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STUDY OF SEMINAL ELECTROPHORETIC PROTEINS TO EVALUATE THE ANTIFERTILITY EFFICACY OF *HIBISCUS ROSA-SINENSIS* IN MALE *MUS MUSCULUS*

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ABSTRACT This electrophoretic study examined the impact of aqueous flower extract of *Hibiscus rosa-sinensis* on seminal proteins in male Swiss albino mice (*Mus musculus*). The animals were divided into control and treated groups. The treated group received orally 0.1 ml of the aqueous flower extract at a dose of 500 mg/kg body weight for 15, 30, 45, and 60 days, while the control group received 0.1 ml of distilled water for the same duration. After the treatment, seminal plasma proteins were separated using polyacrylamide disc gel electrophoresis and categorized into anodic and cathodic based on their relative mobility (Rm). The level of significance was determined using Student's t-test. There was a significant increase ($P < 0.001$) in anodic protein concentration, while cathodic proteins showed a non-significant increase in the seminal plasma of the treated group of mice when compared to the control. Due to a significant increase in anodic protein concentration, the total electrophoretic protein concentration also increased ($P<0.001$) significantly. Overall, *H. rosa-sinensis* extract significantly alters seminal plasma protein composition and may impair male reproductive function, indicating potential antifertility effects.

KEYWORDS : *Hibiscus Rosa-sinensis,* Seminal Proteins, Electrophoresis, Anodic Proteins, Cathodic Proteins, Antifertility, Swiss Albino Mice

INTRODUCTION

Rapid population growth has increased the demand for safe and effective fertility control methods. However, prolonged use of synthetic contraceptives is often associated with adverse effects such as hormonal imbalance, metabolic disturbances, and reproductive disorders. This has led to growing interest in medicinal plants as potential antifertility agents due to their low toxicity, affordability, accessibility, and cultural acceptance. Among them, *Hibiscus rosa-sinensis* Linn. (family Malvaceae), commonly known as China rose, has gained attention for its wide range of medicinal and pharmacological properties.

Seminal plasma proteins are essential regulators of sperm maturation, capacitation, motility, acrosome reaction, and successful fertilization. These proteins interact with sperm membranes and modulate several physiological and biochemical processes required for fertilizing competence. Alterations in seminal plasma protein composition are frequently associated with infertility, reproductive toxicity, and impaired sperm function (Samanta et al., 2018; Caballero et al., 2012). Changes in electrophoretic protein profiles may reflect modifications in seminal plasma constituents and sperm surface proteins that directly influence sperm capacitation, acrosome reaction, and fertilizing ability (Naz and Rajesh, 2004; Jin and Yang, 2017). Kumar et al. (2019) reported that aqueous leaf extract of *Aegle marmelos* selectively modulated seminal plasma proteins and produced a highly significant increase in anodic protein concentration in treated mice. The authors suggested that increased anodic protein fractions may enhance the negative surface charge of spermatozoa, thereby interfering with sperm capacitation and fertilizing ability, ultimately contributing to antifertility effects. However, despite several reports on the reproductive and antifertility effects of *H. rosa-sinensis,* limited information is available regarding the influence of its aqueous flower extract on seminal electrophoretic protein patterns in male albino mice.

Accordingly, the present investigation was undertaken to evaluate the antifertility effect of aqueous flower extract of *H. rosa-sinensis* on seminal electrophoretic protein patterns in male albino mice. The study was designed to examine treatment-induced alterations in seminal plasma proteins and to elucidate the possible biochemical mechanisms associated with plant-mediated reproductive suppression. The findings of the present study may further contribute to the identification of safer, economical, and reversible herbal contraceptive agents for male fertility regulation.

