



ISSN (E): 2320-3862
ISSN (P): 2394-0530
Impact Factor (RJIF): 5.94
www.plantsjournal.com
JMPS 2025; 13(5): 202-205
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Received: 12-07-2025
Accepted: 16-08-2025

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Contraceptive efficacy of aqueous flower extract of *Hibiscus rosa-sinensis* (Linn.) on seminal profile of male *Mus musculus*: A comparative study

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DOI: <https://www.doi.org/10.22271/plants.2025.v13.i5c.1955>

Abstract

Hibiscus rosa-sinensis is a widely available plant in India, traditionally recognized for its medicinal properties. This study evaluated the antifertility efficacy of its aqueous flower extract in male Swiss albino mice (*Mus musculus*). 60 adult male mice were divided into two main groups: Group I (300 mg/kg body weight) and Group II (500 mg/kg body weight). Each group was further subdivided into control and treated group. Control group were fed orally 0.1 ml of glass distilled water, while treated groups were administered 0.1 ml of *H. rosa-sinensis* flower extract orally for 15, 30, 45, and 60 days. Mice were sacrificed and semen samples were obtained from both the cauda epididymis. Seminal parameters like sperm count, sperm motility, sperm mortality, sperm abnormality and seminal pH were assessed. Statistical analysis was done using student t-test. At 300 mg/kg body weight, treated group of mice showed a significant reduction ($p < 0.01$) in sperm count, motility, and seminal pH, while increase in sperm mortality and abnormalities ($p < 0.01$). At 500 mg/kg body weight, these effects were highly significant ($p < 0.001$), indicating a dose-dependent antifertility action. The aqueous flower extract of *H. rosa-sinensis* exerts strong, dose-dependent antifertility effects in male mice and may serve as a safe, reversible, and economical herbal contraceptive for males.

Keywords: *Hibiscus rosa-sinensis*, antifertility, sperm count, sperm motility, sperm mortality, sperm abnormality, *Mus musculus*

Introduction

Population growth is a critical global challenge, particularly in regions where resources are strained and access to family planning methods is limited. Population control refers to the strategic management of population growth to ensure sustainable development, social stability and ecological balance. The male contraceptives such as condoms and vasectomy are effective but they are either limited in application or irreversible underscoring the urgent need for alternative solutions. In recent years, plant-based compounds have gained significant attention as potential agents for developing safe, reversible and affordable contraceptive methods for the human population.

H. rosa-sinensis is one of the highly effective plant-based herbal contraceptives used to manage the human population. The bioactive compounds in *H. rosa-sinensis* shows a promising modulating effect on spermatogenesis and hormonal regulation, thus making it a capable natural male herbal contraceptive. Various research highlights its antifertility properties particularly in males where extracts of *H. rosa-sinensis* have shown the potential to interfere with spermatogenesis and reduces sperm motility. Additionally, its antioxidant and anti-inflammatory properties further enhance its suitability for pharmacological applications, reducing oxidative stress associated with various physiological processes (Mishra *et al.*, 2011) [10]. Beyond antifertility, *H. rosasinensis* exhibits a broad range of therapeutic effects, including antibacterial (Ruban *et al.*, 2012) [14], analgesic (Soni *et al.*, 2011) [15], antiovarulatory (Murthy *et al.*, 1997) [12], anti-implantation (Hadimur *et al.*, 2013) [7] activities. These multifaceted benefits underscore its importance in both traditional and modern medicine. The objective of this experiment was to estimate the contraceptive efficacy of aqueous flower extract of *H. rosa-sinensis* on sperm count, sperm motility, sperm mortality, seminal pH and abnormality of spermatozoa in male *Mus musculus*.

Materials and Methods

Experimental animal

A total of 60 adult Swiss albino mice weighing between 25 and 30 grams and aged between 12 and 14 weeks were chosen for this study from the animal house of the University Department of Zoology at T.M. Bhagalpur University in Bhagalpur. All the mice were kept in a clean, sanitary environment in a well-ventilated room with a temperature of 25 °C and a 10 hours photoperiod. The mice were fed with milk, tap water, bread, seasonal vegetables, and germinated seeds. A control group and a treatment group comprised all of the mice.

Experimental plant

A tropical blooming plant that belongs to the Malvaceae family, *H. rosa-sinensis* is often referred to as the Hawaiian hibiscus, Chinese hibiscus, or shoe flower. In gardens, parks, and indoor containers, it is frequently used as an attractive plant and is well-known for its enormous, beautiful blossoms. Hibiscus has gained popularity all over the world because to its therapeutic and aesthetic qualities.

