

Assessment of the Impact of Fenugreek and Flaxseed Oil Extracts on Male Reproductive Function in Albino Mice and Evaluation of Their Palatability in *Mus musculus*

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ABSTRACT

Some medicinal plants have phytoestrogen compounds that act as natural estrogens. In this study, we investigated the antifertility effect of fenugreek seed and flaxseed oils on male albino mice as a prelude for their use in the control of house mice (*Mus musculus*). Fifteen male albino mice were randomly divided into three groups: one control group and two groups that received oral doses of 200 mg/kg of fenugreek oil or flaxseed oil for 35 days. The body weight, testis weight, sperm count and motility, serum estradiol, testosterone, superoxide dismutase, malondialdehyde, and testis histology were compared with untreated mice. Non-choice and free-choice feeding tests were done on commensal mice for 3 days using plain food and bait formulations containing fenugreek oil or flaxseed oil. The average daily consumption and acceptability were calculated. The oral administration of both oils caused a significant decrease in body weight, sperm count and motility, and serum testosterone, a significant increase in serum estradiol, and deterioration of testis histology. Flaxseed oil caused a significant decrease in testis weight. The acceptability of fenugreek seed oil is higher than the acceptability of flaxseed oil in both non-choice and free-choice feeding tests. We conclude that fenugreek seed oil and flaxseed oil had the same antifertility effects on male albino mice and can be used in the control of house mice.

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MM contributed to study design, performed the experimental work interpretation of results, performed the statistical analysis and critical revision of the manuscript. AWM prepared the figures and tables, and wrote the first draft of the manuscript. RIM contributed to study design, performed the experimental work, critical revision of the manuscript

Key words

Antifertility, Pest control, *Mus musculus*, Plant oils

INTRODUCTION

Mice are vertebrate pests that can transmit diseases like the bubonic plague. Their short lifespan and high reproductive rate make controlling their population challenging. There are traditional methods for controlling their numbers, but these are inhumane and contaminate the environment. In addition, they cannot be used in places where they could potentially harm humans, such as schools and hospitals. And so, new control methods depend on decreasing the number of mouse offspring instead of eliminating live individuals (Mostafa *et al.*, 2021).

Various plants and their parts are being investigated for their antifertility and medicinal properties. Among these, fenugreek (*Trigonella foenum-graecum*) and flax (*Linum usitatissimum*) are widely studied species belonging to the Leguminosae and Linaceae families, respectively. Fenugreek seeds and green leaves improve the flavor, color, and texture of food products, and seeds in particular have a long history of medicinal properties (Sirotkin, 2023; Singh *et al.*, 2022). These properties include cholesterol reduction, lactation stimulation, enhanced gastric activity, appetite regulation, milk production promotion (galactagogue effect), and hepatoprotective activity (Zandi *et al.*, 2015). Fenugreek contains 23–26% protein, 6–7% fat, and 58% carbohydrates, including 25% dietary fiber, by weight. The plant also has a high iron content - it's got 33 mg/100 g dry weight (Wani and Kumar, 2018). Brogi *et al.* (2017) have demonstrated that fenugreek seeds can sterilize albino rats and rabbits by decreasing testis weight, disrupting spermatogenesis, and changing the histological structure of seminiferous tubules.

Flaxseeds (*Linum usitatissimum* L.), often referred to as linseeds, seeds are used in many functional foods, health

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supplements, and skincare products (Mueed *et al.*, 2022; Sanmartin *et al.*, 2020). Flaxseed is rich in fats, proteins, and dietary fiber, and has been recently gaining popularity as a significant source of phytochemicals (Shahzad *et al.*, 2006). These phytochemicals, including phenolic acids, cinnamic acids, flavonoids, and lignans, possess antioxidant properties and are involved in cell growth and viability. They can also alleviate the symptoms of many diseases, such as diabetes, neurological, cardiovascular, and gastrointestinal disorders, and some types of cancers (Parikh *et al.*, 2019).

In this study, we evaluated the antifertility effects of fenugreek seed and flaxseed oils and their palatability as a prelude to controlling the pest population (house mice). We chose these oils because they are environmentally friendly, low-cost, locally available, and contain multiple active compounds that may be more effective.

MATERIALS AND METHODS

Experimental animals

Fifteen male Swiss albino mice were obtained from the National Research Centre (Cairo, Egypt), along with fifteen male commensal mice (wild-caught) supplied by licensed animal dealers. All animals were housed in metal cages (26 × 20 × 6 cm) under controlled environmental conditions (22 ± 2 °C, 40–60% humidity, and a 12-h light/dark cycle). They were acclimatized for one week and provided *ad libitum* access to food and water.

