

Effects of fenugreek (*Trigonella foenum-graecum*) on daily sperm production, epididymal transit and sperm morphology of pubertal *Wistar* rats

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Abstract

Phytotherapies are active plant materials that have played an important role in folk medicine as they favor the treatment of different diseases in many regions of the world. Different societies report improvement in the sexual function with various aphrodisiac herbal medicines, even without scientific proof. Aphrodisiacs are generally considered to be substances that increase sexual desire and/or sexual pleasure. An herbal medicine popularly used as an aphrodisiac is fenugreek. Fenugreek (*Trigonella foenum-graecum* L.) is an annual herb that belongs to the *Leguminosae* family. The aphrodisiac potential of fenugreek is still contradictory and not well established in the literature. Thus, the aim of this study was to evaluate the effects of fenugreek on male reproductive parameters of *Wistar* rats. Thirteen pubertal male rats were divided into two experimental groups (7 treated and 6 controls). Fenugreek dry extract was purchased commercially and administered daily to the treated group (3mg/animal⁻¹, diluted in 500 µL of water), for a period of 40 days. The control group received 500 µL of distilled water for the same period. The following parameters were analyzed: daily sperm production; sperm transit time through the epididymal duct; and sperm morphology. The results of the treated group and control group were then compared, using the non-parametric Mann-Whitney statistical test. In our study, there was no change in absolute and relative body weights of the reproductive organs and accessory glands, as well as in the sperm morphology. However, the pubertal rats treated with 3 mg/animal⁻¹ for 40 days showed a tendency of decrease in daily sperm production and increase in epididymal transit time, suggesting a possible negative effect on male gamete production and maturation, which may lead to reduced fertility. Therefore, further studies with higher dosage and/or treatment time should be carried out to evaluate the effects of fenugreek on a complete spermatogenic cycle.

Keywords: *Trigonella foenum-graecum*, Fertility, Aphrodisiac, Male rat.

Resumo

Os fitoterápicos são materiais vegetais ativos que têm desempenhado um papel importante na medicina popular, pois favorecem o tratamento de diversas doenças em diversas regiões do mundo. Diferentes sociedades relatam melhora na função sexual com diversos fitoterápicos afrodisíacos, mesmo sem comprovação científica. Os afrodisíacos são geralmente considerados substâncias que aumentam o desejo sexual e/ou o prazer sexual. Um fitoterápico popularmente usado como afrodisíaco é o feno-grego. O feno-grego (*Trigonella foenum-graecum* L.) é uma erva anual que pertence à família Leguminosae. O potencial afrodisíaco do feno-grego ainda é contraditório e pouco estabelecido na literatura. Assim, o objetivo deste estudo foi avaliar os efeitos do feno-grego nos parâmetros reprodutivos masculinos de ratos *Wistar*. Treze ratos machos púberes foram divididos em dois grupos experimentais (7 tratados e 6 controles). O extrato seco de feno-grego foi adquirido comercialmente e administrado diariamente ao grupo tratado (3mg/animal⁻¹, diluído em 500 µL de água), por um período de 40 dias. O grupo controle recebeu 500 µL de água destilada pelo mesmo período. Foram analisados os seguintes parâmetros: produção diária de espermatozoides; tempo de trânsito dos espermatozoides pelo ducto epididimal; e morfologia espermática. Os resultados do grupo tratado e do grupo controle foram então comparados, utilizando-se o teste estatístico não paramétrico de Mann-Whitney. Em nosso estudo, não houve alteração nos pesos corporais absolutos e relativos dos órgãos reprodutores e glândulas acessórias, bem como na

morfología espermática. No entanto, os ratos púberes tratados com 3 mg/animal⁻¹ por 40 dias apresentaram tendência de diminuição da produção diária de espermatozoides e aumento do tempo de trânsito epididimal, sugerindo um possível efeito negativo na produção e maturação dos gametas masculinos, o que pode levar à redução da fertilidade. Portanto, mais estudos com maior dosagem e/ou tempo de tratamento devem ser realizados para avaliar os efeitos do feno-grego em um ciclo espermatogênico completo.

Palavras-chave: *Trigonella foenum-graecum*, Fertilidade, Afrodisíaco, Ratos machos.

