effect of *Japa kusumadi yoga* in female albino rats” is planned.

Aim: To evaluate the anti fertility effect of *Japakusumadi yoga*

Objectives:

1. Study the effect of the drug on the estrous cycle of the rats.
2. To evaluate the post coital anti fertility effect

MATERIALS AND METHODS

SOURCE OF DATA:

Animals

Colony-bred female albino rats (wistar strain), weighing about 180-250g were used for the estrous cycle monitoring and anti fertility testing. Adult albino rats of either sex were used for acute toxicity

studies. The animals were procured from the animal house attached to the Pharmacology Laboratory, SDM Centre for Research in Ayurveda and Allied Science, Udyavara. They were exposed to natural day and night cycles with ideal laboratory condition in terms of ambient temperature and humidity. They were fed with rat pellets from Saidurga feeds, Bengaluru and tap water *ad libitum*

The experiment was carried out in conformity with guidelines of the Institutional Animal Ethical Committee (IAEC) after obtaining its permission. ETHICAL CLEARANCE NO- SDMCAU /IAEC/2010/11 **Medicine source:** *Japakusumadi yoga*, the ingredients of which are, *Japakusuma* (*Hibiscus rosa sinensis* flowers), *Purana guda* (Jaggery which is one year old) and *Kanji*.

The *Japakusuma* (flowers of *Hibiscus rosa sinensis*) is collected freshly from the plants of Herbal garden of SDM College of Ayurveda Udupi. The plant was authenticated by Plant expertise, Department of Dravyaguna, SDM College of Ayurveda, Kuthpady, Udupi. *Kanji* was prepared as per the standard method of preparation as given in the classics. *Guda* purchased from local grocery shop (purchased one year prior to the experiment).

Preparation of the trial drug: *Japakusuma*:1.08g and *Guda*:4.32g were taken and a fine paste was prepared. This paste was then mixed with 25 ml of *kanji* and a solution was prepared. 1ml solution/200mg body weight of rats was given.

Methods:

Acute oral toxicity test

The acute oral toxicity study was carried out as per OECD guidelines 425 using AOT software. The experiment was carried out in conformity with guidelines of the Institutional Animal Ethical Committee (IAEC) after obtaining its permission. ETHICAL CLEARANCE NO- SDMCAU /IAEC/2010/11. The *Japakusumadi yoga* was prepared freshly and dosed in the following order 175, 550 and 2000 mg/kg body weights. After the dosing, the animals were observed for 14 days for mortality. The LD50 was determined by using AOT software.

Experimental Models

Post coital antifertility study

Colony bred healthy female wistar albino rats of known fertility weighing about 180-260g were maintained under controlled standard animal house conditions with access to food and water ad libitum. They were randomly divided into 4 groups each with 6 rats. Rats in proestrus phase of cycle were caged with males of proven fertility, in the ratio 2:1. Rats were examined the following morning for evidence of copulation. Rats exhibiting thick clumps of spermatozoa in their vaginal smears were separated and that day was designated as day 1 of pregnancy.

Group 1 considered normal control and treated with normal saline 5ml/kg body weight. **Group II** treated with *Japakusumadi yoga* from day one today six, **Group III** treated with *Japakusumadi yoga* from day seven today eleven, **Group IV** treated with *Japakusumadi yoga* from day twelve today sixteen consecutively. On the 18th day post coitum, the animals were sacrificed under anesthesia. The total no of implantations in both the uterine horns were counted to get an idea of litter size. Post implantation mortality (mortality of implanted embryos) of the embryos was noted. The Anti implantation activity, the post-implantation mortality and anti fertility activity was carried out by standard protocol described by Williamson et al (1996) and Tafesse et al).

Anti-implantation activity: Method followed by Williamson et al (1996) and Tafesse et al is used.

(Number of implants in control –Number of implants in test group/ Number of implants in control) X100

Post-implantation mortality (resorption): Method followed by Williamson et al (1996) and Tafesse et al is used.

(Total number of implanted embryos –Number of Normal embryos% Total number of implanted embryos) x100

Anti fertility activity: Method followed by Williamson et al (1996) and Tafesse et al is used.

