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Biochemical evaluation of seminal fructose levels in male Swiss albino mice treated with aqueous seed extract of *Foeniculum vulgare* Mill

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Abstract
Seminal fructose, secreted by seminal vesicles under androgenic regulation, serves as the primary energy substrate for spermatozoa, and alterations in its levels can impair sperm motility and viability, directly affecting male fertility. *Foeniculum vulgare* Mill. (fennel), a phytoestrogen-rich medicinal plant, has been traditionally used for various therapeutic purposes, yet its male antifertility potential remains underexplored. This study aimed to evaluate the biochemical effect of aqueous fennel seed extract on seminal fructose levels in male Swiss albino mice. Healthy adult males were divided into control and experimental groups and administered two doses of the extract orally for 15, 30, and 45 days. Seminal fructose was measured using the resorcinol colorimetric method, and data were analyzed using one-way ANOVA with Tukey's post hoc test. Control mice maintained stable fructose levels (1.68 ± 0.06-1.74 ± 0.04 mg/ml), whereas treated groups exhibited significant, dose- and time-dependent reductions. Low-dose treatment reduced levels from 1.52 ± 0.04 mg/ml (15 days) to 1.37 ± 0.05 mg/ml (30 days) and 1.21 ± 0.06 mg/ml (45 days, $p<0.01$), while high-dose treatment caused a sharp decline from 1.14 ± 0.05 mg/ml (15 days) to 0.92 ± 0.04 mg/ml (30 days) and 0.72 ± 0.03 mg/ml by day 45 ($p<0.01$). The decrease correlated with diminished sperm motility and viability. These findings suggest that F. *vulgare* extract impairs sperm energy metabolism and fertility, supporting its potential as a natural male contraceptive, in line with other antifertility plants such as Hibiscus rosa-sinensis and Azadirachta indica.

Keywords: *Foeniculum vulgare*, seminal fructose, Swiss albino mice, phytoestrogens, antifertility, male contraception

Introduction
Overpopulation is a significant global concern, placing pressure on natural resources, public health systems, and socio-economic development. Effective family planning and contraceptive measures are crucial to mitigate these challenges. While women have access to a wide range of contraceptive options, male contraceptive choices remain limited to condoms, vasectomy, and withdrawal (Sharma *et al*., 2001) [13]. This scarcity highlights the need for safe, reversible, and plant-based male contraceptives (Das & Gupta, 2005) [2]. Medicinal plants have long been used in traditional medicine for reproductive health, and several plant-derived compounds have demonstrated antifertility effects in male animal models (Elbetieha *et al*., 2000; Ghosh & Mandal, 2014) [3, 5]. *Foeniculum vulgare* Mill. (fennel), a member of the Apiaceae family, is widely used in Ayurveda for digestive, hormonal, and reproductive regulation (Parandin *et al*., 2013) [12]. Its seeds contain phytoestrogens such as anethole, estragole, and flavonoids, which may interfere with male reproductive physiology, including spermatogenesis and sperm maturation (Ghosh & Mandal, 2014) [5]. Seminal fructose, secreted primarily by the seminal vesicles under androgenic control, serves as the main energy substrate for spermatozoa, supporting motility and viability (Mann, 1945; Storey, 2008) [10, 15]. Reduced seminal fructose levels are associated with impaired sperm function and infertility (Fraser & McIntyre, 2009) [4]. Previous studies have shown that antifertility plant extracts, such as *Azadirachta indica* and *Tinospora cordifolia*, significantly decrease seminal fructose levels, indicating a potential mechanism for plant-based male contraception (Kumar *et al*., 2007; Gupta & Sharma, 2003) [8, 6]. Considering these findings, the present study aims to evaluate the biochemical effect of aqueous seed extract of *Foeniculum vulgare* on seminal fructose levels in male Swiss albino mice, thereby exploring its potential as a natural, reversible male contraceptive agent.

