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HISTOLOGICAL AND MORPHOMETRIC ALTERATIONS IN MALE REPRODUCTIVE ORGANS INDUCED BY *RUELLIA TUBEROSA* LINN. ROOT POWDER IN SWISS ALBINO MICE

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ABSTRACT
The current study evaluates the antispermatogenic effects of *Ruellia tuberosa* Linn. root powder on male Swiss albino mice with a focus on organ weights and histoarchitecture of the testis and epididymis. Mice were orally administered 50 mg/day of root powder for 15 and 30 days. Testis and epididymis weights were significantly reduced in treated groups ($p < 0.05$), and histological analysis revealed marked degeneration of seminiferous tubules, exfoliation of germinal cells, and a disrupted epithelial lining in the epididymis. These changes suggest impaired spermatogenesis and altered epididymal function. Recent phytochemical studies highlight bioactive flavonoids and triterpenoids that may mediate oxidative stress in testicular tissue. The effects appear reversible upon cessation, as shown in recovery groups in similar studies (Pardeshi et al. 2016). The findings support the traditional use of *R. tuberosa* in fertility regulation and suggest its potential role in developing reversible, plant-based male contraceptives.

KEYWORDS: *Ruellia tuberosa*, spermatogenesis, seminiferous tubules, testis histology, epididymis, male contraception, Swiss albino mice.

INTRODUCTION
With rising global population pressures, particularly in developing countries, the demand for safe and effective male contraceptive methods has intensified. Current hormonal and surgical options have side effects or irreversibility that limit their widespread use (Shukla et al., 2021). This has prompted interest in exploring plant-derived compounds with antispermatogenic properties.

Ruellia tuberosa Linn. (Acanthaceae), commonly known as Minnie Root or Fever Root, is traditionally used in folk medicine for its anti-inflammatory, antioxidant, and diuretic properties (Khare, 2007; Saranya et al., 2022). Although various pharmacological activities have been reported (Raju et al., 2020), the reproductive effects of its root powder are underexplored.

Preliminary pharmacological investigations suggest that Ruellia tuberosa exhibits antioxidant, anti-inflammatory, hepatoprotective, and antimicrobial activities (Raju et al., 2020; Patel & Desai, 2023). However, its potential role as an antifertility or antispermatogenic agent remains underexplored. Considering the traditional use of Ruellia species in reproductive health and the presence of bioactive phytoconstituents with possible hormonal or gonadotoxic effects, scientific inquiry into its reproductive toxicity is warranted.

This study aims to analyze the morphometric changes and histological alterations in the testis and epididymis of male Swiss albino mice treated with *R. tuberosa* root powder, thereby evaluating its potential as a male contraceptive.

MATERIALS AND METHODS
1. Collection and Preparation of Plant Material
Fresh tuberous roots of *Ruellia tuberosa* Linn. were collected from the campus of Government Vidarbha Institute of Science and Humanities (GVISH), Amravati, Maharashtra, India, during the months of October and November. The botanical identity of the plant material was authenticated by Dr. Mrs. P.V. Bhogaokar, Department of Botany, GVISH. The collected roots were thoroughly washed to remove soil and debris, shade-dried at room temperature ($25 \pm 2°C$), and pulverized using a mechanical grinder. The powdered root material was sieved through a fine mesh and stored in airtight containers for further experimental use.

2. Experimental Animals

Healthy adult male Swiss albino mice (*Mus musculus*), aged approximately 3 months and weighing between 25–30 g, were used for this study. The animals were obtained from a CPCSEA-registered breeding facility and maintained under standard laboratory conditions (temperature: 22 ± 2°C; relative humidity: 50–60%; 12 h light/dark cycle). The study was approved by the Institutional Animal Ethics Committee (IAEC), and the experiments were conducted in accordance with CPCSEA guidelines (Registration No. 1060/ac/07/CPCSEA).

3. Experimental Design

Animals were randomly allocated into the following groups, with 6 mice per group:

3.1 Control Group

Mice in this group received a standard pelleted diet prepared in the laboratory, as per National Institute of Nutrition (NIN), Hyderabad guidelines. Each animal was provided 3 g of feed/day and water *ad libitum*.

