

## **EXPERIMENTAL EVALUATION OF GARBHANIVARANA YOGA IN WISTAR FEMALE ALBINO RATS**

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### **ABSTRACT**

Increasing incidence in population rate should be curtailed. Currently, there are about 51 births per minute in India. So to prove that the present methods of contraception are in use have failed in many aspects and there is the need for a new approach to the problem. The aim of this study is to find contraceptives of Ayurvedic origin. When it comes to Ayurveda as such there is no direct description of contraception in Samhitas, but they try to explain the contraceptive procedures in Ayurvedic grounds by taking the four necessary factors described in Samhitas – *Ritu* (time period), *Kshetra* (uterus), *Ambu* (liquid secretions) and *Beeja* (ovum and sperm). The union of one or two factors is necessary for fertility and if we restrict the union of one or two factors, we can prevent the conception. There are so many *Ekamuliya* (single) drugs as well *Bahumuliya* (combination) drugs which are mentioned in some texts of Ayurveda. It has, therefore become necessary to screen and use biologically active botanical substances as fertility regulating agents which are safe and interfere with the natural patterns of reproduction. Some traditional practitioners used to dispense oral contraceptives mentioned in Ayurvedic classics. we were interested in investigating the effect *Garbhanivarana* Yoga, one such yoga, on different conception related parameters. Ever increasing world's population has severely depleted the natural resources and has forced mankind to developed new fertility regulation methods. Though considerable progress has been made in the development of effective method of fertility control but most the methods developed include chemical formulation being non herbal have several side effects. It has, therefore become necessary to screen and use biologically active botanical substances as fertility regulating agents which are safe and interfere with the natural patterns of reproduction. Some traditional practitioners used to dispense oral contraceptives mentioned in Ayurvedic classics. we were interested in investigating the effect *Garbhanivarana* Yoga, one such yoga, on different conception related parameters. **Objectives:** There are

many formulations used by folklore tradition like nimb taila, Talisapatra added with Gairika with cold water etc. so study of *Garbhanivarana Yoga* on parameters related to conception and its maintenance is done. **Method:** Eighteen wistar albino female rats, were selected and categorized into three groups 1. control group 2. *Garbhanivarana Yoga* (TED) and 3. *Garbhanivarana Yoga* (TEDx2) *Garbhanivarana Yoga* administered for 15 days and then effect on the following parameters was assessed - Estrogen and Progesterone hormone levels, Triglycerides and Cholesterol in Ovary, Histology of ovary and second Anti-implantation activity. **Results:** Correspondingly significant changes in the estrous cycle, hormonal levels, cholesterol and triglycerides in ovary, the weight of the ovaries and the histopathology reports of ovary were appreciated, and the data obtained clearly indicates that Test drug (*Garbhanivarana Yoga*) has good contraceptive effect. **Conclusion:** The data obtained clearly indicated that it has good Anti-fertility, Anti-ovulatory and Anti-implantation activity effect against the endocrinal disruptive activity. Hence may be useful in the preventive measure for family planning as an oral contraception.

**Keywords:** Contraception, *Garbhanivarana Yoga* (*Pippali, Vidanga, Tankana*), Anti-fertility, Anti-ovulatory and Anti-implantation activity.

## INTRODUCTION

Since ages women have been playing a multi-faceted role in our society. The social infrastructure of the family, the community and in turn the mankind revolves around the women. Right from the days of evolution of mankind, women has accompanied man, has always been cooperating with her in built abilities till date. She has always tried to put up with the changing demands of the society by moulding herself, trying to fulfill the demands of her role within the family as a mother or as a wife and outside her family as a working women.

Woman is constantly subjected to drastic physiological and psychological changes throughout reproductive, menopausal and postmenopausal phase of her life, amongst there the Reproductive phase is most important in relation to her health. The population of India has increased 3 times since independence in 1947 and at the present rate of increase; the current population will double in another 33 years.

So, population control is now pressing problem not only in India but also throughout the world.

Control of population rests upon the factors like, the motivation for control and creation of awareness to adopt family planning measures. Some local and oral contraceptives are described in the ancient classics. It will be interesting to know how an ancient healthcare system like Ayurveda dealt with such problems.

