Antifertility effects of aqueous flower extract of *Hibiscus rosa-sinensis* (Linn.) on seminal parameters in male Swiss albino mice.

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Abstract
This study was undertaken to investigate the antifertility effects of aqueous flower extract of Hibiscus rosa-sinensis on seminal parameters in male Swiss albino mice. The treated group of mice was administered 0.1 ml of aqueous flower extract of H. rosa-sinensis orally with a gastric catheter at the dose of 200 mg/kg body weight for 15, 30, 45 and 60 days, while the control group of mice was fed with 0.1 ml of glass distilled water for the same exposure days. The mice were sacrificed through the cervical dislocation method. Various physical parameters including sperm count, sperm motility, seminal pH, sperm mortality and abnormality of spermatozoa were assessed. For statistical analysis of data, the student's t-test was applied.

The result demonstrated a significant decrease ($P<0.001$) in sperm count, sperm motility and seminal pH in the treated group as compared to the control group of mice, while significant ($P<0.001$) increases in sperm mortality and abnormal sperm morphology were also observed in the treated group of mice as compared to the control group of mice. These findings suggest that H. rosa-sinensis has a pronounced antifertility effect in male Swiss albino mice, primarily through its impact on spermatogenesis and hormonal balance. Thus, H. rosa-sinensis can be used as safe, cheaper, herbal, reversible contraceptive agents for males.

Keywords: Antifertility, *Hibiscus rosa-sinensis*, Sperm count, Sperm mortality, Sperm motility, Spermatogenesis.

Introduction

Population growth has been a focal point of global concern, particularly as the world's population continues to rise at an unprecedented rate. In sustainable development, managing population growth has become a priority in development agendas worldwide because of the limited resources, escalating environmental degradation and heightened demands on healthcare and social systems. Overpopulation is a major challenge in many developing countries, leading to increased demand for resources, environmental degradation and social issues. To overcome the problem of population growth, one of the efforts that can be made is developing effective family planning methods[8].

There are several approaches available for control of fertility in females, like condoms, tubectomies, IUDs, birth control pills, implants, etc. But in the case of males, only a few contraceptive methods are available like condoms, withdrawal and vasectomy, but none of them has emerged as the best choice free from side effects. Among the various strategies explored, natural and plant-derived antifertility agents have gained considerable attention due to their perceived safety, lower side effect profiles and potential effectiveness[12].

In particular, plants used in traditional medicine have shown promising antifertility effects in animal studies with various bioactive compounds that can influence spermatogenesis, reduce sperm motility, or inhibit androgen production. By exploring such plants as potential sources for male contraceptives, it aligns with the growing interest in botanical medicine, which seeks safer and more natural approaches to healthcare. In these recent years much attention has been focused on indigenous medicinal plants for possible contraceptive effects in humans since herbal preparations are easily available, cheap and have a natural origin with a higher safety margin and lesser or no side effects[7].

Thus, there is a need to search for such an antifertility agent that is safe, reversible, cheap, herbal, familiar, easily available, specific, indigenous and eco-friendly and without any side effects. One of the effective herbal contraceptives of plant origin to control human population is *Hibiscus rosa-sinensis*[8]. *H. rosa-sinensis*, commonly known as the Hawaiian hibiscus, Chinese hibiscus, or shoe flower, is a tropical flowering plant belonging to the family Malvaceae. It is renowned for its large, showy flowers and is often used as an ornamental plant in gardens, parks and indoor containers. Hibiscus has been embraced globally for both its aesthetic and medicinal properties. It blooms throughout the year in tropical and subtropical climates. Flowers are actinomorphic, radially symmetrical and have prominent stamen and pistil.

H. rosasisensis has a rich history in traditional medicine because phytochemical studies reveal that it is rich in compounds such as flavonoids[14], tannins[1] and polyphenols[1],

which contribute to its antioxidant[5,13,14], anti-inflammatory[15], antifertility[3,7,8] and antibacterial[16] activities. The present study was undertaken in order to estimate the antifertility effects of aqueous flower extract of *H. rosa-sinensis* on sperm count, sperm motility, sperm mortality, seminal pH and abnormality of spermatozoa.