(Number of non pregnant rats /Total number of female rats) X100

Estrous cycle study

Characteristic changes take place in the vaginal epithelium of albino rats at different phases of estrus cycle which can be followed by examination of vaginal smears. The estrous cycle stages are 1. Proestrous stage shows large number of nucleated cells. 2. Estrous stage shows a large number of keratinized cells. 3. Metaestrous stage shows a large number of WBC along with keratinised cells. 4. Diestrous stage shows a good number of WBC, a few epithelial cells and much keratinised cells.

Colony bred female rats of wistar strain showing normal estrous cycle were selected and were maintained under controlled standard house conditions with food and water ad libitum. They were divided into two groups, each containing 6 animals.

Group 1: considered normal control and treated with normal saline 5ml/kg body weight. **Group 2:** treated with *Japakusumadi yoga*. After grouping, the vaginal smear was taken for both control and trial groups in the morning between 9-10 am daily for 15 days followed by administration of respective medication. On the 16st day, the rats were anesthetized and blood was collected by retro-orbital puncture and assigned for biochemical investigation. The uterus and right ovary were excised out from sacrificed animal, weighed and transferred to fixing solution (10% formalin) for Histo pathological examinations. Left ovary from each rat was processed for cholesterol estimation.

Statistical analysis:

All the values were expressed as MEAN± SEM (standard error of mean). One way ANNOVA followed by Dunnet's multiple 't' test as post hoc test was used to analyze the data. The values are expressed as mean ± SEM and p<0.05 was considered as significant. Graph pad Inst 3 was used for this purpose.

RESULTS

Table 1: Results of preliminary phytochemical screening of *Japakusumadi yoga*

Test	Inference
Alkaloid	+
Steroid	+
Carbohydrate	+
Tannin	+
Flavanoids	+
Saponins	-
Coumarins	+
Phenols	+

(+) - present; (-) – negative

Post coital anti fertility effect

Table 4: Effect of trial drug on anti implantation activity and

Duration of pregnancy	Anti implantation activity (%)	Resorption (Post implantation mortality) (%)	Anti fertility activity (%)
1-6 days	67.79	78.94	66.66
-11 days	35.33	13.58	0
12-16 days	52.54	0	52.54

Table 4.1: Effect of trial drug on hormone levels

Group	estrogen		progesterone	
	Control	Trial	Control	Trial
1.D1-D6	38.27±3.75	0.33±0.333***	58.28±1.084	6.41±2.74***
2.D7-D11	58.22±1.77	15.25±2.9***	38.27±3.75	1.46±0.93***
3.D12-16	38.27±3.75	4.78±0.082	58.28±1.08	4.85±3.32***

Values are mean±SEM (n=6) one way ANOVA.*represents significant at p<0.05,** represents highly significant at p<0.01 *** represents very significant at p<0.001 when compared with control

Estrous Cycle Study

Table 5: Effect of trial drug on estrous cycle

Group	Treatment dose	Proestrous (days)	Oestrous (days)	Metaestrous (days)	Diestrous (days)
1	Control	2±0.63	4.5±0.61	5±0.44	1.83±0.60
2	Trial drug	1±0.25	3.66±0.61	4.2±0.37	4.2±0.37**

Values are mean± SEM (n=6) one way ANOVA.*represents significant at p<0.05,** represents highly significant at p<0.01 *** represents very significant at p<0.001 when compared with control

Table 5.1: Effect of trial drug on uterus , ovary weight and cholesterol content

Group	Treatment dose	Uterus weight	Ovary weight	Cholesterol content of ovary
1	Control	0.922±0.10	0.7±0.051	17.83±1.24
2	Trial drug	0.492±0.020***	0.20±0.040***	32.4±4.11**

Values are mean± SEM (n=6) one way ANOVA.*represents significant at p<0.05,** represents highly significant at p<0.01 *** represents very significant at p<0.001 when compared with control.

Histological Examination

Uterus from trial group showed decrease in diameter of the uterus and absence of secondary and tertiary folding. Sub mucous glands were comparatively less in comparison to the control group. There were no significant changes observed in the histology of Ovary.

DISCUSSION

The use of herbo-mineral preparations for preventing the unwanted pregnancy are in practice since ancient time in India. Ayurvedic classics also mention about various such preparations. Among them Japakusumadi Yoga has been taken for evaluating the anti-fertility effect. In the present study anti-fertility effect of the *Japakusumadi yoga* was analysed by studying its effect on the Estrous cycle and Post coital anti fertility activity supported by bio chemical and histological parameters. The preliminary phytochemical analysis and the acute toxicity study of *Japakusumadi yoga* was conducted prior to the efficacy study.

