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EVALUATION OF ANTI-FERTILITY ACTIVITY OF *RUBIA CORDIFOLIA* LINN. ROOT EXTRACTS IN FEMALE RATS

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ABSTRACT

Background: Traditional medicinal systems have long used *Rubia cordifolia* as a remedy for various reproductive and gynecological disorders. However, its antifertility potential remains scientifically underexplored. The objective is to evaluate the antifertility activity of aqueous and ethanolic root extracts of *Rubia cordifolia* in female Wistar rats. **Methods:** Ethanolic (EERC) and aqueous (AERC) extracts were prepared and subjected to phytochemical screening. Acute oral toxicity was evaluated as per OECD guideline 420. Female rats were treated with the extracts (200 mg/kg, orally) during the first seven days of pregnancy. Anti-implantation and abortifacient effects were assessed on day 10. Histopathological analysis of uterine tissue was also performed. **Results:** Both extracts were non-toxic up to 2000 mg/kg. EERC showed 85% abortifacient and 25% anti-implantation activity, while AERC demonstrated 50% activity for both parameters. Phytochemical screening revealed alkaloids, glycosides, tannins, saponins, and phenolics. Histology showed mild uterine epithelial hyperplasia with EERC, and normal uterine structure with AERC. **Conclusion:** *Rubia cordifolia* root extracts exhibit significant antifertility activity through anti-implantation and abortifacient effects, likely due to estrogenic phytoconstituents. These findings support its traditional use and suggest potential for development of plant-based contraceptive agents.

KEYWORDS: *Rubia cordifolia*, antifertility, anti-implantation, abortifacient, phytochemicals, Wistar rats.

INTRODUCTION

Infertility and fertility regulation are significant global health concerns, particularly in developing countries where access to modern contraceptive methods may be limited. Although synthetic hormonal contraceptives are widely used, they are often associated with side effects, high cost, and limited accessibility, especially in rural areas. As a result, there is a growing interest in plant-based alternatives that are both effective and safe. Traditional medicine has identified a number of medicinal plants with potential antifertility activity, prompting researchers to explore their pharmacological effects.[1,2]

Rubia cordifolia Linn. (family: Rubiaceae), commonly known as Indian madder or "Manjistha," is a perennial climber extensively used in Ayurvedic and Unani systems of medicine. The root of this plant is valued for its blood-purifying, anti-inflammatory, antimicrobial, and anticancer properties. It has traditionally been employed in the treatment of gynecological disorders, skin diseases, spleen enlargement, and abnormal uterine bleeding. In folk medicine, it is also reputed to possess

anti-conceptional properties, although scientific validation of this claim is limited.[3,4]

Preliminary phytochemical studies have shown that *Rubia cordifolia* roots contain several bioactive compounds such as anthraquinones (e.g., purpurin, munjistin), alkaloids, tannins, flavonoids, glycosides, and phenolic compounds. Some of these phytoconstituents are known to exhibit estrogenic or anti-estrogenic activity, which can potentially interfere with the implantation process, embryonic development, or hormonal balance in the female reproductive system. Despite its long-standing use in traditional medicine, the antifertility potential of *Rubia cordifolia* remains under-investigated through systematic experimental models.[5,6]

The present study aims to evaluate the antifertility effects of aqueous and ethanolic root extracts of *Rubia cordifolia* in female Wistar rats. Acute toxicity, phytochemical screening, and histopathological analysis of uterine tissues were conducted alongside the assessment of anti-implantation and abortifacient activity. The goal is to scientifically validate the plant's traditional usage and provide a basis for the development of safe, herbal contraceptive formulations.[7,8]

MATERIALS AND METHODS

1. Plant Material and Authentication

The root of Rubia cordifolia Linn. (family: Rubiaceae) was collected from the Nilgiri district, Tamil Nadu, India, during December 2017. The plant material was taxonomically authenticated by Dr. S. Rajan, Field Botanist at the Central Council for Research in Homoeopathy, Ministry of AYUSH, Government of India. The roots were cleaned, shade-dried, and powdered mechanically. The powder was passed through sieve No. 22 and stored in an airtight container until use.