Experimental design

Fifteen adult male mice were randomly divided into three groups, each consisting of five animals: Group 1 (Control): orally received distilled water containing two drops of Tween 80 (emulsifier) daily. Group 2 (Fenugreek-treated): orally received fenugreek seed oil dissolved in distilled water with two drops of Tween 80 at a daily dose of 200 mg/kg for 35 days (Badry *et al.*, 2021). Group 3 (Flaxseed-treated): orally received flaxseed oil dissolved in distilled water with two drops of Tween 80 at a daily dose of 200 mg/kg for 35 days (Abdulshahed and Abdul Ameer, 2023).

Feeding tests

A non-choice feeding test was conducted using fifteen male commensal mice, divided into three groups (n = 5 per group). The control group was fed a diet consisting of 66.5% crushed wheat and 33.5% crushed corn for three consecutive days to establish a baseline for food intake. The two treatment groups received the same basal diet supplemented with the respective test oils. Each mouse was offered 5 g of the assigned diet daily, and food

consumption was quantified by weighing the remaining food after 24 h. The acceptability of the bait formulation was calculated using the equation of Mason *et al.* (1989):

$$\text{Acceptability} = \frac{\text{Average daily consumption of bait formulation (g)}}{\text{Average daily consumption of (bait formulation + plain food) (g)}} \times 100$$

In the free-choice feeding test, each of five mice was simultaneously provided with two food portions for three consecutive days: 5 g of plain diet (66.5% crushed wheat, 33.5% crushed corn) and 5 g of bait containing 10% fenugreek oil. Average daily consumption of each diet was determined as described in the non-choice feeding test. The procedure was then repeated for an additional three days, replacing the fenugreek oil bait with a bait containing 10% flaxseed oil, and consumption was measured in the same manner.

Post-treatment observations and analyses

Body weight was recorded both before and after the treatment period. Following treatment, the testes were carefully extracted and weighed. The epididymis was excised, perfused with 2 mL of isotonic saline solution, and gently cut with scissors to release sperm. The sperm suspension was then incubated at 37 °C for 15 min, after which sperm count and motility were assessed under the light microscope using a hemocytometer as described by Oliveira *et al.* (2014).

Biochemical analysis of blood serum

At the end of the treatment period, blood samples were collected from anesthetized mice, allowed to clot, and then centrifuged. The separated serum was aliquoted and stored at –80 °C for subsequent biochemical analysis.

Testosterone levels were determined in serum samples using an ELISA kit (Diametra, Italy; Catalog No. DKO015), while estradiol concentrations were measured using an ELISA kit from DRG, Germany (Catalog No. 10009).

Biochemical analysis of testis

All tissue and serum samples were preserved at –80 °C until further biochemical analysis. The collected tissues were homogenized in ice-cold phosphate buffered saline to prepare a 10% (w/v) solution. This homogenate was used to evaluate oxidative stress markers, including malondialdehyde (MDA) following the method of El-Maraghi *et al.* (2018), and superoxide dismutase (SOD) activity according to the method of Nishikimi *et al.* (1972).

Histopathological examination

Small portions of the testes were fixed in Bouin's

solution for 24 h. The tissues were then dehydrated using a graded ethanol series of increasing concentrations, followed by immersion in terpineol for three days. Subsequently, the samples were embedded in paraffin wax. The paraffin was changed three times, each for one hour. Paraffin blocks were sectioned at a thickness of 5 μ m and mounted onto smooth glass slides. The sections were stained using Harris hematoxylin and eosin.

Statistical analyses

The statistical analysis was performed using the Instat Program GraphPad (San Diego, USA), version 3.6. The results were represented as mean $\pm$ SEM. The data distribution was tested with the Kolmogorov-Smirnov test. The statistical analysis was performed by using one-way ANOVA. The *P* values lower than 0.05 were accepted as significant.

RESULTS

Body weight, testis weight and semen analysis

Table I summarizes the effects of the two extracted oils on body weight, testis weight, sperm count and motility, hormonal levels in blood serum, and oxidative stress markers in the testes of treated mice.

Table I. Effect of fenugreek seed oil and flaxseed oil on body weight, testis weight, sperm count, sperm motility, estradiol, total testosterone, superoxide dismutase, and Malondialdehyde.