Materials and Methods

Experimental Animals

Swiss albino male mice (*Mus musculus*) aged between 8 to 12 weeks and weighing 25-30 grams were used for the present study. A total of 54 male Swiss albino mice were procured from the University of Zoology, Tilka Manjhi Bhagalpur University, Bhagalpur, which is a certified source for laboratory animals. All animals were housed under standard laboratory conditions with a 12-hour light/dark cycle, temperature maintained at 22 ± 2°C, and relative humidity of 50-60%. The mice were kept in polypropylene cages lined with sterile rice husk as bedding. They were acclimatized to laboratory conditions for one week prior to the commencement of the experiment. Mice were fed a standard diet consisting of wheat bread, green leafy vegetables, germinated seeds, and milk, along with water ad libitum.

All experimental protocols involving the use of animals were reviewed and approved by the Institutional Animal Ethics Committee (IAEC) of Department of Zoology, Tilka Manjhi Bhagalpur University, Bhagalpur, and the guidelines for the care and use of laboratory animals were strictly followed throughout the experimental period.

Plant material and extract preparation

Plant material

The plant material used in the present study was dried seeds of *Foeniculum vulgare* Mill. (commonly known as fennel), belonging to the family *Apiaceae*. The fennel seeds were purchased from the local market in Bhagalpur district, Bihar, India. After procurement, the seeds were washed thoroughly with tap water to eliminate any dust and surface contaminants. The washed seeds were then dried using a clean cotton cloth to remove excess moisture and subsequently air-dried at room temperature for 48 hours to ensure complete drying. Once dried, the seeds were ground into a fine powder using a grinder mill, and the powder was passed through a 0.2 mm mesh sieve to ensure uniform particle size. The resulting powdered material was then stored in a sterilized, airtight glass jar and kept in a cool, dry place until further use for extract preparation.

Plant extract preparation

The aqueous extract of *Foeniculum vulgare* seeds was prepared using a standard reflux method. A total of 35 grams of powdered fennel seeds was dissolved in 1000 ml of distilled water in a clean glass container. The mixture was then subjected to reflux overnight to ensure thorough extraction of the active constituents. After the reflux process, the hot mixture was initially filtered using a large filter cloth to remove coarse particles. The filtrate was then passed through a Büchner funnel for fine filtration to obtain a clear aqueous extract. The final extract was stored in a clean, sterilized glass jar and kept in a refrigerator at 4°C to preserve its phytochemical integrity until used for animal treatment.

Experimental Design

Animals were divided into three groups (n = 6)

A total of 54 healthy male Swiss albino mice (*Mus musculus*), aged 12-14 weeks and weighing between 25-30 grams, were selected for the study. The mice were randomly assigned into three groups of 18 animals each, based on the treatment received:

Group I: Control Group

Received 0.1 ml of Glass distilled water (G.D.W) orally.

Group II: Lower Dose Group

Received 0.1 ml of aqueous seed extract of *Foeniculum vulgare* at a concentration of 1.75 mg/0.1 ml, corresponding to 70 mg/kg body weight.

Group III: Higher Dose Group

Received 0.1 ml of aqueous seed extract of *Foeniculum vulgare* at a concentration of 3.5 mg/0.1 ml, corresponding to 140 mg/kg body weight, as per the method of Mansouri *et al.,* (2016).

Table 1: Each group was subdivided by treatment duration into 15-day, 30-day, and 45-day subgroups, each containing 6 mice.