Birth control is also known as contraception and fertility control. Fertility control involves sexual health care. Contraception is the prevention of conception. But fertility control involves not only contraception but also methods of interception such as post coital contraception and termination of pregnancy. In that fertility control if contraception is practiced thoroughly then there is no need of termination of pregnancies. There are well established contraceptive drugs and measures have been evaluated in existing modern medicine but the hormonal contraceptives cannot be used for long duration due to their severe side effects. In Ayurveda there are so many herbs which are giving good results without side effects and those *dravya* (medicine) are

easily available. So, many studies on the contraception in Ayurveda are going on, In order to evaluate the contraceptive effect and to provide safe economic contraceptive formulation, an attempt was made in this study.

### AIMS AND OBJECTIVES

1. To evaluate effect of *Garbhanivarana yoga* on Ovulation.
2. To evaluate effect of *Garvhanivarana yoga* on Hormonal changes.
3. To evaluate Anti - implantation effect.

### MATERIALS AND METHODS

#### TEST DRUG

*Garbhanivarana yoga*<sup>1</sup> (*Pippali*<sup>2</sup>, *Vidanga*<sup>3</sup>, *Tankana*<sup>4</sup> in equal quantity) with milk as *Anupana*.

#### METHOD OF PREPERATION OF MEDICINE FOR ADMINISTRATION

*Pippali* (*Piper longum* Linn.), *Vidanga* (*Embelia ribes* Burm.f.) fruits was taken in clean and dry *khalwa yantra*, pounded well and fine powder was prepared by *vastra galana* method. *Tankana bharjan* done and fine powder was prepared and mixed with above made powder. Thus prepared fine powder stored in clean and air tight container.

Fine powder of these drugs were taken and mixed well into 10 ml of *Godugdha*, shaken vigorously in test tube and then the uniform suspension was fed to albino rats orally by a syringe<sup>6</sup>.

#### DOSAGE CALCULATION

Human dose of *Garbhanivarana yoga* is 3 to 4g is as per the *Bhaisyajya Ratnavali* 5 calculated by converting human dose into rat dose using standard conversion method by following Pagets and Burns table.

### FORMULA

Rat dose = Human dose x Body surface area constant of the rat x 5 = 4g x 0.018 x 5/kg body weight = 0.36 g/kg body weight = 0.36 mg/g body weight and double TED dose = 0.72 mg /g body weight

This dose was further calculated according to the body weight of each rat.

### EXPERIMENTAL ANIMALS

Eighteen wistar albino rats were obtained from animal house attached to Pharmacology and toxicology laboratory at SDM centre for research in Ayurveda and allied Science Udupi, India with ethical committee clearance No (CPCSEA/IAEC/SDMCRA/PT-01) on 16/02/16. All the experimental animals were maintained under standard laboratory conditions, fed with commercial pallet diet (*pranav* agro Industry, Pune) and water ad libitum and darkness maintained in animal house with temperature 18 -25 ° c. Different groups of animals placed separately in polypropylene metabolic cage.

### METHODS OF SELECTION OF ANIMALS

#### Inclusion criteria

- Healthy fertile female albino rats of child bearing age with normal estrous cycle
- Body weight 150 to 200g
- Healthy fertile male rats were taken for mating.

#### Exclusion criteria

- Unhealthy fertile female albino rats of Body weight less than 150 g and more than 200 gm.
- Sterile male and female rats.

## PARAMETERS FOR EXPERIMENTAL STUDY

1. In vivo investigations: Estrous cycle monitoring.
2. Biochemical parameter: Cholesterol and Triglyceride levels in the ovary.
3. Hormonal Assay: Serum Estradiol, Progesterone.
4. Histopathology of Ovary

### 1) Estrous cycle monitoring

Study of antioviulatory effects was determined by observing vaginal smear daily between 9 to 10 AM continuously for 15 days. Vaginal smear were prepared by introducing a drop of distilled water into the vagina with the help of a dropper, collecting back and placing it on a clean slides, concentrated solution of methylene blue was used as a dye. The prepared smear was examined microscopically under low power for different phases of Estrous cycle<sup>7</sup>.