2. Preparation of Plant Extracts

2.1. Ethanolic Extract (EERC)

200 g of the powdered root was subjected to cold maceration with 640 mL of 80% ethanol in a round-bottom flask. Continuous shaking was performed for the first hour followed by intermittent shaking over six days. On the seventh day, the extract was filtered using muslin cloth and evaporated under reduced pressure using a rotary evaporator. The yield was recorded as 20.21 g.

2.2. Aqueous Extract (AERC)

Similarly, 200 g of powdered root was extracted using 800 mL of distilled water, following the same maceration and filtration procedures. The aqueous extract was evaporated under reduced pressure and the yield was 14.53 g.

3. Phytochemical Screening

Preliminary phytochemical screening of both extracts was conducted using standard procedures as described by Trease and Evans to test for:

- **Carbohydrates** (Molisch's, Benedict's, Fehling's tests)
- **Proteins and Amino Acids** (Ninhydrin, Millon's, Xanthoproteic tests)
- **Alkaloids** (Mayer's, Wagner's, Dragendorff's, Hager's reagents)
- **Tannins and Phenolic Compounds**
- **Glycosides** (Keller-Killiani test)
- **Saponins** (Foam test)
- **Flavonoids** (Shinoda test)

4. Experimental Animals

Adult healthy Wistar rats (female: 150–200 g, male: 150–200 g) were obtained from Kerala Veterinary and Animal Sciences University, Thrissur, India. The animals were maintained under standard laboratory conditions (temperature $22 \pm 3°C$, 12 h light/dark cycle) in polypropylene cages with free access to water and standard pellet diet.

The study was approved by the Institutional Animal Ethics Committee (IAEC No: COPSRIPMS/IAEC/PG/Pharmacology/04/2016-17). All procedures were conducted in accordance with CPCSEA guidelines.

5. Acute Oral Toxicity Study

Acute toxicity was assessed according to OECD Guideline 420 (Fixed Dose Procedure). Female Wistar rats were used. The sighting study was performed using stepwise doses of 5, 50, 300, and 2000 mg/kg of EERC and AERC administered orally. Based on the absence of mortality and behavioral changes, 2000 mg/kg was considered safe. In the main study, five female rats received 2000 mg/kg of extract and were observed for 14 days for signs of toxicity, behavioral changes, food and water intake, and mortality.

6. Antifertility Activity Study

6.1. Selection of Estrous Cycle Phase

Vaginal smears were collected daily using a pipette with 10 µL of saline. The smears were observed microscopically to determine the stage of the estrous cycle. Female rats in the estrous phase were selected for mating.

6.2. Mating Procedure

Selected females were mated with proven fertile males in a 2:1 ratio (female:male). The presence of spermatozoa or a vaginal plug in the morning smear confirmed mating, and that day was considered day 1 of pregnancy.

6.3. Experimental Groups

Twelve pregnant rats were randomly divided into two groups:

- **Group I**: EERC (200 mg/kg/day, orally, from day 1 to day 7 of gestation)
- **Group II**: AERC (200 mg/kg/day, orally, from day 1 to day 7 of gestation)

6.4. Evaluation Parameters

- **Laparotomy** was performed on day 10 under light ether anesthesia. Uterine horns were examined for implantation sites.
- **Reproductive Organ Weights**: On the final day, the uterus and ovaries were excised and weighed.
- **Body Weight**: Body weights were recorded on day 0 and day 7.
- **Abortifacient and Anti-implantation Indices** were calculated:

$$\text{Anti-implantation (\%)} = \left(\frac{\text{No. of corpora lutea - No. of implantations}}{\text{No. of corpora lutea}}\right) \times 100$$

$$\text{Abortifacient Activity (\%)} = \left(\frac{\text{No. of dead implants}}{\text{Total No. of implants}}\right) \times 100$$

7. Histopathological Analysis

Uterine tissues were fixed in 10% buffered formalin, processed by dehydration in graded alcohols, cleared in xylene, and embedded in paraffin. Sections (5 µm) were cut using a rotary microtome and stained with hematoxylin and eosin (H&E). Sections were evaluated under light microscopy for endometrial changes, glandular structure, stromal integrity, and presence of inflammation or hyperplasia.