Preparation of plant extract

H. rosa-sinensis flowers were gathered from the Bhagalpur district's local garden. After being cleaned with tap water, the flowers were left to dry in the shade. After that, the dried flowers were ground into a fine powder using a motor and pestle. Until the extract was prepared, the flower powder was stored in sterile, closed glass containers.

A dose of 300 mg/kg body weight was prepared by combining 1000 ml of glass-distilled water with 75 grams of powdered *H. rosa-sinensis* flower. Additionally, this mixture was soaked in a beaker at room temperature for the entire night. The mixture was filtered through filter paper. The experiment was conducted using the resultant filtrate (Jana *et al.*, 2013) [9]. To prepare the plant extract for a dosage of 500 mg/kg body weight, 125 grams of powdered *H. rosa-sinensis* flowers were dissolved in 1000 ml of glass distilled water, and the combination was then left to soak in the beaker at room temperature for the entire night. Filter paper was used to filter the mixture. After the filtrate was utilized for treatment, the remaining filtrate was kept in a glass jar in the refrigerator at 4 °C for later use. (Jana *et al.*, 2013) [9].

Design of the experiment

All the male Swiss albino mice were divided into Group I and Group II which were further divided into control and treated groups. The Group I mice in the treated group were fed 0.1 ml of *H. rosa-sinensis* flower extract orally through a gastric catheter at a dose of 300 mg/kg body weight for 15, 30, 45, and 60 days, whereas the mice in the control group were fed

0.1 ml of glass distilled water over the same exposure days. Group II mice in the treatment group were given 0.1 ml of *H. rosa-sinensis* flower extract orally at a dosage of 500 mg/kg body weight for 15, 30, 45, and 60 days. During the same exposure days, the mice in the control group were given 0.1 ml of glass distilled water. Six mice from each group were sacrificed through the cervical dislocation procedure after 15, 30, 45, and 60 days of exposure. The cauda epididymis was removed and crushed in 2 ml of normal saline. To prevent further tissue contamination, the seminal fluid was sieved using a metallic filter. The filtrate was used to study various seminal parameters. The sperm count was done after the method of Eliasson (1975) [4]. The study of seminal pH, sperm motility, sperm mortality, and abnormalities was done after the method of Tijee and Oentoeng (1968) [16].

Statistical analysis

For every sample, the mean, standard deviation, and standard error were determined. The Mean \pm SEM was used to express the data. To determine the degree of significance between the treated group and the control group, student t-test was used.

Results

The administration of aqueous flower extract of *H. rosa-sinensis* to Group I (300 mg/kg body weight) resulted in a significant decrease ($p < 0.01$) in sperm count, sperm motility, and seminal pH over the course of 15 to 60 days of exposure. The treatment also led to a significant increase ($p < 0.01$) in sperm abnormalities and sperm mortality as shown in Table-1 and Figure-1. These changes imply that, even at the lower dosage, the extract had a notable negative impact on male reproductive indicators.

In Group II (500 mg/kg body weight), the effects were more pronounced and significant. The sperm count, motility, and seminal pH exhibited a sharper decline compared to control groups. Meanwhile, sperm mortality and sperm abnormalities rose significantly ($p < 0.001$) and more rapidly with continued treatment as indicated in Table-2 and Figure-2. These alterations were not only duration-dependent but also clearly dose-dependent, showing a stronger intensity at the higher concentration of the extract. Overall, the present investigation demonstrated that aqueous flower extract of *H. rosa-sinensis* caused significant impairment of seminal quality in male Swiss albino mice. Both treatment groups showed detrimental effects on sperm physiology, but the higher dose (500 mg/kg body weight) resulted in the most severe disruptions. These findings suggest that *H. rosa-sinensis* exerts a potent antifertility effect, with the magnitude of alterations in seminal parameters being directly proportional to both dose and duration of treatment.

Table 1: Effect of aqueous flower extract of *H. rosasinensis* on seminal quality of mice at the dose 300mg/kg bodyweight