3.2 Experimental Group

Mice received 50 mg of *Ruellia tuberosa* root powder mixed in 3 g of feed per day. This group was further subdivided into two:

- **Experimental Group I**: Treatment with root powder for 15 days.
- **Experimental Group II**: Treatment with root powder for 30 days.

3.3 Positive Control Group

Mice received crude cotton seed oil at a dose of 25 µL/mouse/day mixed in 3 g of feed. This group was subdivided into:

- **Positive Control Subgroup I**: Treated for 15 days.
- **Positive Control Subgroup II**: Treated for 30 days.

4. Schedule of Sacrifice

At the end of the respective treatment periods, mice were weighed and sacrificed by cervical dislocation. The scrotal sac was incised, and the testes and epididymides were dissected, cleaned of fat and connective tissue, and processed for further analysis.

5. Body and Organ Weights

Initial and final body weights of the animals were recorded. After sacrifice, the weights of the testes and epididymides were measured using a precision digital balance to assess any treatment-related changes.

6. Histopathological Analysis

Tissue specimens from testes and epididymis were fixed in 10% neutral buffered formalin, dehydrated through ascending grades of alcohol, and embedded in paraffin wax. Sections of 5 µm thickness were cut using a microtome and stained with hematoxylin and eosin (H&E) for histological examination under a light microscope.

RESULTS

1. Body and Organ Weights

No significant difference in overall body weight was observed between the experimental, positive control, and control groups throughout the study period. However, a significant reduction in testicular weight was recorded in both the *Ruellia tuberosa*-treated and positive control groups.

On day 15, the experimental group treated with *Ruellia tuberosa* root powder showed an average 8.0 mg reduction in testis weight compared to control ($p < 0.05$), while on day 30, a 10.0 mg reduction was observed ($p < 0.02$), indicating a 10–30% decrease relative to control. The positive control (cotton seed oil-treated) group showed a more pronounced decrease in testicular weight—13.2 mg (15 days) and 15.4 mg (30 days), both statistically significant ($p < 0.05$). However, the difference between the experimental and positive control groups was not statistically significant.

A similar trend was observed in epididymal weight. Treatment with *Ruellia tuberosa* caused a significant reduction in the weight of epididymis at both 15 and 30 days ($p < 0.01$). The positive control group showed even greater reductions ($p < 0.05$), and the differences between the experimental and positive control groups were also statistically significant.

Table No. 1: Effect of Root powder of *Ruellia tuberosa* on weight of Testis.

Animal groups	15 Days treatment (Weight in mg)	30 Days treatment (Weight in mg)
Control	98.6 ± 4.39	97 ± 6
Experimental (Treatment: Root powder of *Ruellia tuberosa*)	90.6 ± 4.92*	87±1.41*
Positive Control (Treatment: Cotton seed oil)	87.8±10.52**	83.2 ± 7.46**

*=Statistically significant (p< 0.05) **= moderately significant (p<0.01) ***=Highly significant (p<0.001)

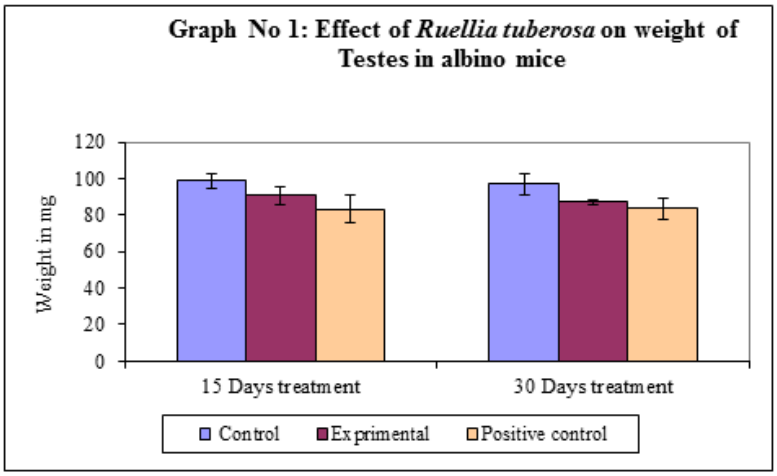
*: Significance compared with Control $: Significance capered with Positive control group