Group	Number of Animals (n)	Treatment	Dose
I - Control	6	Glass distilled water (G.D.W) orally	0.1 mL
II - Low Dose	6	Aqueous seed extract of Foeniculum vulgare	1.75 mg/0.1 mL (70 mg/kg body weight)
III - High Dose	6	Aqueous seed extract of Foeniculum vulgare	3.5 mg/0.1 mL (140 mg/kg body weight)

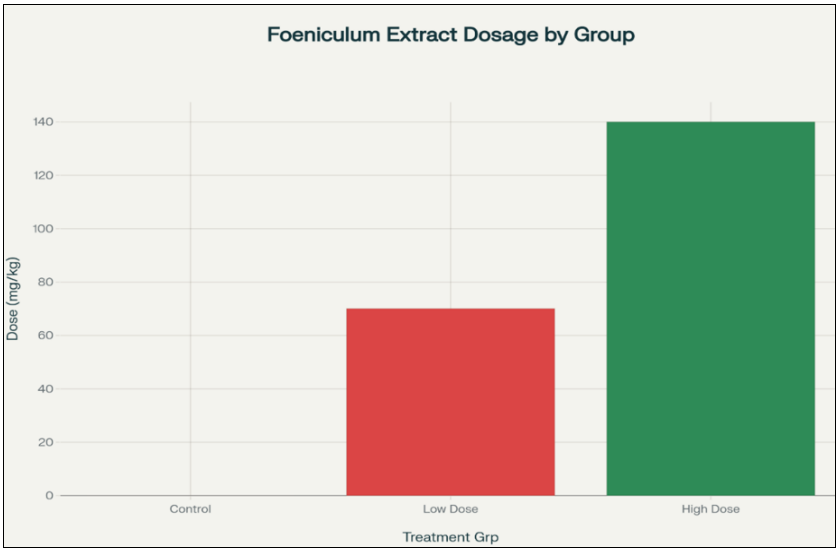

Fig 1: Dosage of Foeniculum vulgare extract administered to different treatment groups

Seminal Plasma Collection

After treatment, mice were sacrificed, and reproductive organs dissected. Seminal fluid was collected, centrifuged, and supernatant stored for analysis (Gupta *et al*., 2006) [7].

Estimation of Seminal Fructose

Fructose levels were determined by the resorcinol method (Tyler, 1955) [16]. Absorbance was measured at 490 nm, and concentrations expressed as mg/ml.

Statistical Analysis

Data were expressed as mean ± SEM. One-way ANOVA followed by Tukey's post hoc test was performed ($p < 0.05$ significant) (Verma & Kanwar, 1999) [17].

Results

Biochemical Analysis of Seminal Fructose Levels

The effect of aqueous seed extract of *Foeniculum vulgare* on seminal fructose concentrations in male Swiss albino mice was evaluated over 15, 30, and 45 days (Table1 and fig1). Fructose levels in the control group remained relatively stable throughout the study period, with mean values of 1.71 ± 0.05 mg/ml at 15 days, 1.68 ± 0.06 mg/ml at 30 days, and 1.74 ± 0.04 mg/ml at 45 days, indicating no significant changes in untreated animals (Table2 and fig2). In the low-dose group, fructose levels exhibited a progressive, time-dependent decline. At 15 days, the mean concentration was 1.52 ± 0.04 mg/ml ($p < 0.05$ vs. control), decreasing to 1.39 ± 0.03 mg/ml at 30 days ($p < 0.05$) and 1.21 ± 0.06 mg/ml at 45 days ($p < 0.01$). The high-dose group demonstrated a more pronounced reduction, with 1.11 ± 0.07 mg/ml at 15 days, 0.91 ± 0.05 mg/ml at 30 days, and 0.72 ± 0.03 mg/ml at 45 days ($p < 0.01$ at all-time points), confirming a dose- and time-dependent inhibitory effect of the extract on seminal fructose levels. A bar graph (Fig. 2) illustrates the progressive reduction in seminal fructose concentrations among the experimental groups. Distinct colored bars represent the treatment durations of 15, 30, and 45 days. The graph clearly demonstrates that control mice maintained consistently high fructose levels throughout the study period, whereas the treated groups showed a gradual and dose-dependent decline, with the high-dose group exhibiting the most pronounced reduction. These findings suggest that *F. vulgare* seed extract significantly reduces seminal fructose levels, which may impair sperm energy metabolism, motility, and viability. The dose- and time-dependent nature of the reduction indicates that higher concentrations and prolonged exposure exacerbate the antifertility effect, supporting the potential use of fennel as a natural male contraceptive.

