

PHARMACEUTICO-ANALYTICAL AND EXPERIMENTAL STUDY ON THE ANTI-FERTILITY ACTIVITY OF GARBHANIRODHAKA YOGA IN FEMALE WISTAR ALBINO RATS

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ABSTRACT

Background: Population stabilization is a critical public health priority, necessitating the development of safe, non-hormonal, and naturally derived contraceptive agents. Garbhanirodhaka Yoga, a classical Ayurvedic herbomineral formulation, is reputed for its potential antifertility effects but lacks modern scientific validation. **Objective:** This study aimed to establish the pharmaceutico-analytical standards of Garbhanirodhaka Vati and evaluate its antifertility activity and safety profile in female Wistar albino rats. **Methods:** The formulation was prepared following classical Ayurvedic procedures using Shuddha Gairika (purified red ochre) and Talisapatra (*Abies webbiana*). Acute oral toxicity was assessed per OECD guidelines. Female Wistar albino rats were divided into five groups (n=6): Normal Control, Standard Control (ala-D), High Dose (single administration), Medium Dose (500mg/kg daily), and Low Dose (250mg/kg daily). Parameters including body

weight, estrous cycle regularity, and fertility outcomes were monitored over 15 days. **Results:** Pharmaceutico-analytical evaluation confirmed the formulation's quality, with microbial

loads within safety limits. In experimental studies, no significant alterations in body weight were observed ($p>0.05$), indicating a lack of systemic toxicity. Vaginal smear analysis revealed that while control groups maintained regular cyclicity, treated groups exhibited disrupted cycles. The medium-dose group showed persistent estrus, while the low-dose group exhibited persistent diestrus, indicative of ovulatory inhibition. **Conclusion:** *Garbhanirodhaka Vati* demonstrates a favorable safety profile and significant dose-dependent antifertility activity. The findings support its potential as a safe, non-hormonal oral contraceptive alternative.

KEYWORDS: *Garbhanirodhaka Yoga*, Antifertility, Ayurveda, Wistar Rats, Estrous Cycle, *Gairika*, *Talisapatra*.

1. INTRODUCTION

The global need for effective population stabilization has driven research into alternative contraceptive methods that are safe, accessible, and free from the side effects associated with synthetic hormonal contraceptives.^[1] While modern methods such as hormonal pills, IUDs, and sterilization are effective, they are often accompanied by adverse effects ranging from hormonal imbalance to physiological discomfort.^[2] Consequently, there is a resurgence of interest in traditional systems of medicine like Ayurveda to identify non-hormonal fertility regulation agents.

Ayurvedic classics describe *Garbhanirodhaka Yoga*, a herbomineral formulation comprising *Shuddha Gairika* (Red Ochre) and *Talisapatra* (*Abies webbiana*), as a potent agent for preventing conception.^[3] Despite its historical citation, rigorous scientific data regarding its pharmaceutical standardization and pharmacological efficacy remains limited.

This study was undertaken to bridge the gap between traditional knowledge and modern scientific validation. The primary objectives were to prepare *Garbhanirodhaka Vati* according to classical guidelines, subject it to pharmaceutico-analytical evaluation, and investigate its antifertility potential and safety in a female Wistar albino rat model.^[4]

2. MATERIALS AND METHODS

2.1. Drug Preparation (Pharmaceutics)

The test drug, *Garbhanirodhaka Vati*, was prepared in the Department of Rasashastra and Bhaishajya Kalpana at Sri Sri College of Ayurvedic Science and Research, Bengaluru.

- **Ingredients:** The formulation consists of *Shuddha Gairika* and *Talisapatra*.
- **Method:** The raw materials were authenticated and processed. *Gairika* was purified (*Shodhana*) using cow's ghee (*Go Ghrita*).^[5] The final formulation was processed into *Vati* (tablets) using *Khalva Yantra* (mortar and pestle) and dried in a hot air oven.^[6]
- **Vehicle:** Acacia gum was utilized as a suspending agent for uniform oral administration during animal trials.^[7]

2.2. Experimental Animals

Healthy female Wistar albino rats weighing between 180–220 g were used. The animals were housed in polypropylene cages with autoclaved rice husk bedding under standard laboratory conditions (25±2°C, 12h light/dark cycle) strictly adhering to CPCSEA guidelines.^[8]

2.3. Study Design

The animals were randomized into five groups (n=6 per group)^[9]