Material and Methods

Preparation of Plant Extract: The flowers of *H. rosa-sinensis* were collected from the local garden of Bhagalpur district. The flowers were washed in tap water and dried in the shade. Then, the dried flowers were pulverized in the mortar and pestle to a fine powder. The flower powder was kept in clean, closed glass containers until the extract preparation. The preparation of plant extract for a dose of 200 mg/kg body weight was done by dissolving 50 grams of *H. rosa-sinensis* flower powder into 1000 ml of glass distilled water and the mixture was soaked overnight in the beaker at room temperature. The mixture was filtered with the help of filter paper. The filtrate was used for treatment and the leftover filtrate was stored in a glass jar at 4°C in the refrigerator for further use[3,4].

Experimental Animal: 30 adult Swiss albino mice (age 12 to 14 weeks) weighing 25 to 30 grams were selected for this investigation from the animal house of the University Department of Zoology, T. M. Bhagalpur University, Bhagalpur. All mice were maintained under clean and hygienic conditions in a well-ventilated room with a 10-hour photoperiod along with a 25°C temperature. The mice were provided with bread, seasonal vegetables, germinated seeds, milk and tap water *ad libitum*.

Experimental design: All mice were divided into two groups, a control group and a treated group. The treated group of mice was administered 0.1 ml of aqueous flower extract of *H. rosa-sinensis* orally with a gastric catheter at the dose of 200 mg/kg body weight for 15, 30, 45 and 60 days, while the control group was fed with 0.1 ml of glass distilled water for the same exposure days. 6 mice of each group were sacrificed after the exposure of 15, 30, 45 and 60 days. The mice were sacrificed through the cervical dislocation method. For physical parameter assessment, both the cauda epididymis was taken out and teased in 2 ml of normal saline. Seminal contents were sieved by a metallic filter to avoid any other tissue contamination. The sample was then stained with eosin and counting of sperm was done with the help of a hemocytometer followed by the method of Eliasson[6].

During the experiment, sperm motility, mortality, abnormality and seminal pH were also observed and studied. The motility of spermatozoa was observed according to the method of Tijee and Oentoeng[18]. Seminal pH was measured with the help of pH paper manufactured by Merck Limited, Worli, Mumbai. The abnormality of spermatozoa was studied after the same method, by forming a thin film of semen on slides. The film on slides was fixed in methanol. Then the slides were stained in eosin for 40 minutes. After staining, the slides were washed under tap water and then kept for drying. Then the dried slides were observed under a microscope to study the abnormality of spermatozoa. For the statistical analysis of data, the student's t-test was applied.

Results

Sperm count: In the present investigation, the mean sperm count in the control group of male Swiss albino mice observed was 221.16± 4.60×10^4 sperm/ml, while after treatment with aqueous flower extract of *H. rosa-sinensis*, the sperm count got reduced to 184.67± 2.22×10^4 sperm/ml, 161.33± 2.95×10^4sperm/ml, 143.00± 2.52×10^4 sperm/ml and 114.50± 4.98×10^4 sperm/ml during 15, 30, 45 and 60 days of exposure respectively.

Sperm motility: The mean motility percent of spermatozoa was observed to be 74.16± 1.17 in the control group of male mice, but after treatment with aqueous flower extract of *H. rosa-sinensis*, the mean motility percent was reduced from 69.33± 1.82 to 38.60± 1.54 during 15 to 60 days of treatment.

Seminal pH: The mean seminal pH observed in the control group of mice was 7.4±0.06, but after treatment with aqueous flower extract of *H. rosa-sinensis*, the pH decreased to 6.7±0.09, 6.1±0.07, 5.8±0.11 and 5.5±0.12 in the treated group of mice for 15, 30, 45 and 60 days respectively. As indicated in table 1, sperm count, seminal pH and sperm motility show a significant decrease during 15 to 60 days ($P < 0.001$) among treated groups of mice with aqueous flower extract of *H. rosa-sinensis* compared to the control group.