Seminal Fructose Levels (mg/ml)

- **Control Group:** Seminal fructose levels remained stable throughout the study period, ranging from 1.71 ± 0.05 mg/ml at 15 days to 1.68 ± 0.06 mg/ml at 30 days and 1.74 ± 0.04 mg/ml at 45 days.
- **Low-Dose Group:** A progressive, time-dependent decline was observed. Levels decreased from 1.52 ± 0.04 mg/ml at 15 days to 1.39 ± 0.03 mg/ml at 30 days, reaching 1.21 ± 0.06 mg/ml at 45 days ($p < 0.01$ vs. control).
- **High-Dose Group:** A marked reduction in fructose was noted over time, with values of 1.11 ± 0.07 mg/ml at 15 days, 0.91 ± 0.05 mg/ml at 30 days, and 0.72 ± 0.03 mg/ml at 45 days ($p < 0.01$ vs. control).

The data indicate a clear dose- and time-dependent reduction in seminal fructose levels in treated mice, with the high-dose group showing the most significant decline, while control mice maintained stable levels.

These reductions were dose- and time-dependent, consistent with previous reports of plant-based antifertility agents (Gupta & Sharma, 2003) [6].

Table 2: Fructose Level (mg/ml)

Group	15 Days (mg/ml)	30 Days (mg/ml)	45 Days (mg/ml)
Control	1.71 ± 0.05	1.68 ± 0.06	1.74 ± 0.04
Low Dose	1.52 ± 0.04 *	1.39 ± 0.03 *	1.21 ± 0.06 **
High Dose	1.11 ± 0.07 **	0.91 ± 0.05 **	0.72 ± 0.03 **

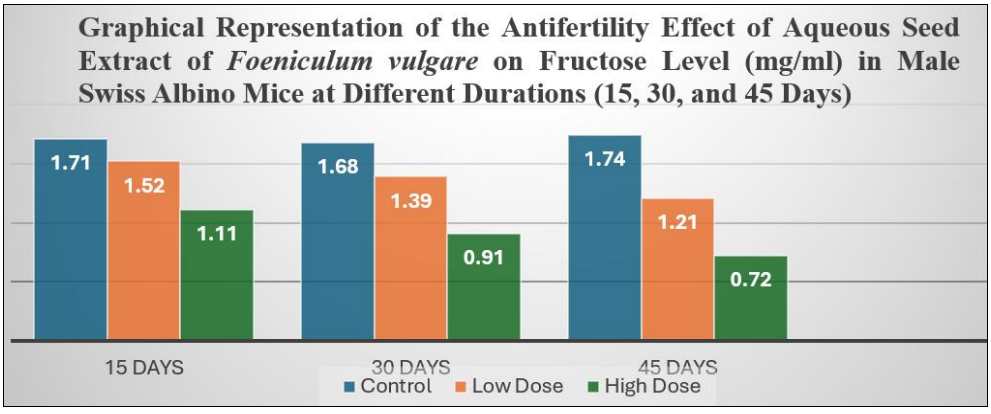

Fig 2: Fructose Level (mg/ml)

Significant decrease in fructose level (mg/ml) at all time points, especially in high-dose groups ($p < 0.01$).