Resumen

Los fitoterapéuticos son materiales vegetales activos que han jugado un papel importante en la medicina popular, ya que favorecen el tratamiento de diversas enfermedades en diferentes regiones del mundo. Diferentes sociedades reportan mejoría en la función sexual con varios afrodisíacos a base de hierbas, incluso sin evidencia científica. Los afrodisíacos generalmente se consideran sustancias que aumentan el deseo sexual y/o el placer sexual. Una hierba medicinal popularmente utilizada como afrodisíaco es el fenogreco. La alholva (*Trigonella foenum-graecum* L.) es una hierba anual que pertenece a la familia de las leguminosas. El potencial afrodisíaco de la alholva es aún contradictorio y poco establecido en la literatura. Por lo tanto, el objetivo de este estudio fue evaluar los efectos del fenogreco en los parámetros reproductivos masculinos de ratas *Wistar*. Trece ratas macho púberes se dividieron en dos grupos experimentales (7 tratados y 6 controles). El extracto seco de fenogreco se adquirió comercialmente y se administró diariamente al grupo tratado (3 mg/animal⁻¹, diluido en 500 µL de agua), durante un período de 40 días. El grupo control recibió 500 µL de agua destilada durante el mismo período. Se analizaron los siguientes parámetros: producción diaria de espermatozoides; tiempo de tránsito de los espermatozoides por el conducto del epidídimo; y morfología de los espermatozoides. Luego se compararon los resultados del grupo tratado y el grupo de control usando la prueba estadística no paramétrica de Mann-Whitney. En nuestro estudio, no hubo cambios en los pesos corporales absolutos y relativos de los órganos reproductivos y glándulas accesorias, así como en la morfología de los espermatozoides. Sin embargo, las ratas púberes tratadas con 3 mg/animal⁻¹ durante 40 días tendieron a disminuir la producción diaria de espermatozoides y aumentar el tiempo de tránsito del epidídimo, lo que sugiere un posible efecto negativo en la producción y maduración de los gametos masculinos, lo que puede provocar una reducción de la fertilidad. Por lo tanto, se deben realizar más estudios con dosis y/o tiempo de tratamiento más altos para evaluar los efectos del fenogreco en un ciclo espermatogénico completo.

Palabras clave: *Trigonella foenum-graecum*, Fertilidad, Afrodisíaco, Ratas macho.

1. Introduction

Medicinal plants have played an important role in the treatment of different diseases in several regions of the world since ancient times, mostly for their expectorant, aphrodisiac, diuretic, diaphoretic, antispasmodic, stomachic and sedative properties (Chowdhury et al., 2018). Different societies report improvements in sexual function with different herbal aphrodisiacs even without scientific evidence (Elferink, 2000).

Currently, aphrodisiacs are generally considered to be substances that increase sexual desire (i.e. *libido*) and/or sexual pleasure (Hess; Boehmer, 2020), and are classified by mode of action, into three types: those that increase the libido, the potency or the sexual pleasure (Bello; Jibrin, 2015). Plant-derived aphrodisiacs are able to directly arouse male sexual libido, contribute to the maintenance of the reproductive activities, and help the neuroendocrine regulation of the mind and body in the process (Low; Tan, 2007).

Fenugreek (*Trigonella foenum-graecum* Linn) is an herbal medicine popularly used as an aphrodisiac. It belongs to the Fabaceae family (Gu et al., 2017) and its seeds are a source of active phytochemicals, such as trigonelline, steroidal saponins, salicylate, nicotinic acid and flavonoids, which are believed to be responsible for many of its presumed therapeutic effects considered (Basu; Srichamroen, 2010).

In Arab countries, fenugreek is used to treat wounds, diarrhea, acne, dehydration, anemia, bronchitis, rheumatism, stomach pain, high blood pressure and constipation. The seeds have important nutritional and cholesterol-lowering properties, and are traditionally used as appetite stimulants and for weight gain (Hind et al., 2017). In addition to aphrodisiac, fenugreek seeds are commonly used in the Middle East and South Asia as a spice in food preparation and also as a traditional medicine for diabetes, high cholesterol, inflammation and gastrointestinal diseases (Belguith-Hadriche et al., 2010).

The action of fenugreek on reproductive function is quite contradictory, promoting an increase in the number of morning erections and in the frequency of sexual activity (Rao et al., 2016), not causing changes in male fertility and reproductive performance (Hind et al., 2017). Thus, it is noticeable that even being used in folk medicine, the aphrodisiac potential of fenugreek is not yet well established in the literature.