Sperm mortality: The mean mortality percent observed in the control group of male mice was 15.33 ± 1.45, but it increased from 31.50 ± 1.73 to 61.00 ± 1.37 between 15 days and 60 days of exposure to aqueous flower extract of *H. rosa-sinensis* in the treated group of male mice when compared to the control group. Sperm mortality in caudal semen showed a significant increase after 15 to 60 days ($P<0.001$) of treatment compared to the control group of mice.

Sperm abnormality: The mean sperm abnormality percent increased significantly ($P<0.001$) from 30.67± 3.47 to 67.16± 1.96 from 15 to 60 days of exposure in the treated group of mice as compared to the control group (19.50±2.14) of mice. As indicated in table 1, sperm mortality and sperm abnormality show a significant increase during 15 to 60 days ($P<0.001$) among treated groups of mice with aqueous flower extract of *H. rosa-sinensis* compared to the control group of mice.

Discussion

The present investigation shows antifertility effects of aqueous flower extract of *H. rosa-sinensis* on seminal parameters in male Swiss albino mice at the dose of 200 mg/kg bw.

Table 1
Effects of aqueous flower extract of *H. rosa-sinensis* on seminal quality of male Swiss albino mice

Experimental variant group	Sperm count ($\times 10^4$ ml) sperm/ml	Sperm motility (in %)	Sperm mortality (in %)	Sperm abnormality (in %)	Seminal pH
	Mean ±SE	Mean%±SE	Mean%±SE	Mean%±SE	Mean±SE
Control (6)	221.16±4.60	74.16±1.17	15.33±1.45	19.50±2.14	7.4±0.06
15 Days Treatment (6)	184.67±2.22*	69.33±1.82*	31.50±1.73*	30.67±3.47*	6.7±0.09*
30 Days Treatment (6)	161.33±2.95**	61.00±1.37**	43.83±1.20**	42.67±1.82**	6.1±0.07**
45 Days Treatment (6)	143.00±2.52**	52.30±1.61**	49.17±1.70**	56.50±2.22**	5.8±0.11**
60 Days Treatment (6)	114.50±4.98***	38.60±1.54***	61.00±1.37***	67.16±1.96***	5.5±0.12***

The symbols *,**,*** shows significance at 0.05, 0.01, 0.001 respectively with value in control. Number within parenthesis shows number of samples. Mean ±SE (Mean±Standard Error).