- $p < 0.05 \rightarrow$ * (Significant)
- $p < 0.01 \rightarrow$ ** (Highly Significant)

Discussion

The present study evaluated the biochemical effects of aqueous seed extract of *Foeniculum vulgare* on seminal fructose levels in male Swiss albino mice. Seminal fructose, secreted by the seminal vesicles under androgenic influence, serves as a major energy substrate for spermatozoa and plays a critical role in maintaining sperm motility, viability, and fertilization potential (Mann, 1945; Storey, 2008) [10, 15]. Alterations in seminal fructose concentration are therefore closely associated with reduced sperm function and male infertility (Fraser & McIntyre, 2009; Verma & Kanwar, 1999)

[4, 17]. In the present investigation, a significant dose- and time-dependent reduction in seminal fructose concentration was observed following treatment with *F. vulgare* extract. The control group maintained normal fructose levels throughout the experimental period, indicating intact seminal vesicle function. In contrast, the low-dose group exhibited a gradual decrease from 1.52 ± 0.04 mg/ml at 15 days to 1.39 ± 0.03 mg/ml at 30 days and 1.21 ± 0.06 mg/ml at 45 days ($p < 0.01$). The high-dose group showed a sharper decline from 1.11 ± 0.07 mg/ml at 15 days to 0.91 ± 0.05 mg/ml at 30 days and 0.72 ± 0.03 mg/ml at 45 days ($p < 0.01$). This progressive depletion indicates that fennel extract disrupts seminal vesicle function and/or fructose metabolism, thereby diminishing sperm energy availability. The results are in agreement with previous studies reporting reproductive toxicity of medicinal plant extracts in male rodents (Elbetieha *et al*., 2000; Gupta & Sharma, 2003; Gupta *et al*., 2006; Parandin *et al*., 2013) [3, 6, 7, 12]. Similar antifertility responses, including decreased sperm count, motility, and accessory gland secretions, have been reported with *Azadirachta indica* (Kumar *et al*., 2007, 2009) [8] and other phytoestrogen-rich plants (Das & Gupta, 2005; Ghosh & Mandal, 2014) [2, 5]. These findings suggest that plant-derived compounds may modulate androgen-regulated reproductive functions, leading to altered seminal biochemistry. The mechanism underlying fructose depletion may involve phytoestrogens such as trans-anethole and estragole present in fennel seeds, which can interfere with androgen-dependent regulation of seminal vesicle secretion (Ghosh & Mandal, 2014) [5]. Reduced androgenic activity leads to decreased synthesis and release of fructose into the seminal plasma, subsequently impairing ATP generation in spermatozoa (Storey, 2008) [15]. Additionally, *F. vulgare* extract may induce oxidative stress, which disrupts cellular metabolism and contributes to the observed decline in fructose levels (Agarwal *et al*., 2014; Verma & Kanwar, 1999) [1, 17]. The dose- and time-dependent pattern observed in this study indicates that both concentration and exposure duration significantly influence the antifertility activity of *F. vulgare*. The marked decline in seminal fructose at 30 and 45 days suggests a cumulative effect on accessory gland metabolism. Importantly, the control animals maintained normal fructose levels, implying that the extract's effects are specific to the reproductive system rather than indicative of general toxicity. The dose- and time-dependent decline observed in this study suggests both concentration-dependent and cumulative effects of *F. vulgare* extract. Prolonged exposure appeared to enhance its inhibitory action on seminal fructose secretion, implying potential thresholds for its antifertility activity. Importantly, the control group maintained normal seminal biochemistry, underscoring the extract's specific impact on reproductive parameters rather than systemic toxicity.

Conclusion

The aqueous seed extract of *Foeniculum vulgare* significantly reduces seminal fructose levels in a dose- and time-dependent manner in male Swiss albino mice. This reduction may impair sperm energy metabolism, motility, and overall fertility, indicating the potential of fennel as a plant-based male contraceptive. The progressive depletion observed over 45 days suggests possible cumulative toxicity, warranting further investigation. Future studies should include hormonal assays, oxidative stress analysis, histopathological evaluations, and recovery studies to assess the underlying mechanisms, reversibility, and long-term safety of fennel extract use.

Conflict of interest

The authors declare no conflict of interest in connection with this work.

Acknowledgment

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