MATERIALS AND METHODS

Experimental Animals

Healthy adult male Swiss albino mice (*Mus musculus*) weighing 25–30 g and aged 10–12 weeks were selected for the study from the animal house of the University Department of Zoology, T. M. Bhagalpur University, Bhagalpur. All animals were kept in polypropylene cages and maintained under hygienic conditions in a well-ventilated room, with a 10-hour photoperiod (7:00 a.m. to 5:00 p.m.) at room temperature. The mice were fed twice daily with a diet consisting of bread, daliya (porridge), green vegetables, milk, and germinated seeds, along with ad libitum access to tap water.

Preparation of Aqueous Flower Extract

Fresh flowers of *H. rosa-sinensis* were collected from the garden of B.N. College, Bhagalpur; washed thoroughly; shade-dried; and powdered. The aqueous flower extract for the dose of 500 mg/kg body weight was prepared by dissolving 125 grams of *H. rosa-sinensis* flower powder in 1000 ml of glass-distilled water and left to soak overnight at room temperature. Filtration was performed using filter paper to obtain the aqueous flower extract. (Jana et al., 2013).

Experimental Design

Animals were divided into two groups: a control and a treated group, each consisting of six animals. The treated groups received an aqueous flower extract of *H. rosa-sinensis* at a dose of 0.1 ml (500 mg/kg body weight/day) for 15, 30, 45, and 60 days. The control group was administered 0.1 ml of distilled water for the same exposure days (Jana et al., 2013). After the treatment period, all animals were sacrificed. The cauda epididymis was carefully removed from the testes and mixed with 2 ml of normal saline. Each cauda was then minced, and the seminal content was filtered using a metallic filter. The resulting seminal fluid was centrifuged for electrophoretic analysis. Polyacrylamide disc gel electrophoresis was performed following the method described by Ivor Smith (1976).

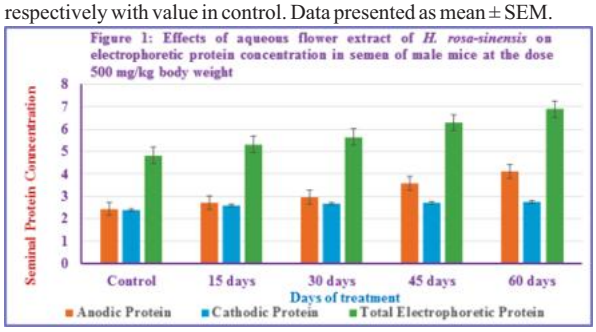
Relative mobility (Rm) refers to the distance moved by polypeptide bands relative to the movement of the marker Bromophenol Blue (BPB) in the gel. Protein bands migrating toward the cathode were identified as cathodic proteins, while those moving toward the anode were classified as anodic proteins. Protein concentrations of the bands were estimated using known concentrations of bovine serum albumin (BSA). The results were expressed as mean ± standard error of the mean. Statistical analysis was performed using Student's t-test to compare data and determine the level of significance.

RESULT

Table 1: Effect of Aqueous Flower Extract of *H. rosa-sinensis* on Electrophoretic Protein Concentration at a Dose of 500 mg/kg Body Weight of the Treated Group of Mice in Comparison with the Control.

Experimental variant group	Cathodic protein (mg/ml)	Anodic protein (mg/ml)	Total electrophoretic protein (mg/ml)
Control	2.38±0.08	2.45±0.04	4.83±0.12
15 days treatment	2.60±0.05	2.72±0.08*	5.32±0.10*
30 days treatment	2.67±0.06	2.98±0.11**	5.65±0.15**
45 days treatment	2.71±0.04	3.58±0.24***	6.29±0.05***
60 days treatment	2.77±0.03	4.12±0.06***	6.89±0.08***

The symbols *,**,*** shows significance at 0.05, 0.01, 0.001 respectively with value in control. Data presented as mean ± SEM.



The quantitative analysis of different seminal electrophoretic proteins showed a marked difference in the concentration of cathodic, anodic, and total electrophoretic protein concentration in the treated group of mice when compared to the control group.

Cathodic Protein Concentration: Table 1 indicates that the concentration of cathodic protein remained insignificant in the treated group of male mice when compared to the control group at 15, 30, 45, and 60 days of exposure.