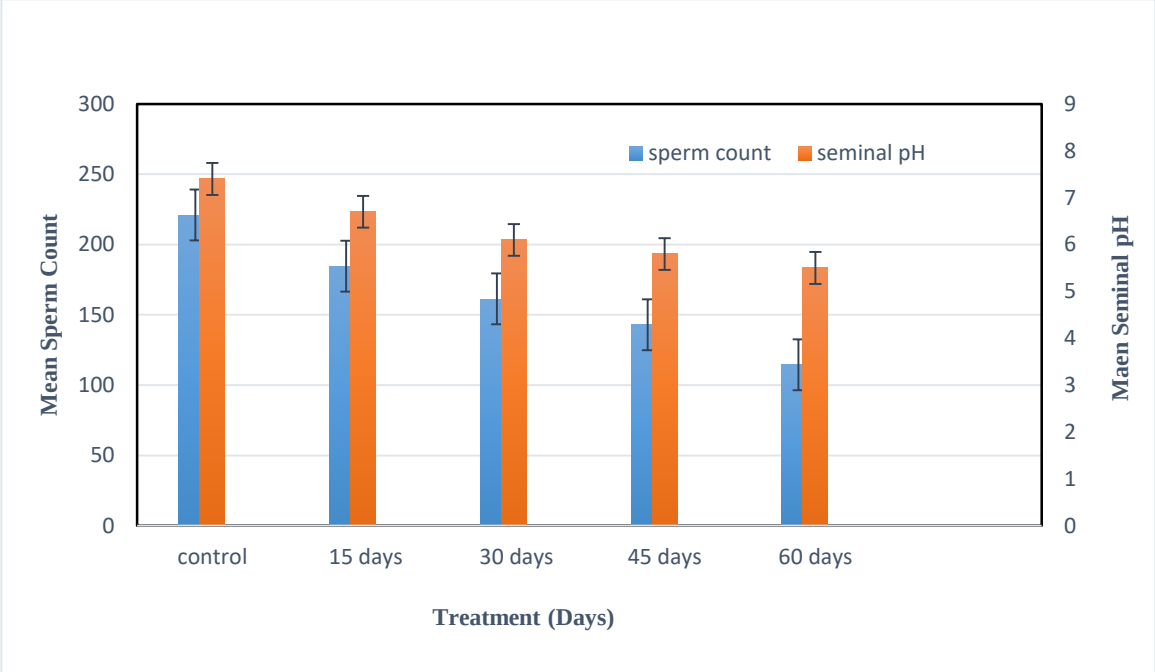

Figure 1A: Histogram showing effects of aqueous flower extract of *H. rosa-sinensis* on mean sperm count and mean seminal pH

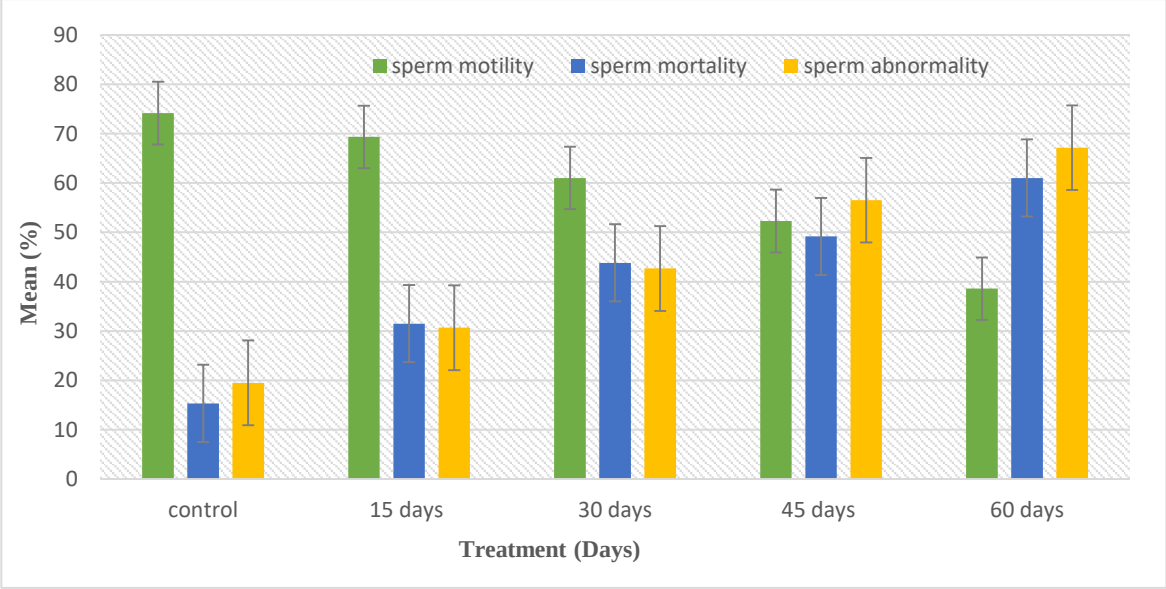

Figure 1B: The histogram showing effects of aqueous flower extract of *H. rosa-sinensis* on sperm motility, sperm mortality and abnormality of sperm in mice

