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Anti-spermatogenic Effect of Hydro-ethanol Extract of Caesalpinia pulcherrima Leaves in Albino Rats

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Abstract

In this work investigates the anti-spermatogenic potential of the hydro-ethanol extract of *Caesalpinia pulcherrima* leaves (HEECPL) in male albino rats. Two groups of adult rats were created: the control group and the group that received HEECPL treatment (20 mg/100 g of body weight, orally once daily for 28 days). After completion of treatment, sperm count, motility, viability, and acrosome cap status were decreased significantly ($p < 0.05$), along with a decrease in acrosome cap status compared to the control. These results imply that HEECPL impairs spermatogenesis and sperm function, possibly through phytoconstituent-mediated action of the extract. The study supports the possibility for the development of male contraceptive agent from *C. pulcherrima* as a natural and plant based effective birth control measure covering male which is not consider for this purpose like female.

Keywords: *Caesalpinia pulcherrima*; Anti-spermatogenic; Sperm viability; Male contraceptive

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1. Introduction

Uncontrolled population growth remains one of the major global challenges, exerting pressure on food supply, healthcare systems, and socio-economic development (Jain et al., 2023). Family planning and fertility regulation are therefore essential strategies for maintaining population balance. Although several synthetic contraceptive drugs are currently available, but their prolonged use is often accompanied by adverse effects such as hormonal imbalance, reduced libido, and infertility after withdrawal (Bhatt and Deshpande, 2021). Hence, the search for safer, effective, and reversible plant-based contraceptive agents has gained increasing attention in recent years (Kumar et al., 2017).

Medicinal plants are valuable sources of bioactive compounds capable as anti-fertile agent. Several plant-derived phytochemicals, such as alkaloids, saponins, flavonoids, and tannins, have been reported to possess antifertility or anti-androgenic activities in rat (Ramgir et al., 2022). Among these, *Caesalpinia pulcherrima* (L.) Sw. (family: Fabaceae), commonly known as "Pride of Barbados" or "Dwarf Poinciana," has long been utilised in ayurvedic and traditional medicine to treat respiratory conditions, fever, and inflammation (Pandey et al., 2023). In our earlier in-vitro investigations, the hydro-ethanol extract of *C. pulcherrima* leaves (HEECPL) demonstrated significant effects on sperm viability and function, supported by LC–MS analysis revealing the presence of bioactive phytoconstituents such as flavonoids, alkaloids, and phenolic compounds (Lohar et al., 2025). Building upon these findings, the present in-vivo study was designed to evaluate the anti-spermatogenic efficacy of HEECPL in male albino rats. The study specifically assesses key spermiological sensors i.e., sperm count, motility, viability, and acrosome cap status to elucidate the potential anti-spermatogenic effects of the extract. This investigation aims to provide experimental evidence supporting the role of *C. pulcherrima* as a natural, plant-derived candidate for male contraception. From our previous pilot study, it has been noted that 20 mg/100 g body weight most effective. To know the mechanism of action of the extract the said dose was used for investigates in details.

2. Materials and Methodologies

2.1. Plant collection and extraction

Mature freshly leaves of *Caesalpinia pulcherrima* were obtained from the Vidyasagar University, authenticated by a botanist, and shade-dried for seven days. The dried leaves were powdered and extracted using distilled water and 100 percent ethanol (3:2) for 48 hours. The extract was concentrated under reduced pressure using a rotary evaporator and stored at 4°C until use.

2.2. Experimental animals

Twelve adult male albino rats were used for this experiment. The animals were kept in a typical laboratory setting with a temperature of 25 $\pm$ 2°C, a 12-hour light/dark cycle, and unlimited access to water and a standard pellet diet. The Institutional Animal Ethics Committee

authorised all experimental procedures, which were carried out in accordance with CCSEA norms.

2.3. Experimental design

Twelve rats were split into two groups at random ($n = 6$ each):

Group I (Control): For 28 days, distilled water (0.5 ml/100 g body weight/day) was administered orally once a day.

Group II (HEECPL-treated): Received HEECPL (20 mg/100 g of body weight/day, orally once daily) for 28 days.

After the treatment period, animals were anesthetized, and cauda epididymis were collected for spermatogenic sensors analysis.

2.4. Sperm count

Spermatozoa were collected from the cauda epididymis and counted using a Neubauer hemocytometer chamber. The sperm count was expressed in millions per milliliter (Pant and Srivastava, 2003).

2.5. Sperm motility

Sperm motility was analyzed microscopically using glass slides covered with a coverslip. For each field, 100 spermatozoa were evaluated under a light microscope, and the percentage of motile sperm cells expressed (Zemjanis, 1970).

2.6. Sperm viability

Eosin-nigrosin staining was used to assess sperm viability. On a spotless glass slide, equal amounts of sperm solution and eosin-nigrosin stain were combined and applied. The slides were inspected under a microscope following air drying. Dead spermatozoa absorbed the eosin stain, whereas live spermatozoa did not. The percentage of viable sperm was calculated from a count of at least 200 spermatozoa (World Health Organization, 1999).