Parameters	Control	Fenugreek seed oil	Flaxseed oil
Body weight (g)	33.3 $\pm$ 0.62 (31.8-35)	29 $\pm$ 0.73* (27.3-32.7)	29.5 $\pm$ 0.81* (26.6-32.4)
Testis weight (g)	0.08 $\pm$ 0.003 (0.08-0.1)	0.08 $\pm$ 0.002 (0.08-0.1)	0.05 $\pm$ 0.008** (0.04-0.06)
Sperm count $\times 10^6$	9.8 $\pm$ 0.66 (8-12)	4.2 $\pm$ 0.86** (2-7)	2.6 $\pm$ 0.5** (1-4)
Sperm motility %	70 $\pm$ 3.5 (60-80)	26.4 $\pm$ 0.71** (20-35)	14 $\pm$ 0.6** (10-20)
Serum estradiol (pg/ml)	49.6 $\pm$ 0.92	265.2 $\pm$ 1.65**	280.2 $\pm$ 2.08**
Total serum testosterone (pg/ml)	4.53 $\pm$ 0.1	2.81 $\pm$ 0.01**	2.89 $\pm$ 0.07**
SOD in testis tissue (U/mg protein)	4.40 $\pm$ 0.01	4.39 $\pm$ 0.06	4.44 $\pm$ 0.07
MDA in testis tissue (nmol/mg protein)	0.84 $\pm$ 0.01	0.83 $\pm$ 0.01	0.85 $\pm$ 0.007

Data are expressed as Mean $\pm$ SEM followed by range in (parentheses), n=5. *Significant difference ($p \leq 0.05$); ** Highly significant ($p \leq 0.01$).

Treatment with both oils resulted in significant reductions in body weight compared to the control group. Fenugreek seed oil produced a mild decrease in testis weight, whereas flaxseed oil caused a more pronounced reduction. Both oils also led to a highly significant decline in sperm count and motility relative to the control group. The two extracted oils caused a highly significant decrease in serum testosterone levels in treated mice compared with controls, while simultaneously inducing a notable increase in estradiol levels. Both oils exhibited an insignificant effect on SOD and MDA activities, suggesting limited antioxidant response alterations.

When the SOD of the testis decreases, it causes histopathological effects and decreases the fertility of mice. When the MDA of the testis increases, it causes impairment of sperm and decreases the fertility of mice.

Histological examination

Figures 1B and C illustrate the effects of the two extracted oils on the histological structure of the testes. Microscopic examination of the testes from the control group revealed a normal histological architecture, with well-organized seminiferous tubules containing spermatogonia, primary and secondary spermatocytes, spermatids, and spermatozoa, as well as normal Sertoli and Leydig cells (Fig. 1A).

Treatment with fenugreek seed oil (200 mg/kg) induced histopathological alterations in the testes, including pyknotic nuclei in spermatocytes, loss of basophilic nuclei, and the presence of vacuolated spermatocytes (Fig. 1B). Similarly, mice treated with flaxseed oil (200 mg/kg) exhibited degeneration of seminiferous tubules, necrosis, and vacuolation in spermatocytes (Fig. 1C).

Feeding tests on *Mus musculus*

In the feeding test, the acceptability of fenugreek seed oil and flaxseed oil bait formulations was evaluated in non-choice and free-choice feeding tests. In non-choice feeding tests, the fenugreek seed oil and flaxseed oil bait formulations were presented to commensal males separately. The average daily consumption of fenugreek seed oil bait formulation (3.2 g per individual) was higher in males compared to flaxseed oil bait formulation (2.4 g per individual). Therefore, the acceptability of fenugreek seed oil was higher than that of flaxseed oil, at 48.3% and 40.6% respectively. Based on the results of feeding trials in free choice tests, plain food was most accepted by commensal male mice, followed by fenugreek seed oil and flaxseed oil baits (Table II).