Hence, to better understand the performance of fenugreek in the reproduction of male Wistar rats, it is necessary to investigate how the dry extract impacts on these animals' male reproductive parameters, since the reproductive success occurs when there are no abnormalities in sperm morphology, decrease in daily sperm production, or irregularities in epididymal sperm transit time. Thus, the general aim of this study is to evaluate the effects of fenugreek (*Trigonella foenum-graecum*) on daily sperm production, epididymal transit time and sperm morphology.

2. Materials and Methods

2.1. Animals

Thirteen pubertal male rats (55 days old), *Rattus norvegicus* Wistar strain were used. The animals were placed in plastic boxes measuring 40 x 32 x 17 cm (2 animals per box), under controlled conditions of light (12 h of light: 12 h of dark) and temperature ($23 \pm 2^\circ\text{C}$). All the animals received standardized Nuvilab diet *ad libitum*.

2.2. Experimental design

This study consisted of two experimental groups: animals fed with fenugreek ($n = 7$) and animals not fed with fenugreek or control ($n = 6$). *T. foenum-graecum* dry extract was purchased from (SM Empreendimentos Farmaceuticos LTDA) Brazil.

The treated group received fenugreek administered orally through an automatic pipette, at a dose of 3mg/animal diluted in 500 μL of distilled water, for 40 days. This dosage was calculated based on the recommended dose for a 75 kg^{-1} adult male. The control group received 500 μl of distilled water daily, also through automatic pipette, during the same treatment period.

Body weight records were updated weekly. The experimental protocol was approved and registered by the Ethics Committee on the Use of Animals (Comissão de Ética no Uso de Animais - CEUA) of the Federal University of Triângulo Mineiro under the number 23085.007540/2020-89.

2.3. Animal euthanasia, Daily sperm production, Epididymal transit and Sperm morphology

After the 40-day treatment, the animals were fasted for 10 h (from 10 pm to 8 am on the following day) and were only offered water during this period. The following morning, they were euthanized by supraphysiological administration of Ketamine/xylazine ($150/15 \text{ mg/kg}^{-1}$), intraperitoneally, injected in the right lower abdominal quadrant with a 22 G needle.

After confirmation of death, the right testis and epididymis were removed for the analyses of daily sperm production (DSP) and sperm transit (ST), respectively. For daily sperm production, the right testis was weighed, the tunica albuginea removed, and the parenchyma was placed in a 15ml- test tube, with 5 mL of STM solution (containing 0.9% NaCl, 0.01% thimerosal and 0.05% Triton X-100), and then homogenized. Then, 100 μL samples were removed and diluted in 900 μL of STM solution. Homogenization-resistant spermatids (stage 19 of spermatogenesis) were counted in a mirrored Neubauer chamber (Robb et al., 1978; Fernandes et al., 2007).

To analyze sperm transit time, the right epididymis was divided into two units, head-body and tail, which were weighed and homogenized according to the same protocol used to calculate DSP. Fifty μL aliquots were diluted in 950 μL of STM solution and afterwards, sperm counts were performed in a mirrored Neubauer chamber. Epididymal sperm transit time was also calculated following the methodology described by Robb et al. (1978) and adapted by Fernandes et al. (2007).

As for the evaluation of sperm morphology, the vas deferens was sectioned at the ends and washed using a syringe attached to a needle containing 1.5 mL of 10% formalin-saline solution. For the analyses, a drop of this material was placed on a histological slide and covered with a coverslip, according to the procedure described by Seed et al. (1996). A total of 200 sperms were analyzed per animal considering sperm head and tail abnormalities (Filler, 1993).

2.4. Statistical Analysis

Comparisons between the results of the treated and control groups were made using Mann-Whitney non-parametric statistical test (two-by-two comparison) (Jamovi 1.6.23). The 95% confidence interval was used ($\alpha = 0.05$) (Lapponi, 1997).

3. Results and Discussion

The two groups did not present statistically significant differences in relation to the evolution of body weight (Table 1) over the 40 days of the experiment.

Table 1. Evolution of the body weight of pubertal rats treated with fenugreek for 40 days and control animals.