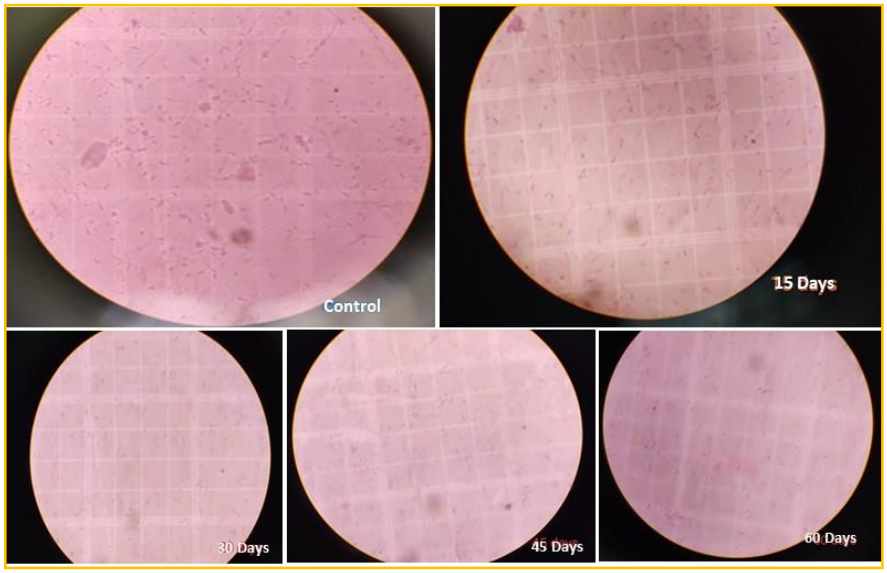

Figure 1C: Images showing decline in sperm count when compared to control after treatment with aqueous flower extract of *H. rosa-sinensis* in male Swiss albino mice

The aqueous flower extract is rich in bioactive compounds such as flavonoids, alkaloids and saponins which are hypothesized to interfere with spermatogenesis and sperm function[1]. The significant decline (P<0.001) in sperm count between 15 and 30 days of treatment with aqueous flower extract of *H. rosa-sinensis* may be attributed to the disruption of the seminiferous epithelium, affecting the production and maturation of spermatogenic cells. These antifertility effects may be linked to the presence of phytoconstituents that may inhibit androgen synthesis or block receptor binding[9].

Sperm motility, which is a crucial factor for fertility, was significantly reduced (P<0.001) in the treated group of mice. The reduction in sperm motility would have resulted from alterations in the function of the epididymis or changes in the biochemical composition of seminal plasma. Flavonoids and saponins are thought to induce oxidative stress, which reduces sperm motility[17]. During the treatment between 15 and 30 days, sperm mortality also significantly increased (P<0.001) in the treated group of mice. This might have resulted from oxidative damage to the sperm membrane or reduced mitochondrial function, essential for sperm viability[17]. The phytochemicals present in the extract may act as natural spermicides by disrupting sperm membrane integrity[12].

A significant increase (P<0.001) in sperm abnormalities suggests that *H. rosa-sinensis* extract may interfere with spermiogenesis. Additionally, chromosomal aberrations induced by certain phytochemicals could contribute to an increase in sperm abnormalities[15]. The *H. rosa-sinensis* extract causes seminal pH to be acidic in the treated group of mice, which makes the sperm highly fragile and increases sperm mortality. Similar findings also reported that treatment of cow urine[10] and seeds of *Carica papaya*[11] on Swiss albino mice exhibits a significant decline (P < 0.001) in sperm count, sperm motility and seminal pH, whereas there is a significant increase (P < 0.001) in sperm abnormality and sperm mortality when compared to the control group of mice.

Udoh et al[19] also reported that treatment of *Carica papaya* seed extract on Wistar rats caused pronounced hypertrophy of pituitary gonadotrophs and degeneration of Leydig cells, which ultimately decrease spermatogenesis. Another study conducted by Aladakatti et al[2] on leaves of *Azadirachta indica* demonstrated a decrease and regression in the number of Leydig cells and their nuclear diameter, indicating androgen deficiency.

Conclusion

The present study concludes that aqueous flower extract of *H. rosa-sinensis* shows antifertility effects in male mice by significantly decreasing (P<0.001) sperm count, sperm motility and seminal pH and significantly increasing mortality and abnormality of spermatozoa (P<0.001). Therefore, aqueous flower extract of *H. rosa-sinensis* can be used as a safe, reversible, cheap, oral male contraceptive for birth control among the human population.

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