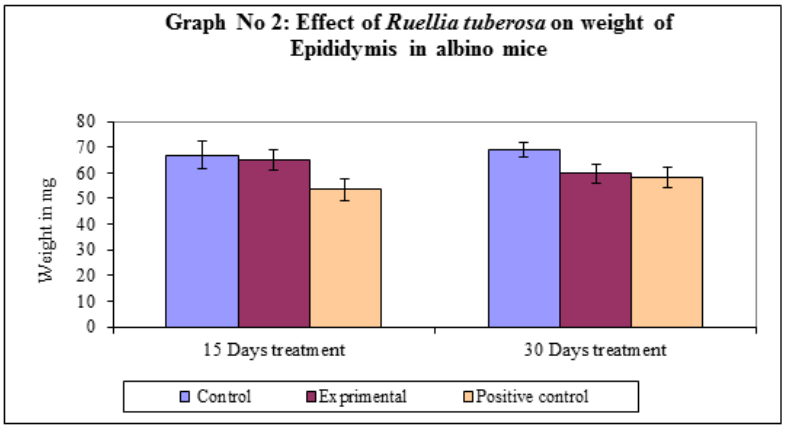
Table No. 2: Effect of Root powder of *Ruellia tuberosa* on weight of Epididymis.

Animal groups	15 days treatment (Weight in mg)	30 days treatment (Weight in mg)
Control	66.8 ± 2.86	68.8 ± 2.86
Experimental	64.8 ± 4.02**	59.6 ± 3.44**$$

(Treatment: Root powder of *Ruellia tuberosa*)		
Positive Control (Treatment: Cotton seed oil)	53.4 ± 4.04**	58.4 ± 3.91**

*=Statistically significant (p< 0.05) **= moderately significant (p<0.01)
***=Highly significant (p<0.001)*: Significance compared with Control
$: Significance capered with Positive control group





2. Histological Observations

2.1 Testis

15-DaysTreatment(ExperimentalGroup):

Transverse sections (T.S.) of the testis showed decreased spermatogenic activity. Seminiferous tubules were depleted of spermatids, and secondary spermatocytes were abundant in comparison to primary spermatocytes. Spermiogenesis was impaired, and exfoliation of germ cells was observed in some tubules. The lumen contained immature spermatogenic cells.

- **Fig. 1A:** Seminiferous tubule devoid of spermatids.
- **Fig. 1B:** Disorganized tubule with exfoliated cells and immature spermatids.

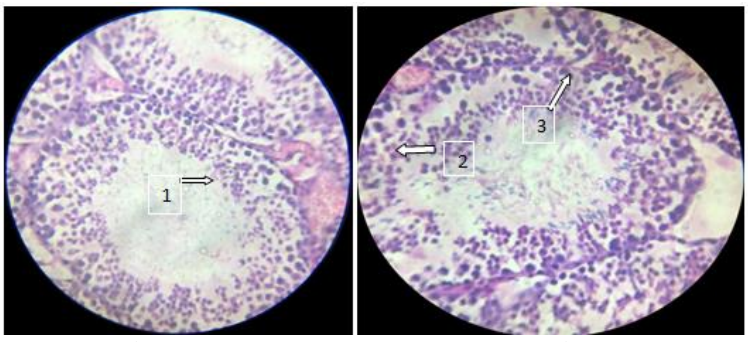


Fig. 1A **Fig. 1B**

Fig. 1: T.S. of Testis-Experimental group treated with root powder *of Ruellia tuberosa* for 15 days.

A: Seminiferous tubule showing absence of spermatids and spermiogenic cells.
B: Seminiferous tubule showing exfoliation, disorganisation and immature spermiogenic cells in the lumen.
1: Secondary spermatocyte
2: Spermatid
3: Spermiogenic Cells

30-Day Treatment (Experimental Group)
Marked reduction in the number of cells per tubule was observed, with degenerating spermatids and eosinophilic material in the luminal space. The number of both primary and secondary spermatocytes was lower than in the 15-day group, indicating progressive degeneration. Leydig cells retained normal morphology.

- **Fig. 2A & 2B:** Degenerated spermatids and disrupted tubules.