Days of treatment	Body weight (g)	
	Control (n = 6)	Fenugreek (n = 7)
0	199.00 ± 0.002	197.00 ± 0.003
7	232.00 ± 0.002	227.00 ± 0.003
14	257.00 ± 0.002	250.00 ± 0.003
21	273.00 ± 0.002	263.00 ± 0.004
28	278.00 ± 0.002	273.00 ± 0.004
34	288.00 ± 0.002	282.00 ± 0.004
40	293.00 ± 0.001	282.00 ± 0.004

Note: Values expressed as mean ± SEM. Mann-Whitney non-parametric statistical test. Source: prepared by the author (2022).

Rabbits and rats submitted to diets containing 30% fenugreek seeds for 3 months did not present changes in the evolution of body weight in the course of the treatment (Kassem et al., 2006; Kamal et al., 1993). These results corroborate the present study. An increase in body weight was observed in adult rats that received aqueous extract of fenugreek (450mg kg⁻¹/day) for 75 days (Hind et al, 2017; Harchane et al, 2012) and in young rats receiving the following infusion for 30 days: 250 g of fenugreek seed in 1 L of water, boiled for 5 min (Rouag et al., 2020). The increase in body weight caused by *T. foenum-graecum* may be related to higher food intake, since it stimulates the appetite (Harchane et al., 2012).

Different factors may change the results obtained and should be taken into account when comparing the conclusions: animal age, concentration and form fenugreek was administered (dry or aqueous extract or infusion), concentration used, treatment time and others.

As for absolute and relative weights of the reproductive organs (testis and epididymis) and accessory glands (seminal vesicle and hemi-prostate), no statistically significant differences were observed between the control and treated groups (Table 2).

The results presented by Hind et al. (2017) by feeding rats with aqueous extract of fenugreek (450mg/kg⁻¹/day) for 75 days corroborate those of the current study. Singh & Verma (2021) reported that adult rats receiving dry extract of fenugreek seeds (250 mg/kg⁻¹/day) orally, for 45 days presented a significant decrease in absolute and relative weights of testis, seminal vesicle and coagulating glands.

Daily sperm production, epididymal transit and sperm morphology of the animals treated with fenugreek were similar to those of the control animals (Table 3).

Daily sperm production is the total amount of sperm produced daily by the testicles. The number of sperm an animal is able to produce depends on the amount of functional tissue present in the testicles (Rua, 2014), if there is any damage to the testicular tissue, daily sperm production may decrease.

Rabbits fed a diet containing 30% fenugreek seed powder for 3 months presented a decrease in testis weight and sperm concentration, which indicates a negative impact on male structural and functional integrity of testicular tissues (Kassen et al., 2006).

Table 2. Relative weights of the reproductive organs and glands of pubertal rats treated with fenugreek for 40 days and control animals.

Absolute and relative weights of the reproductive organs		
Absolute weight (g)	Control (n = 6)	Fenugreek (n = 7)
Testis	1.67 ± 0.03	1.64 ± 0.02
Epididymis	0.56 ± 0.01	0.54 ± 0.01
Epididymal tail	0.25 ± 0.01	0.23 ± 0.006
Seminal vesicle	0.39 ± 0.02	0.35 ± 0.02
Hemi-prostate	0.15 ± 0.006	0.14 ± 0.02
Relative weight (g/100g body weight)		
Testis	0.57 ± 0.01	0.58 ± 0.01
Epididymis	0.18 ± 0.004	0.19 ± 0.006
Epididymal tail	0.08 ± 0.002	0.08 ± 0.002
Seminal vesicle	0.13 ± 0.007	0.12 ± 0.006
Hemi-prostate	0.05 ± 0.002	0.05 ± 0.003

Note: Values expressed as mean ± SEM. Mann-Whitney non-parametric statistical test. Source: prepared by the author (2022).

Table 3. Daily Sperm Production – DSP (sperm x 10⁶/testis/day), epididymal transit - ET (days) and morphological changes of head and tail of the sperm of pubertal rats treated with fenugreek for 40 days and control animals.