The anti fertility effect the test drug was evaluated by assessment of the anti implantation effect, post implantation mortality and anti fertility activity. The anti-implantation effect observed in the group where the drug is administered for 1-6 days of pregnancy might be due to the disturbance of endocrine-endometrial synchrony which is dependent on estrogen and progesterone balance. As evident from the observations in study, there is a significant decrease in the estrogen and progesterone levels. This disturbed hormonal balance in turn alters the receptivity of the endometrium. Endometrial changes by hormonal imbalance provide the non receptive conditions which prevent the implantation of blastocyst. The cause for the loss of implantation may be due to antizygotic, blastocytotoxic or anti-implantation activity⁸. The possible reason for the Post implantation mortality (resorption) could be attributed to either direct effect or indirect effect on the corpus luteum affecting the synthesis and /or secretion of progesterone⁹.

The anti fertility activity exhibited by the trail drug could be because of its estrogenic nature. The preliminary phyto chemical analysis of the test formula-

tion has demonstrated the presence of flavonoids which are the phytoestrogens. Discussing the role of phyto estrogens, Turner et al opines that the flavinoides exert their effects primarily by binding to the estrogen receptor and are reported for their estrogenic activity responsible for anti fertility¹⁰.

Administration of the test drug showed a statistically significant increase in the duration of diestrus phase and a considerable decrease in the duration of proestrous, estrous and metaestrus phase, when compared to the control group. Thus a disruption of the estrous cycle is observed which could be because of non availability of estrogen, leading to the imbalance in estrogen and progesterone ratio¹¹.

Ovary consists of aggregation of three endocrine tissues, the stroma, the follicles and corpus luteum. The net weight of the ovary constitutes the sum of weight of these tissues constitutes .During the estrous cycle the weight of the ovarian tissue increases under the influence of gonadotrophic and steroidal hormones. The significant decrease in the weight of the ovaries in the trial group may be due to the non-availability of gonadotrophic or steroidal hormones or both for conversion of cholesterol into estrogen. Simultaneous increase in the Cholesterol content of the ovary, which is the precursor of steroidogenesis also support this view¹².

The significant decrease in the weight of uterus and abnormal histo architecture in the trial group could be due to the non availability of the hormone estrogen which could be because of the anti estrogenic activity of the trial drug which blocks the production or utilization of the estrogen or inhibits their effect. The results are in agreement with those of Kholkute et al (1976)¹³

The analysis of the data generated during the study shows that the test drug *Japakusumadi yoga* disrupts the estrous cycle and posses antifertility effect. The cause for the disruption of the estrous cycle could be because of the imbalance of the endocrine milieu. The significant decrease in the estrogen and the progesterone hormone has caused the prolongation of the diestrus phase. The Post coital anti fertility effect brought about was mainly due to the anti implantation activity of the trial drug and also partly due to the resorption (post implantation loss) of the

embryo. This could be because of the disturbance in estrogen: progesterone balance, which is very much essential for the regulation of estrous cycle, initiation and maintenance of the pregnancy. The atypical cytoarchitecture of the uterus in the study group also could be another important cause of the non implantation of the embryo. There was significant decrease in the weight of reproductive organs i.e. uterus and ovary with a significant increase in cholesterol content which signifies its non utilization for steroidogenesis.

CONCLUSION

This experimental study was undertaken to assess the effect of test formulation *Japa kusumadi yoga* on different parameters related to female reproductive system. It was observed that the test drug possesses anti implantation activity. The study on the Estrous cycle showed that the duration of Estrous phase, meta estrous and proestrous phase was shortened and diaestrus phase was significantly prolonged. The serum biochemistry revealed that there was significant increase in cholesterol content, decrease in estrogen and progesterone levels. The histopathology study of the uterus showed an abnormal cytoarchitecture but no significant changes in ovarian architecture.

In conclusion, the result of the present study showed that administration of *Japa kusumadi yoga* demonstrated antifertility activity which could be because of the presence of phytochemicals like flavinoids, steroids and tannins.

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