Dissection procedure:

At the end of the study, i.e. on the 16th day, 24 hour after the last dose the rats were sacrificed. The rats were anesthetized then blood collected for the hormonal assay and incision over the abdomen was taken which was extended above the neck and below the vagina, the organ ovary were excised out from sacrificed animal, freed from extra deposition, and weighed. Right ovary from each rat was processed for cholesterol estimation and another sent for histopathological study (fixing solution - 10 % formalin)

### 4) Anti implantation test

The female rats in proestrous phase were mated with male rats of known fertility in the ratio of 2:1 in the evening. Vaginal smears were examined in the following morning for the presence of sperms to confirm mating. Female rats exhibiting thick clumps of spermatozoa in the vaginal

smear were chosen for the study and that day was considered as day one of pregnancy. The obtained dose for each rat was administered according to their body weight from 6th to 15th day of pregnancy which is the period of organogenesis.

### Dissection procedure:

At the end of the study, i.e. on 19th day of pregnancy the rats were sacrificed. The rats were anesthetized then incision over the abdomen was taken which was extended above the neck and below the vagina, then the number of implantation sites, live fetuses and dead fetuses were noted in both horns of the uterus. The observations of the drug treated groups were compared with normal control group.

Percentage of abortion was calculated by the formula:

Percentage of abortion =  $\frac{(\text{Number of implantations} - \text{Number of live fetuses}) \times 100}{\text{Number of implantations}}$ .

### Statistical analysis

The data obtained were be analyzed by employing one way ANOVA followed by Dunnet's multiple t-test as post hoc test. A p value of less than 0.05 would be considered to indicate statistically significant.

## OBSERVATION AND RESULTS

The data related to the effect of test formulation on estrous cycle can be found in Table-1. Proestrus cycle was found to be decreased in a moderate manner in both the test drug administered groups, which was statistically non-significant. The oestrus cycle was found to be significantly ( $p < 0.5$  with TED dose and  $p < 0.01$  with TED x 2 dose) shortened in both the doses of test formulation in a dose dependent manner in comparison to the control group values.

Metaestrus phase was found to be non-significantly prolonged however, significant prolongation of di-estrus phase was observed.

From the data depicted in Table-2 it can be observed that the test drug at higher dose level produced significant decrease in cholesterol and triglyceride content of the ovary. The effect was not significant with the lower dose. Both serum estrogen and progesterone level were found to be moderately elevated in test drug administered group but the observed elevation was found to be non-significant in comparison to control group. Weight of ovary was found to be remarkably decreased at both the dose level ( $p < 0.01$ )

In pregnant rats, the data related to the effect of test drug on hormonal changes shows in Table-3 serum estrogen and progesterone was found to be decreased and increased in moderate manner in both the test groups. The effect was not significant with the lower dose.

The data shows in Table-4, in both the test drug administered groups found that decrease no. of live litter and increase no. of dead litter which was significant in comparison to control group.

#### **Histological examination:**

The sections of the ovary from different groups were examined under microscope to note down the differences in the organ cytoarchitecture among different groups.

The sections from control group- exhibited normal cytoarchitecture with different components in normal condition. However, increased leucocyte presence was observed at many sites. Follicles in different stages of development and maturation were observed. No change could be observed in primary germinal epithelium, the cortex and medulla size and proportion were found to be normal.

The sections from TG I administered group - exhibited increased leucocytosis. The number of mature follicles was less. Some of the mature follicles showed degenerative changes.

The sections from TG II administered group also exhibited increased leucocyte infiltration. Some sections exhibited normal profile of the follicles but majority follicles showed features of degenerative changes.

#### **DISCUSSION**

In this anti - fertility and anti-implantation study, results of test groups were compared with control group. This can be compiled by the results obtained from the experimental study which was carried out on three groups. Study has showed that *Garbhanivarana Yoga* Test drug treated groups' shows structural and functional changes in the reproductive system and on the hormonal levels.