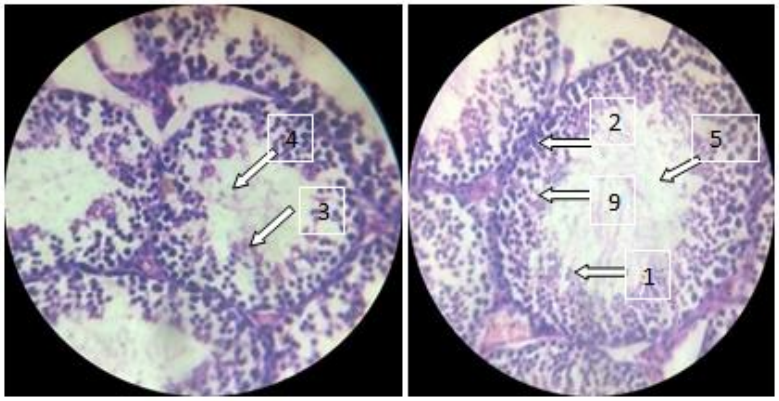


Fig. 2A **Fig. 2B**

Fig: 2 T.S. of Testis-Experimental group treated with root powder *of Ruellia tuberosa* for 30 days.

A: Seminiferous tubule showing exfoliation and disorganisation
B: Seminiferous tubule showing spermatids but no spermiogenic cells.
1: Secondary spermatocyte
2: Spermatid
3: Space in germinal epithelium showing disorganisation
4: Degenerating spermatogenic cells
5: Eosinophilic material in the lumen.

Positive Control (Cotton Seed Oil)
At both 15 and 30 days, there was a significant reduction in spermatogenic cells, disorganization, exfoliation of secondary spermatocytes, and disrupted basement membranes. After 30 days, spermatogonia near the basement membrane were significantly reduced.

- **Fig. 3A–3B, 2A–2B:** Testicular atrophy, exfoliation, and degeneration.

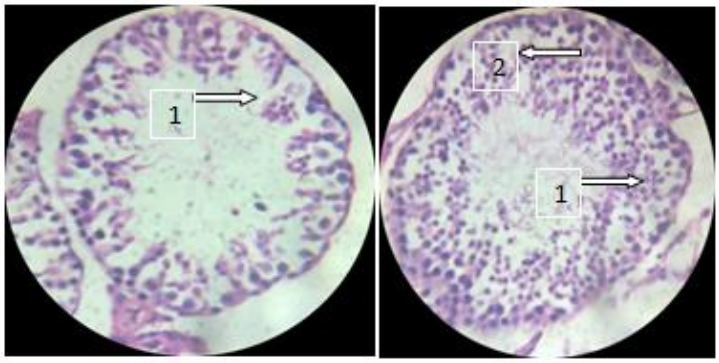


Fig. 3A **Fig. 3B**

Fig 3: T.S. of testis- Positive control group treated with cotton seen oil for 15 days.

A: Seminiferous tubele showing exfoliation of secondary spermatocytes.
B: Seminiferous tubele showingDisorganisation og germ cells.
1: Exfoliation of secondary spermatocytes
2: Space in germinal epithelium showing disorganisation

2.2 Epididymis
Control Group
Epididymal tubules exhibited healthy pseudostratified epithelium with abundant spermatozoa in the lumen.

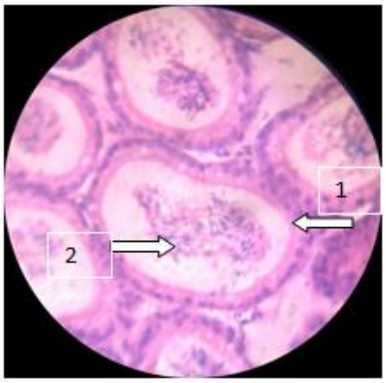


Fig. 4: T. S. of Epididymis-Control Group.

1: Psudoepithelial lining
2: Spermatozoa in the lumen

Experimental Group (15-Day)
Spermatozoa were sparse or absent in the lumen; the tubal epithelium appeared disturbed with signs of degenerative changes.

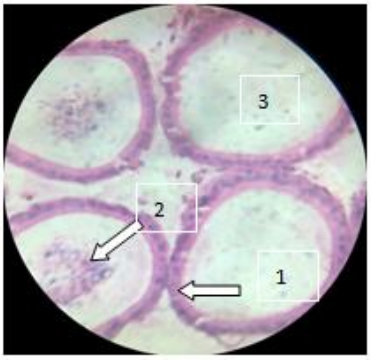


Fig. 5: T.S. of Epididymis- Experimental group treated with root powder of *Ruellia tuberose* for 15 days.