| Experimental variant group | Sperm count ($\times 10^4$ ml) | Sperm motility (in%) | Sperm mortality (in%) | Sperm abnormality (in%) | Seminal pH |
|----------------------------|---------------------------------|----------------------|-----------------------|-------------------------|-------------------|
| | Mean \pm SE | Mean% \pm SE | Mean% \pm SE | Mean% \pm SE | Mean \pm SE |
| Control (6) | 226.67 \pm 2.24 | 76.33 \pm 1.43 | 10.50 \pm 1.41 | 14.17 \pm 1.65 | 7.3 \pm 0.06 |
| 15 Days Treatment (6) | 176.17 \pm 3.08* | 63.67 \pm 2.11* | 35.83 \pm 2.54* | 37.50 \pm 1.34* | 6.4 \pm 0.05* |
| 30 Days Treatment (6) | 150.36 \pm 3.58** | 50.83 \pm 1.35** | 47.33 \pm 3.71** | 49.83 \pm 2.53** | 5.6 \pm 0.13** |
| 45 Days Treatment (6) | 128.17 \pm 7.36** | 45.33 \pm 0.88** | 58.00 \pm 2.99** | 62.67 \pm 2.87** | 5.2 \pm 0.12** |
| 60 Days Treatment (6) | 94.33 \pm 1.77*** | 31.17 \pm 1.08*** | 69.33 \pm 2.34*** | 69.17 \pm 2.46*** | 4.8 \pm 0.16*** |

The symbols *, **, *** shows significance at 0.05, 0.01, 0.001 respectively with value in control. Number within parenthesis shows number of samples

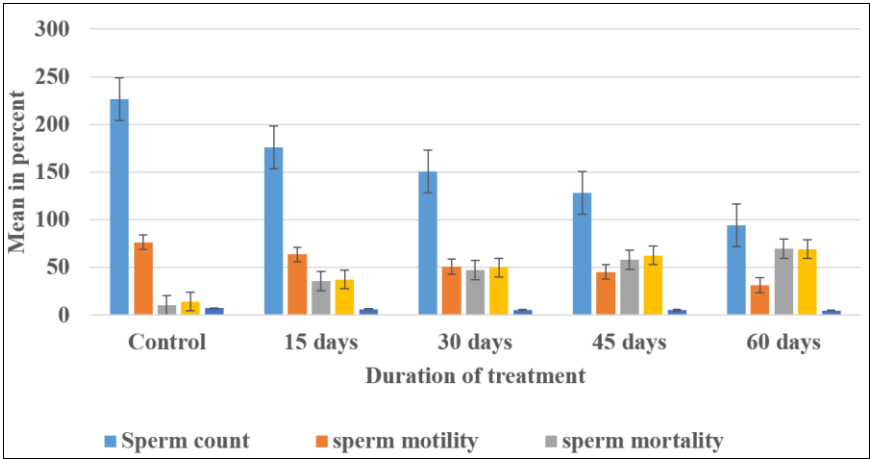


Fig 1: Histogram showing effects of aqueous flower extract of *H. rosasinensis* on mean sperm count, sperm motility, sperm mortality, abnormality of sperm, and mean seminal pH at 300mg/kg body weight

Table 2: Effect of aqueous flower extract of *H. rosasinensis* on seminal quality of mice at the dose 500mg/kg bodyweight

| Experimental variant group | Sperm count (×10 ⁴ ml) | Sperm motility (in%) | Sperm mortality (in%) | Sperm abnormality (in%) | Seminal pH |
|----------------------------|-----------------------------------|----------------------|-----------------------|-------------------------|-------------|
| | Mean ± SE | Mean%±SE | Mean%±SE | Mean%±SE | Mean ± SE |
| Control (6) | 230.17±4.38 | 75.42±1.42 | 13.50±0.88 | 12.67±0.88 | 7.5±0.06 |
| 15 Days Treatment (6) | 161.50±3.01* | 58.33±2.16* | 41.36±1.59* | 43.29±1.95* | 6.0±0.10* |
| 30 Days Treatment (6) | 136.17±3.74** | 42.50±0.88** | 56.48±2.13** | 57.32±2.95** | 5.1±0.22** |
| 45 Days Treatment (6) | 104.67±4.49*** | 35.48±1.67*** | 67.53±2.65*** | 69.83±1.56*** | 4.6±0.13*** |
| 60 Days Treatment (6) | 76.23±6.68*** | 26.50±1.81*** | 78.56±1.99*** | 77.50±2.54*** | 4.2±0.06*** |

The symbols *, **, *** shows significance at 0.05, 0.01, 0.001 respectively with value in control. Number within parenthesis shows number of samples.