The duration of Estrous cycle was observed to be shorten in both test groups, the Estrous phase showed shortening of duration double than the normal group, there was even significant increase in the diaestrous phase, this suggests that there was irregularity in duration of proestrous and metestrous phase interfering with the normal proliferation and maturation of the follicles leading to ovulation inhibition, and the ovaries revealed abnormal decreased stimulation of folliculogenesis.

In another observation it was noted that *Garbhanivarana Yoga* (TED) and *Garbhanivarana Yoga (TED x 2)* also led to decrease in weight of ovaries which suggested non-availability of gonadotrophic or steroidal hormones for proper conversion of cholesterol into estrogen, in the same group decreased levels of cholesterol than normal control group were noted which suggested there was no cho-

lesterol for estrogen conversion this leads to anovulatory cycle. To support this observation the decreased levels of estrogens in both the groups, proves that required amount of estrogen was not produced for follicular development. The progesterone levels not increased sufficiently in both the groups may be due absence of corpus luteum which suggests anovulation. This all points show that *Garbhanivarana Yoga* act as contraception.

In other group of study estrogen and progesterone levels should be increased during pregnancy but in both *Garbhanivarana Yoga* (TED) and *Garbhanivarana Yoga* (TED x 2) groups no such changes observed. No. of live litter were decreased than the control group and No. of dead litter has been increased this all suggests there is no proper nourishment and development of fetuses. During the study shows that the test drug *Garbhanivarana yoga* has definite pregnancy hindrance effect. This effect might be due to the changes it brings about in the ovarian function. How, it is achieved remains to be elucidated- it may be through affecting the Estrogen/Progesterone balance.

It is possible that the test formulation may be modulating the activity of the above factors at different levels and the other by interfering with the effect of test drug on pituitary.

The data obtained clearly indicated that it has good Anti-fertility, Anti-ovulatory and Anti-implantation effect against the endocrinal disruptive activity with both Test groups, hence may be useful in the preventive measure for family planning as an oral contraception.

## CONCLUSION

Possible action of *Garbhanivarana Yoga* may be that all ingredients are *pitta vardhak*, as *pitta* and *rakta* have same origin, *Rakta* produced

from *Rasa* dhatu and *Aratva* is *upadhatu* of *Rasa* and *Rakta*.

The *Ushna* and *Tikshna guna* of *pitta* are increased which irritates the *Garbhashaya* and may lead to *Garbhapata* (Abortion) as well as it decreases the *picchila guna* of *kapha* leading to *Aratvakshaya* due to vitiated *doshas* this may lead to irregular menstrual cycles, and probable have antioviulatory effect.

*Pippali*, *Vidanga* & *Tankana* have shown the presence of saponins, steroids, flavanoids & tannins, benzene, chloroform and aqueous extract of this fruits shows antifertility activity, anti- oestrogenic and anti ovulatory effect . Especially steroids & saponins are used as raw material for preparation of medically useful steroids & sex hormones like progesterone, oestradiol, & testosterone. Thus steroids, saponins might have contributed in the contraceptive activity of the drugs. Significant anti implantation & pregnancy interruption activity was noted in all the Groups indicating the contraceptive activity of the test drugs. The presence of Steroids, Saponins, flavanoids & Tannins might have contributed in contraceptive activity.<sup>8,9,10</sup>

## Scope for further research

This is an animal based experimental study but the promising results of this study necessitate a well designed randomized clinical research before the test drug is recommended for clinical practice.

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**Table1:** Effect of *Garbhanivarana Yoga* on Estrous cycle

| Estrous cycle phases | No. of days   |                                    |  | Percentage %  |                                    |  |
|----------------------|---------------|------------------------------------|--|---------------|------------------------------------|--|
|                      | Control group | <i>Garbhanivarana yoga</i> ( TED ) | <i>Garbhanivarana yoga</i> ( TED x 2 ) | Control group | <i>Garbhanivarana yoga</i> ( TED ) | <i>Garbhanivarana yoga</i> ( TED x 2 ) |
| Proestrous phase     | 1.83 ± 0.30   | 1.16 ± 0.30                        | 1.5 ± 0.22                             | -             | 36 ↓                               | 18 ↓                                   |
| Estrous phase        | 3 ± 0.36      | 1.5 ± 0.56                         | 0.66 ± 0.33 **                         | -             | 50 ↓                               | 34 ↓                                   |
| Metestrous phase     | 3.83 ± 0.83   | 4.5 ± 0.42                         | 5 ± 0.63                               | -             | 17 ↑                               | 30 ↑                                   |
| Diaestrous phase     | 5.8 ± 0.58    | 8.4 ± 0.67 *                       | 8.2 ± 0.48 *                           | -             | 44 ↑                               | 41 ↑                                   |