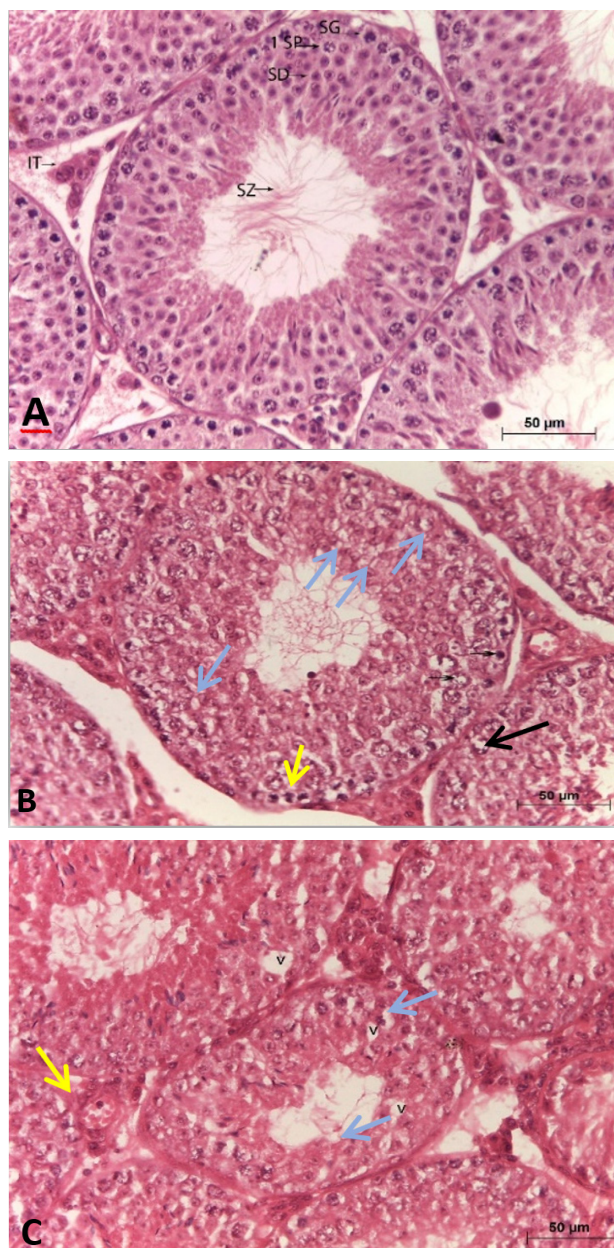


Fig. 1. Transverse section of the testis of a male albino mouse A, control, B, treated with 200 mg/kg of fenugreek seed oil and C, treated with 200 mg/kg of flaxseed oil.

A shows average-sized seminiferous tubules (T) with average basement membranes, SG: spermatogonia, 1°sp: primary spermatocyte, SD: spermatid, SZ: spermatozoa, IT: interstitial cell. B shows spermatocytes' pyknotic nuclei (yellow arrow) as well as loss of basophilic nuclei (black arrow), and vacuolated spermatocytes (blue arrows). C shows degeneration of seminiferous tubules evident through pyknotic nuclei (blue arrows), vacuolation of spermatocytes (v), and congested blood vessels (yellow arrows). H and E, X 400.

Table II. Average daily consumption and acceptability of oil formulations and plain food in free-choice feeding tests.

No.	Free choice feeding trials	Average daily consumption (g/individual)	Acceptability (%)
1	Fenugreek seed	2.8	53.8
	Plain food	2.4	46.2
2	Flax seed	1.6	35.5
	Plain food	2.9	64.5

DISCUSSION

Body weight, testis weight, and semen analysis

The decrease in body weight in mice receiving fenugreek seed oil may be due to substances such as protodioscin and galactomannan, which inhibit weight gain. Factors such as the cholesterol-lowering property of fenugreek and the content of fiber that inhibits carbohydrate digestion also contribute to this effect (Al-Ashban *et al.*, 2010). The decrease in body weight in mice that received flaxseed oil might have resulted from its ingredients of antioxidant nature, such as alpha-linolenic acid, which support fat metabolism and are involved in the increase of enzyme activity. Additionally, flaxseed oil increases the fecal fat proportion and hence the intestinal fat uptake will be further reduced (Helal *et al.*, 2023). The reduction of testis weight of mice by the treatment of flax seed oil may be a result of the increase in 17-beta-estradiol levels, which inhibits Leydig cell division. The cells are vital for testosterone production, and the decrease in their proliferation will naturally lead to lower levels of testosterone, which are insufficient to sustain a normal weight of the testis (Kamal *et al.*, 1993). Moreover, the estrogen-like substances present in flaxseed oil are assumed to reduce the proliferation of Sertoli cells, contributing to the decrease in testicular weight (Helal *et al.*, 2023). The reduction in sperm count and motility in mice treated with fenugreek seed oil may be due to its negative effect on the secretion of sialic acid in the epididymis, which is essential for the survival of the sperm. Additionally, fenugreek induces oxidative stress in the testes by decreasing the activity of antioxidant enzymes, such as superoxide dismutase and catalase, thereby impairing spermatogenesis and reducing sperm count (Singh *et al.*, 2022). Further, estrogen levels in the organ can be boosted by fenugreek, and at the same time, the testosterone is reduced, potentially leading to the disruption of the sperm generation process (Al-Yahya, 2013). The decrease in sperm count and motility in mice treated with flaxseed oil may be due to lowered testosterone levels, aligning with

Helal *et al.* (2023). Safari *et al.* (2022) agreed on sperm count but found motility unaffected. Conversely, Mohsen *et al.* (2019) reported increases in both sperm count and motility with flaxseed oil.