1: Psudoepithelial lining
2: Spermatozoa in the lumen
3: Tubules without spermatozoa

Experimental Group (30-Day)
Marked depletion of luminal spermatozoa, significant epithelial disruption, and lowered epithelial lining were observed.

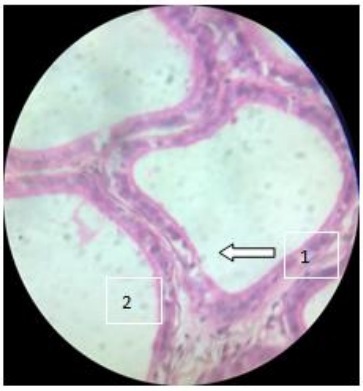


Fig. 6: T.S. of Epididymis- Experimental group treated with root powder of *Ruellia tuberosa* for 30 days.

1: Psudoepithelial lining
2: Tubules without spermatozoa

Positive Control Group
Significant epithelial damage and absence of spermatozoa were evident after 15 and 30 days, with disrupted basement membranes and empty lumens.

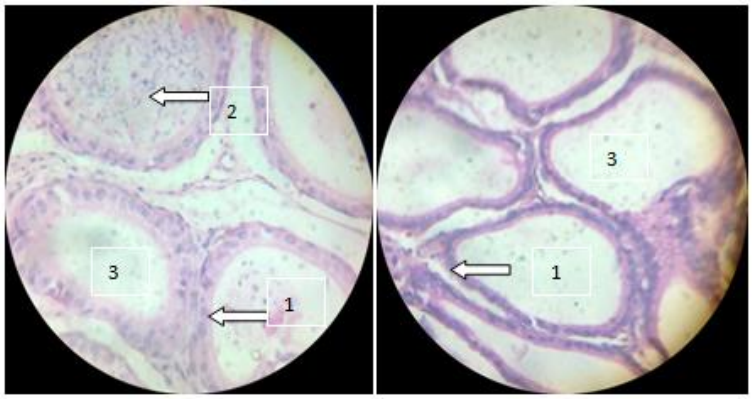


Fig. 7: T.S. of Epididymis- Positive control group treated with cotton seed oil for 15 days.
Fig. 8: T.S. of Epididymis- Positive control group treated with cotton seed oil for 30 days.

1: Psudoepithelial lining
2: Spermatozoa in the lumen
3: Tubules without spermatozoa

4. DISCUSSION

Significant reductions in testis and epididymis weight after *R. tuberosa* treatment indicate suppressed spermatogenesis. Organ weight loss is a recognized index of reproductive toxicity (Hess & de Franca, 2020).

Histological findings confirmed disruption in the normal spermatogenic cycle. Exfoliation of germ cells, loss of spermatids, and empty lumens point toward cytotoxicity against Sertoli and germ cells. Sertoli cell disruption is known to lead to sloughing of germ cells and impairment of spermiogenesis (Sharpe, 2020).

The epididymis, essential for sperm maturation and motility, also showed significant structural alterations. Empty lumens and degenerated pseudostratified epithelial lining may compromise the sperm storage and transit function, as seen in other herbal antifertility models (Oliveira et al., 2021).

Interestingly, the similarity between effects observed in experimental and positive control groups (cotton seed oil, a known antifertility agent) reinforces the bioactivity of *R. tuberosa* root powder as an antispermatogenic agent. However, the relatively milder histopathological changes in the 15-day treatment group compared to 30-day treatment suggest a progressive but potentially reversible effect, aligning with earlier findings in recovery studies (Pardeshi et al., 2016).

Phytochemicals such as flavonoids (e.g., luteolin, apigenin) present in *R. tuberosa* are known to modulate oxidative stress pathways and disrupt hormonal signaling, contributing to germ cell apoptosis (Saranya et al., 2022; Ahmad et al., 2023).

5. CONCLUSION

This study demonstrates that oral administration of *R. tuberosa* root powder causes significant morphometric and histological changes in the testis and epididymis of male Swiss albino mice. These effects appear dose- and duration-dependent, suggesting a potential role for *R. tuberosa* in male contraception.

Future studies should include hormonal profiling and fertility recovery trials to confirm reversibility and safety. The minimal systemic toxicity reported in other studies further supports its application as a safe herbal contraceptive candidate.

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