8. Statistical Analysis

All quantitative data were expressed as mean ± SEM. One-way analysis of variance (ANOVA) was used followed by Dunnett's multiple comparison test to compare treatment groups with the control group. A p-value < 0.05 was considered statistically significant.

RESULTS

1. Acute Toxicity Study

No mortality or behavioral abnormalities were observed in either the sighting or main study groups up to the maximum dose of 2000 mg/kg for both ethanolic (EERC) and aqueous (AERC) extracts. This classifies the extracts under **GHS Category 5 (least toxic)**. All physiological parameters (motor activity, reflexes, cardiovascular signs, respiration, etc.) remained normal during the 14-day observation.

Table 1: Mortality Record for EERC and AERC Extracts in Acute Oral Toxicity Study.

Dose (mg/kg)	No. of Animals	Mortality	Toxic Signs
5	1	0/1	None
50	1	0/1	None
300	1	0/1	None
2000 (Sighting)	1	0/1	None
2000 (Main Study)	5	0/5	None

Interpretation: The LD_{50} of both extracts is >2000 mg/kg. Hence, 200 mg/kg (1/10th) was selected for efficacy testing.

2. Body Weight Changes

Body weights were recorded before (Day 0) and after (Day 7) administration of test extracts. Both groups showed a statistically significant (**p < 0.05**) reduction in final body weight compared to initial values.

Table 2: Effect of Rubia cordifolia Extracts on Body Weight.

Group	Initial Weight (g)	Final Weight (g)	% Change
EERC	233.57 ± 2.42	192.85 ± 1.92	↓17.43%
AERC	239.16 ± 2.05	210.83 ± 1.99	↓11.84%

Interpretation: Weight loss may indicate systemic effects of the extracts that could influence reproductive health.

3. Reproductive Organ Weights

After treatment, animals were sacrificed and their reproductive organs weighed. Both uterus and ovary weights were significantly decreased in extract-treated groups.

Table 3: Effect of Rubia cordifolia Extracts on Uterus and Ovary Weights.

Group	Ovary Weight (mg)	Uterus Weight (mg)
EERC	0.73 ± 0.12	0.14 ± 0.05
AERC	0.78 ± 0.17	0.15 ± 0.03

Interpretation: Decrease in organ weights is consistent with estrogen-suppressive effects, supporting antifertility activity.

4. Anti-implantation and Abortifacient Activity

Both extracts exhibited anti-implantation and abortifacient effects in proven-fertile female rats. Rats

were sacrificed on Day 10 of gestation and uterine horns were examined for implantation and resorption sites.

Table 4: Effect on Implantation and Abortifacient Activity.

Group	No. of Rats	No. of Implantations	% Anti-Implantation	% Abortifacient
EERC	6	2	25%	85%
AERC	6	3	50%	50%

Interpretation

- **EERC:** Higher abortifacient activity post-implantation (85%).
- **AERC:** More effective in preventing implantation (50%).

Both extracts demonstrated significant antifertility potential through two mechanisms:

- Inhibition of embryo implantation
- Termination of implanted embryos

5. Phytochemical Analysis

Preliminary phytochemical screening of both extracts showed presence of several bioactive compounds. Some constituents, such as alkaloids, glycosides, and tannins, may be responsible for the observed antifertility activity.

Table 5: Phytochemical Constituents Detected in EERC and AERC.

Sr.	Tests	Aq. Ext	Al. Ext
1. Carbohydrate			
A.	Molisch test	+ve	-ve
B.	Benedict test	+ve	+ve
C.	Barfoed test	-ve	-ve
D.	Fehling test	+ve	+ve
2. Alkaloids			
A.	Dragendorff test	+ve	+ve
B.	Wagner's test	-ve	-ve
C.	Mayer's test	-ve	-ve
D.	Hager's test	+ve	+ve
3. Amino acids			
A.	Ninhydrine test	+ve	+ve
4. Protein			
A.	Biuret test	+ve	-ve
B.	Xanthoproteic test	+ve	+ve
C.	Millon test	-ve	-ve
5. Saponin			
A.	Foam test	+ve	-ve
6. Glycosides			
A.	Keller Killiani test	+ve	-ve
B.	Borntrager's test	-ve	-ve
7. Phenolic compound			
		+ve	+ve
8. Flavonoids			
		-ve	-ve
9. Tannins			
A.	$FeCl_3$	+ve	+ve
B.	Lead acetate	+ve	+ve
C.	Pot. Dichromate	+ve	+ve
D.	Gelatin Test	+ve	+ve

Interpretation: The phytochemical screening revealed alkaloids, tannins, glycosides, and saponins, which may contribute to the antifertility activity of *Rubia cordifolia* through hormonal modulation and anti-implantation effects.