Biochemical assays

The decrease in testosterone levels observed in mice treated with fenugreek seed might be associated with the impact of saponins on serum cholesterol, an essential component for testosterone synthesis in Leydig cells. Several studies have indicated that saponins reduce serum cholesterol levels, subsequently influencing both testosterone production and spermatogenesis (Badry *et al.*, 2021). While flaxseed oil reduced testosterone levels in treated mice, it increased their estrogen levels. Estrogen inhibits the growth of Leydig cells by stopping their reproduction and inhibiting the enzymes required for testosterone synthesis. There may be a lack of testosterone as a result of these actions (Helal *et al.*, 2023). Flaxseed oil causes an increase in estradiol levels in mice since it contains lignans, phytoestrogens, cyanogenic glycosides, anthocyanins, phytic acid, and minerals (Tanideh *et al.*, 2021).

Elevated cholesterol levels from dietary oils can also raise estrogen and progesterone levels (Noaman *et al.*, 2022). The elevation of estradiol levels in mice treated with fenugreek seed oil may be caused by compounds called phytoestrogens, which are plant-based substances that can mimic the effects of estrogen in the body. The most commonly known phytoestrogen in fenugreek is called diosgenin, and it is thought to be responsible for the estrogenic activity. Thus, when ingested, these phytoestrogens will activate the estrogen receptors, leading to an elevated level of estradiol in the body (Khushboo *et al.*, 2023).

The insignificant decrease in SOD activity may be attributed to the dose of fenugreek oil or the duration of treatment, which may have triggered a feedback mechanism that limited its antioxidant effect. In contrast, other studies have reported an increase in SOD activity, likely due to the presence of polyphenols and flavonoids in fenugreek, which are known to enhance the activity of antioxidant enzymes (Tewari *et al.*, 2020). The decline in MDA, a marker of oxidative stress and lipid peroxidation, indicates that fenugreek oil might also help in lowering the lipid peroxidation in the cells (Hameed *et al.*, 2024). Al-Madhagy *et al.* (2023) suggests that the beneficial effects of flaxseed may be attributed to its high content of omega-3 polyunsaturated fatty acids. These fatty acids can replace the oxidized polyunsaturated fatty acids in cell membranes that have been damaged by reactive oxygen species (ROS).

Histology examination of testes

The fact that there was a marked decrease in PCNA-positive germ cells compared to the controls with fenugreek seed implies that the cells may have undergone a process of apoptosis (Singh *et al.*, 2022). PCNA is an essential glycoprotein for DNA replication during the late G1→S-phase in cells, its reduced expression can lead to apoptosis (Paunesku *et al.*, 2001). Additionally, fenugreek components may directly harm androgen synthesis cells (Kassem and Al-Mamary, 2006).

The active phytochemicals in flaxseed, particularly lignans acting as phytoestrogens, led to the degeneration of tubules and loss of stages of spermatogenesis (Abdulshahed and Abdul Ameer 2023). The disruption of spermatogenesis is probably not related to the hypothalamus-pituitary-testis axis and may be due to the impairment of estrogen autocrine and paracrine actions in the testes (Assinder *et al.*, 2007).

CONCLUSIONS AND RECOMMENDATIONS

Fenugreek seed oil and flaxseed oil exhibited similar negative effects on the male reproductive system of albino mice: decreasing sperm concentration, sperm motility, and serum testosterone, increasing serum estradiol, and causing histopathological effects on the testes. By adding food additives to the two oils, we can make them more palatable and, hence, use them to control house mice.

Fenugreek and flaxseed oils show promise as plant-based antifertility agents for rodent control. Further studies should optimize dose and duration, assess effects on both sexes, and determine reversibility of reproductive changes. Enhancing bait palatability and evaluating ecological safety, particularly for non-target species, will be essential for effective and responsible field application.

DECLARATIONS

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IRB approval

All experimental procedures were reviewed and

approved by the Institutional Animal Care and Use Committee of the Department of Zoology, Faculty of Science, Ain Shams University, and were conducted in accordance with the guidelines for the care and use of laboratory animals of Research Ethics committee of Ain Shams University.

Ethical statement

All albino mice were treated according to the guidelines of the National Institute of Health (NIH publication No. 86-23, revised 1985) for the care and use of laboratory animals. The study protocol was reviewed and approved by the Research Ethics Committee of the Faculty of Science, Ain Shams University (Approval code: ASU-SCI/ZOOL/2025/3/2) before the commencement of the experiment.

Generative AI and AI-assisted technology statement

The authors declare that no generative AI and AI assisted technology was used in the creation of this manuscript.

Statement of conflict of interests

The authors have declared no conflict of interest.

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