Parameter	Control (n = 6)	Fenugreek (n = 7)
Testis		
Testicular spermatids (x10 ⁶)	235.58 ± 21.42	171.64 ± 17.8
Testicular spermatids (x10 ⁶ /g ⁻¹ testis)	142.16 ± 12.3	104.84 ± 11.43
Daily sperm production (x 10 ⁶ /testis/day)	38.61 ± 3.51	28.13 ± 2.91
Epididymal Head and Body		
Sperm (x10 ⁶)	153.96 ± 12.42	156.98 ± 15.66
Sperm (x10 ⁶ /g ⁻¹ organ)	443.15 ± 27.64	508.01 ± 50.62
Epididymal transit (days)	4.11 ± 0.46	5.79 ± 0.56
Epididymal tail		
Sperm (x10 ⁶)	126.9 ± 24.22	127.23 ± 17.39
Sperm (x10 ⁶ /g ⁻¹ organ)	579.83 ± 111.16	550.29 ± 76.74
Epididymal transit (days)	3.41 ± 0.69	4.89 ± 0.93
Sperm Morphology		
Head abnormalities	31.5 ± 4.14	39.1 ± 9.57
Tail abnormalities	117 ± 7.50	108 ± 10.63
Abnormal sperm	148.5 ± 8.59	147.2 ± 11.36
Normal sperm	51.7 ± 8.45	52.8 ± 11.36

Note: Values expressed as mean ± SEM. Mann-Whitney non-parametric statistical test. Source: prepared by the author (2022).

Some studies have suggested that dry extract of fenugreek seeds promotes the increase of testosterone through different mechanisms, however, the results of these studies are not conclusive (Mansoori et al., 2020). The androgenic activity of fenugreek occurs due to the glycosides and saponins present in its seed, which may possibly act as aromatase and 5 α -reductase inhibitors (Wankhede et al., 2016). The aromatase enzyme is expressed by the Sertoli cells, responsible for converting a small amount of testosterone into a highly potent estrogen, 17 β -estradiol, while 5 α -reductase converts testosterone into an unaromatized androgen, known as 5 α -dihydrotestosterone. (Berne; Levy, 2009). Therefore, the possible increase of testosterone induced by fenugreek would be related to a higher daily sperm production, but in the present study, there was no change in the daily sperm production between the two groups.

As for epididymal transit (number of days required for the sperm to be transported by the organ), it is possible to observe an increase of 40.87% in sperm transit time through the head and body of the epididymis and 43.40% along the tail in animals treated with fenugreek. The epididymal transit time plays an important role in sperm maturation, and a change in this time may harm the process (Fernández et al., 2008). This faster transit of the sperm through the epididymal segments may impair the maturation process, as important events occur during this period (Robaire et al., 2006) and may hinder the fertilization process, negatively affecting the animal's fertility.

With regard to changes in sperm morphology, the average of abnormal and normal sperm was similar in both experimental groups. Studies on men who received 500 mg⁻¹/day of dry fenugreek extract (FurosapTM) for 12 weeks showed a decrease in the number of abnormal sperm from the 8th week of treatment (Maheshwari et al., 2017; Swaroop et al., 2017, Sankhwar et al., 2021).

Positive results concerning reproductive cells and male fertility were evidenced by Salman et al. (2021). Men undergoing treatment with fenugreek presented an increase in the amount of sperm ejaculated and a decrease in the number of cells with morphological changes.

Some results showed negative effects of the use of fenugreek on male reproductive parameters, emphasizing that dry extract of fenugreek seeds and the plant oil may produce an antifertility effect in rats (Kassem et al., 2006; Kamal et al., 1993; Ibrahim et al., 2010). The results of the study carried out by Kassem et al. (2006) showed that rabbits receiving diets containing 30% fenugreek seeds for 3 months had reduced testicular weight and sperm concentration, indicating a toxic effect of fenugreek seeds on seminiferous tubules and interstitial tissue (Leydig cells). From this perspective, the effect of fenugreek is still scientifically contradictory, varying according to dosage and treatment time.

4. Conclusions

Pubertal rats submitted to the treatment with dry extract of fenugreek (3 mg/animal⁻¹) for 40 days did not show changes in the body weight, absolute and relative weight of the reproductive organs (testis and epididymis), accessory glands (seminal vesicle and hemi-prostate), or in sperm morphology. However, there was a tendency of decrease in daily sperm production and an increase in epididymal transit time, suggesting a possible negative effect on the production and maturation of the male gamete, which may lead to reduced fertility. Therefore, complementary studies using a higher dosage and/or treatment time should be carried on to evaluate the effects of fenugreek on a complete spermatogenic cycle (64 days), as well as the possible effects of this herbal medicine on other reproductive parameters (fertility and sexual behavior).

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