2.7. Acrosome cap status

Thin, evenly coated gelatin glass slides were used to evaluate the acrosomal status. Sperm samples were diluted using a D-glucose solution prepared in phosphate-buffered saline. The prepared slides were examined under a bright-field microscope. Spermatozoa with intact acrosomes displayed a halo around the head region, indicating a positive acrosomal response (Gopalkrishnan, 1995).

2.8. Statistical analysis

ANOVA followed by "Multiple Comparison Student's one-tail 't'-test" was performed as a statistical study. All data were expressed as Mean $\pm$ SEM. Diminutions were considered sig-

nificant at $p < 0.05$ (Lohar et al., 2025).

3. Results and Discussion

Administration of the HEECPL for 28 consecutive days showed a marked anti-spermatogenic activity of Wister albino rats when compared with the control group. A significant ($p < 0.05$) reduction in sperm count and motility were observed in the HEECPL-treated rats (Figure 1A,B), indicating suppression of spermatogenesis and reduced sperm production. Decrement of sperm motility also suggesting impaired maturation and compromised epididymal function of the rats (Lohiya et al., 2000).

Furthermore, sperm viability assessment revealed a significant decline ($p < 0.05$) in the percentage of viable spermatozoa in the treated group (Figure 1C), implying membrane-damaging effects of the extract on sperm cells. Evaluation of acrosome cap status also showed a higher percentage of acrosome-reacted or damaged spermatozoa in the HEECPL-treated rats compared to controls (Figure 1D). Damage to the acrosomal membrane typically reflects disruption of sperm fertilizing ability and reduced structural stability, which further supports the anti-spermatogenic potential of the extract (Dahan and Breitbart, 2022).

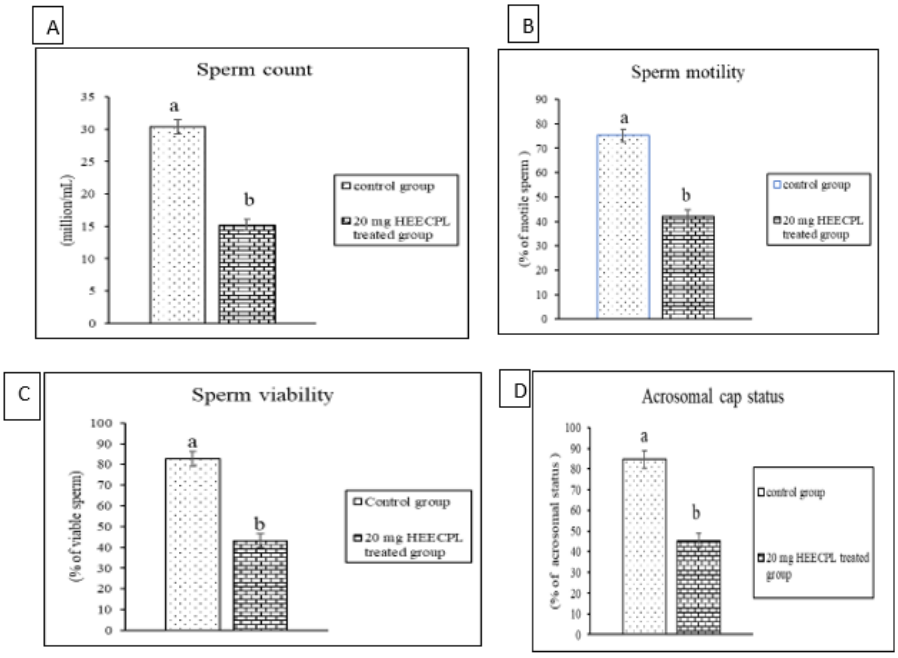

Figure 1: Effects of treatment with the HEECPL on sperm (A) count, (B) motility, (C) viability and (D) acrosome cap status. Column diagram was prepared using Mean ± SEM (n = 6). ANOVA followed by "Multiple Comparison Student's one-tail 't'-test" was performed as a statistical study. Columns with superscripts marked as a and b differ from one another significantly, $p < 0.05$.

The observed alterations may be attributed to the presence of active phytoconstituents detected in our previous in-vitro publication through liquid chromatography-mass spectrometry analysis, which revealed flavonoids, alkaloids, terpenoids, and phenolic compounds as major

bioactive constituents of HEECPL (Lohar et al., 2025). These secondary metabolites are known to interfere with testicular spermatogenesis through oxidative stress induction, hormonal imbalance, or direct inhibition in germ cell proliferation. Similar antifertility and anti-spermatogenic effects have been reported for other medicinal plants containing comparable phytochemicals (Verma and Yadav, 2021). These findings confirm the anti-spermatogenic efficacy of HEECPL in in-vivo condition, supporting our earlier in-vitro observations. The extract significantly impairs sperm production, motility, and viability, and damages acrosome status which are the key indicators of male fertility potential.

4. Conclusion

Above results validate the candidature use of HEECPL as a male fertility-suppressing agent and highlight its promise as a candidate for the development of a plant-based male contraceptive formulation. Though, further studies are essential to understand the exact mechanism of action of the extract from hormonal, genomic, and histological viewpoints, along with safety before clinical application.

Conflict of Interest Statement

The authors declare that they have no conflict of interest.

Ethical Approval Statement

This study was ethically permitted from Institutional Animal Ethics Committee (VU/IAEC/10/7/2022).

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