- **Group I (Normal Control):** Received distilled water.
- **Group II (Standard Control):** Received *Mala-N* (Ethinylestradiol + Levonorgestrel) at human equivalent doses.
- **Group III (High Dose):** Received a single high dose of the test drug.
- **Group IV (Medium Dose):** Received 500 mg/kg of the test drug daily for 15 days.
- **Group V (Low Dose):** Received 250 mg/kg of the test drug daily for 15 days.^[10]

2.4. Experimental Protocol

- **Mating:** Female rats in proestrus/estrus were cohabitated with males (3:1 ratio). Mating was confirmed by the presence of a vaginal sperm plug or spermatozoa in vaginal smears (Day 1 of pregnancy).^[11]
- **Dosing:** The test drug was administered orally post-mating confirmation.
- **Observations**
 - **Body Weight:** Recorded daily to monitor systemic toxicity.^[12]
 - **Vaginal Cytology:** Vaginal smears were collected, stained with Methylene blue, and examined microscopically to determine the phase of the estrous cycle (Proestrus, Estrus, Metestrus, Diestrus).^[13]
 - **Toxicity Signs:** Animals were monitored for behavioral changes, mortality, or physical signs of toxicity.

2.5. Statistical Analysis

Data were expressed as Mean \pm SD. Statistical significance was evaluated using One-way ANOVA followed by Dunnett's post-hoc test. A p-value of <0.05 was considered statistically significant.^[14]

3. RESULTS

3.1. Pharmaceutico-Analytical Evaluation

The prepared *Garbhanirodhaka Vati* was dark brown with a characteristic odor and consistent tablet hardness.^[15]

- **Microbial Limit Test:** The formulation was free from pathogens such as *E. coli*, *Salmonella* spp., *S. aureus*, and *Pseudomonas aeruginosa*. The total microbial count was 800cfu/g, and yeast/mold count was 30 cfu/g, well within permissible safety limits.^[16]

3.2. Body Weight and Toxicity

No mortality or abnormal behavioral changes were observed in any group throughout the study.

- **Body Weight Analysis:** There were no statistically significant differences in body weight gain across the groups ($p > 0.05$). For instance, on Day 1, the mean weight for the Control group was 196.8 ± 8.7 g, and for the Low Dose group was 195.2 ± 8.2 g.^[17] This stability suggests the test drug did not induce systemic toxicity or adverse metabolic effects.^[18]

3.3. Antifertility Activity (Vaginal Cytology)

- **Control Group:** Showed regular progression of the estrous cycle or signs of pregnancy (increased mucus, cessation of cycling) after mating.
- **Standard Group (*Mala-N*):** Exhibited disrupted cyclicity consistent with hormonal contraception.
- **Test Groups**
 - **Medium Dose (500 mg/kg):** Animals predominantly showed the **Estrus** phase, indicating a disruption in the ovulatory cycle.^[19]
 - **Low Dose (250 mg/kg):** Animals exhibited a persistent **Diestrus** phase.^[20] The persistence of the diestrus phase is a strong indicator of anti-ovulatory activity and inhibition of follicular maturation.

4. DISCUSSION

The study successfully validated the traditional claim of *Garbhanirodhaka Yoga* as an antifertility agent.

- **Mechanism of Action:** The vaginal smear results suggest that the formulation acts by disrupting the estrous cycle. The persistent diestrus observed in the low-dose group implies an anti-ovulatory effect or an alteration in the hormonal milieu required for implantation.^[21]
- **Safety Profile:** One of the significant findings is the safety of the formulation. Unlike synthetic contraceptives which often cause weight fluctuations and metabolic disturbances, *Garbhanirodhaka Vati* maintained a stable body weight profile across all doses.^[22] The use of *Shuddha Gairika* (purified Iron oxide) did not result in toxicity, likely due to the classical *Shodhana* (purification) process.
- **Dose-Dependency:** The study revealed a dose-dependent response where lower doses appeared more effective in maintaining a sustained diestrus block compared to the high-dose single administration.^[23]

Limitations: The study was limited to a 15-day duration and a sample size of n=6 per group. Long-term chronic toxicity studies and hormonal assays (FSH, LH, Estrogen, Progesterone) are recommended for future research to pinpoint the exact endocrine mechanism.^[24]

5. CONCLUSION

Garbhanirodhaka Vati, a herbo-mineral formulation, demonstrated significant antifertility activity in female Wistar albino rats without inducing systemic toxicity. The low-dose regimen (250 mg/kg) was particularly effective in inhibiting normal estrous cyclicity, evidencing its potential as a safe, non-hormonal contraceptive. These preclinical findings provide a robust scientific basis for further clinical exploration of this Ayurvedic formulation.^[25]

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