Data expressed as MEAN ± SEM, \*P<0.05, \*\*P<0.01 in comparison to normal control group

**Table2:** Effect of *Garbhanivarana Yoga* on Biobchemical changes and Weight of ovary

| Tests                         | Values        |                                    |  | Percentage %  |                                    |  |
|-------------------------------|---------------|------------------------------------|--|---------------|------------------------------------|--|
|                               | Control group | <i>Garbhanivarana yoga</i> ( TED ) | <i>Garbhanivarana yoga</i> ( TED x 2 ) | Control group | <i>Garbhanivarana yoga</i> ( TED ) | <i>Garbhanivarana yoga</i> ( TED x 2 ) |
| Triglycerides in ovary(mg/dl) | 21.16 ± 3.25  | 17.2 ± 2.08                        | 10.26 ± 2.28 *                         | -             | 23 ↓                               | 51 ↓                                   |
| Cholesterol in ova-           | 17.83 ±       | 14.2 ± 2.33                        | 9.33 ± 1.38 **                         | -             | 20 ↓                               | 47 ↓                                   |

|                        |                |                  |                 |   |      |       |
|------------------------|----------------|------------------|-----------------|---|------|-------|
| ry(mg/dl)              | 1.24           |                  |                 |   |      |       |
| Estrogen(pg/ml)        | 26.88 ± 10.98  | 15.43 ± 2.82     | 20.49 ± 10.16   | - | 42 ↑ | 23 ↑  |
| Progesterone(ng/ml)    | 9.44 ± 4.29    | 15.43 ± 2.82     | 19.27 ± 8.08    | - | 63 ↑ | 104 ↑ |
| Weight of ovaries(gms) | 0.046 ± 0.0049 | 0.022 ± 0.0058** | 0.02 ± 0.0036** | - | 52 ↓ | 56 ↓  |

## IN PREGNANT RATS

**Table 3:** Effect of *Garbhanivarana Yoga* on Serum Hormonal levels in Pregnant Rats

| Hormones           | Values        |                                    |  | Percentage %  |                                    |  |
|--------------------|---------------|------------------------------------|--|---------------|------------------------------------|--|
|                    | Control group | <i>Garbhanivarana yoga</i> ( TED ) | <i>Garbhanivarana yoga</i> ( TED x 2 ) | Control group | <i>Garbhanivarana yoga</i> ( TED ) | <i>Garbhanivarana yoga</i> ( TED x 2 ) |
| Estrogen level     | 38.27 ± 3.75  | 37.89 ± 3.32                       | 39.53 ± 4.28                           | -             | 10 ↓                               | 3 ↑                                    |
| Progesterone level | 58.28 ± 1.08  | 59.92 ± 0.046                      | 37.35 ± 11.10                          | -             | 2 ↑                                | 35 ↓                                   |

**Table 4:** Effect of *Garbhanivarana Yoga* as Anti - implantation activity

| No. of implantations | Values        |                                    |  | Percentage %  |                                    |  |
|----------------------|---------------|------------------------------------|--|---------------|------------------------------------|--|
|                      | Control group | <i>Garbhanivarana yoga</i> ( TED ) | <i>Garbhanivarana yoga</i> ( TED x 2 ) | Control group | <i>Garbhanivarana yoga</i> ( TED ) | <i>Garbhanivarana yoga</i> ( TED x 2 ) |
| No. of live litter   | 92.66 ± 4.52  | 50.27 ± 7.38**                     | 41.25 ± 2.93**                         | -             | 45↓                                | 55↓                                    |
| No. of dead litter   | 7.33 ± 4.52   | 52.22 ± 5.46**                     | 58.74 ± 2.93**                         | -             | 61↑                                | 70↑                                    |

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