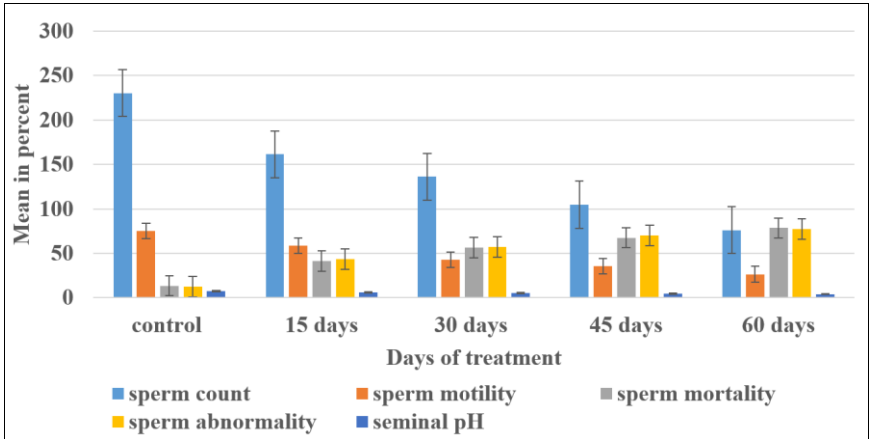


Fig 2: Histogram showing effects of aqueous Flower extract of *H. rosasinensis* on mean sperm count, sperm motility, sperm mortality, abnormality of sperm and mean seminal pH at 500mg/kg body weight

Discussion

The results of this study showed that aqueous flower extract of *H. rosa-sinensis* has a significant dose-dependent antifertility effect in male Swiss albino mice. These effects were more noticeable at 500 mg/kg body weight than at 300 mg/kg body weight and were accompanied by a decrease in sperm count, motility, and seminal pH, as well as an increase in sperm mortality and abnormalities. These results are consistent with more recent findings showing that both aqueous and non-aqueous (e.g. benzene or methanolic) plant extracts exert spermatotoxic and reproductive-inhibitory effects in animal models (e.g. Gupta *et al.*, 2024) [5]. Another recent study found that *H. rosa-sinensis* has a significant effect on male reproductive function as evidenced by the dose-dependent reduction in seminal fructose, an energy substrate essential for sperm motility. (Anand and Kumar, 2025) [1]. These biochemical changes point to a

disturbance in the secretory function of seminal vesicles, which may be caused by anti-androgenic phytochemicals like flavonoids, tannins, alkaloids, and saponins. Such classes of compounds have been implicated in the antifertility mechanisms of *Hibiscus rosa-sinensis* (Kholkute *et al.*, 1976) [19]. These findings are consistent with past studies employing extracts from leaves and flowers. Studies have found that male albino mice treated with benzene leaf extract had decreased fertility and spermatogenesis, but there was no discernible hematological or histopathological toxicity (Gupta *et al.*, 2024) [5]. In rat models, flower extract has also been demonstrated to have spermatotoxic effects and reduce sperm motility (Jana *et al.*, 2013) [9]. Furthermore, studies have looked into antifertility effects of other hibiscus parts, like the roots. In female rats, an ethanol extract of the root exhibited estrogenic and anti-implantation activity, suggesting a

potential hormonal pathway interfering with fertility. All together these results highlight the strong, dose-dependent antifertility effects of *H. rosa-sinensis* in males, which include disruption of sperm viability, energy provision, and accessory gland function. The presence of phytochemicals such as flavonoids, tannins, alkaloids, and saponins probably contributes to anti-androgenic or spermatotoxic effects.

Similar effects on antifertility have been noted in a number of other medicinal plants whose phytochemical components disrupt the physiology of male reproduction. Sperm count, sperm motility, sperm mortality, and seminal pH have all been demonstrated to be significantly reduced by *Curcuma longa*, suggesting spermatotoxic effects (Hembrom *et al.*, 2015) [8]. Similarly, it has been shown that aqueous extracts of *Piper betel* reduce seminal quality by decreasing sperm concentration and increasing the percentage of abnormal sperm forms (Verma *et al.*, 2015) [18]. Furthermore, treatment with *Aegle marmelos* has also been linked to changes in spermatogenesis and a gradual deterioration in sperm characteristics like sperm motility, sperm morphology, and overall fertility index (Kumar *et al.*, 2017) [11]. Collectively, these results lend credence to the mounting evidence that a variety of bioactive chemicals originating from plants may have male contraceptive effects by altering spermatogenic and seminal characteristics.

Conclusion

The aqueous flower extract of *H. rosa-sinensis* significantly impairs seminal quality in male Swiss albino mice, as evidenced by reduced sperm count, decreased sperm motility and seminal pH, and increased sperm abnormalities as well as sperm mortality. The findings strongly support the potential of *H. rosa-sinensis* as a natural male contraceptive agent.

Conflict of interest

There are no conflicts of interest.

Acknowledgment

The authors gratefully acknowledge the Department of Zoology, B.N. College, Bhagalpur, and the University Department of Zoology, T.M. Bhagalpur University, for providing the necessary laboratory and library facilities.

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