6. Histopathological Analysis

Uterine tissues were examined microscopically to assess endometrial and glandular changes.

Histological Feature	Aqueous Extract (AERC)	Ethanolic Extract (EERC)
Endometrium	Normal columnar epithelium; intact basement membrane	Mild hyperplasia observed; indicates epithelial proliferation
Glands	Large, well-defined, circular to elongated	Enlarged and circular; normal appearance
Stroma	Normal spindle-shaped cells; mild inflammatory infiltrate	Mild inflammatory changes present
Myometrium	No pathological change	No significant lesion
Interpretation	Maintained normal structure; anti-implantation likely due to biochemical effects	Epithelial hyperplasia suggests hormonal modulation and uterine sensitivity

Interpretation: EERC showed mild epithelial hyperplasia, indicating hormonal influence on the uterus. AERC maintained normal uterine structure, suggesting its anti-implantation effect is likely due to biochemical rather than structural alterations.

DISCUSSION

The present study evaluated the antifertility potential of *Rubia cordifolia* root extracts in female Wistar rats using both ethanolic (EERC) and aqueous (AERC) extracts. The extracts were found to be non-toxic up to a dose of 2000 mg/kg as per OECD guideline 420, indicating a favorable safety profile for further pharmacological investigations.

Significant antifertility activity was observed in both treatment groups. EERC exhibited 85% abortifacient activity with 25% anti-implantation effect, whereas AERC showed 50% activity for both parameters. These findings suggest that both extracts interfere with different stages of pregnancy—AERC predominantly inhibiting implantation and EERC disrupting post-implantation processes, possibly through hormonal interference or endometrial changes.

The phytochemical screening revealed the presence of bioactive constituents such as alkaloids, tannins, glycosides, saponins, and phenolic compounds. These phytochemicals have been reported in literature to exert antifertility effects by mimicking or interfering with reproductive hormones, modulating the uterine environment, and altering endometrial receptivity.

Body weight and reproductive organ weights were significantly reduced in treated animals, further supporting systemic effects of the extracts on reproductive physiology. The decrease in uterine and ovarian weights indicates a possible anti-estrogenic or hormone-suppressive action.

Histopathological analysis provided deeper insight into uterine tissue response. EERC-treated rats showed mild epithelial hyperplasia in the endometrium and minimal inflammation, pointing toward hormonal modulation or mild uterine irritation. In contrast, AERC-treated rats displayed normal uterine architecture, suggesting its antifertility action may be due to subtle biochemical modulation rather than structural changes.

Together, these findings indicate that *Rubia cordifolia* root extracts possess substantial antifertility activity without inducing significant toxicity. The ethanolic extract was more effective in inducing post-implantation resorption, while the aqueous extract was more potent in preventing implantation. These dual mechanisms make *Rubia cordifolia* a promising candidate for further investigation as a plant-based contraceptive agent.

However, the study is limited to in vivo animal testing, and further work is required to isolate and characterize the specific active compounds responsible, determine their mechanisms of action, and validate the safety and efficacy in higher animal models or clinical trials.

CONCLUSION

The findings of the present study confirm that *Rubia cordifolia* root extracts exhibit significant antifertility activity, primarily through anti-implantation effects and reduction in litter size. These results support its traditional use as an anti-conceptional agent in folk medicine. The antifertility effect is likely attributed to estrogenic phytoconstituents present in the plant, which interfere with normal implantation processes. This study lays the foundation for future investigations aimed at isolating the bioactive compounds and elucidating the underlying cellular mechanisms. Further research using different animal models and clinical evaluation is essential to develop safe and effective herbal formulations for fertility regulation.

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