Anodic Protein Concentration: The treatment of aqueous flower extract of H. rosa-sinensis at the dose of 500 mg/kg body weight in male mice caused a significant ($P < 0.01$) increase in anodic protein concentration after 15 and 30 days of exposure when compared to the control group of mice. However, a highly significant increase ($P<0.001$) in anodic protein concentration was obtained after the exposure of 45 and 60 days.

Total Electrophoretic Protein Concentration: Table 1 and figure 1 clearly depict that the concentration of anodic protein increased in the seminal plasma of the treated group of male mice. The total electrophoretic protein concentration also increased significantly ($P<0.001$) after 15, 30, 45, and 60 days of exposure when compared to control group of mice.

DISCUSSION

The present investigation demonstrates that aqueous flower extract of *H. rosa-sinensis* significantly alters seminal electrophoretic protein concentration (cathodic, anodic, and total) in male albino mice, indicating marked effects on seminal plasma biochemistry and sperm physiology, supporting its antifertility potential. Similar antifertility effects of medicinal plants through disruption of spermatogenesis and seminal biochemistry have been widely reported (Gupta and Yadav, 2024). Significant changes in cathodic and anodic protein concentration suggest disruption of proteins essential for sperm membrane stability, capacitation, motility, and fertilization (Samanta et al., 2018). Increased anodic proteins ($P < 0.001$) may enhance negative sperm surface charge, impairing capacitation and fertilization, consistent with findings in Aegle marmelos-treated mice (Kumar et al., 2019).

Vengaiah et al. (2015) reported that administration of *Piper betel* leaf stalk at a dose of 50 mg/kg body weight/day for 15 days in male albino rats significantly increased total protein levels in all reproductive organs, including the testes, epididymis, seminal vesicle, and prostate gland. Similarly, Verma et al. (2016) observed that treatment of male albino mice with *Piper betel* leaf stalk extract at 50 mg/kg for up to 50 days produced a significant increase ($P < 0.001$) in total electrophoretic and anodic seminal proteins, while cathodic protein levels remained comparatively unchanged. The insignificant increase in cathodic proteins may indicate depletion or alteration of fertility-associated proteins essential for sperm viability, membrane integrity, and metabolic activity, as also observed in H. rosa-sinensis treated rodents (Kumar et al., 2013; Anand and Kumar, 2025). Increased seminal plasma protein concentration has been associated with impaired sperm maturation, reduced motility, oxidative stress, and altered accessory sex gland secretions, ultimately contributing to male infertility (Samanta et al., 2018; Kholkute, 1977; Tan, 1983). Earlier investigations demonstrated that H. rosa-sinensis extracts can interfere with spermatogenesis and accessory reproductive organ function, producing anti-spermatogenic and antifertility effects in experimental animals (Kholkute et al., 1977; Tan, 1983). These reproductive alterations are likely mediated by bioactive phytochemicals such as flavonoids, alkaloids, tannins, glycosides, saponins, and phenolic compounds, which are known to disrupt androgen biosynthesis, testicular steroidogenesis, sperm metabolism, and epididymal function (Vasudeva and Sharma, 2008; Gupta and Yadav, 2024). Overall, the electrophoretic changes in seminal proteins suggest that *H. rosa-sinensis* exerts antifertility activity through modulation of seminal plasma composition, impairment of sperm function, and disruption of reproductive physiology, thereby supporting its potential utility in the development of plant-based male contraceptives (Anand and Kumar, 2025; Vasudeva and Sharma, 2008).

CONCLUSION

The aqueous flower extract of *H. rosa-sinensis* significantly altered seminal electrophoretic proteins in male Swiss albino mice. These findings indicate that changes in seminal electrophoretic proteins are closely associated with impaired male reproductive function. Therefore, *H. rosa-sinensis* may possess potential reversible antifertility activity and could be used as an herbal contraceptive agent.

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Conflict of Interest

The authors declare that there is no conflict of interest.

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