

## Effect of the Steroidal Extract of Fenugreek (*Trigonella foenum graecum*) Seeds on some aspects of Reproductive functions in Male Mice

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### Abstract

The current study was designed to determine the role of effective dose of steroidal extract of *Trigonella foenum graecum* to produce infertility on male reproductive mice and compared with the effect of administered estrogen in mice. Thirty adult male mice 8-10 weeks old and their weight ranged between 25-35 gm were randomly divided into 3 equal groups 10 mice for each group. 100 mg/ kg B.W. of steroidal extract of fenugreek was administered orally to the first treated group (T1) daily for 38 days, where as 10 µg/ kg B.W. of estrogen was drenched orally (T2), and Control group was daily received ethyl acetate 20% orally. The results of the current study showed significant decrease ( $P<0.05$ ) in body weight changes of group (T1) treated with 100 mg/ kg B.W. steroidal extract of fenugreek as compared with control group, while the group treated with 10 µg/ kg B.W. of estrogen (T2) revealed significant increase ( $P<0.05$ ) in body weight changes in comparison with control group. It has been also recorded significant decrease ( $P<0.05$ ) in testicular weight for both T1 and T2 as compared with control. The results of this study illustrated also significant decrease ( $P<0.05$ ) in sperms viability percentages in both T1 and T2 as compared with control. The results of this study registered significant decrease ( $P<0.05$ ) in both T1 and T2 groups in the following parameters (sperm concentration, testosterone hormone levels) when compared with control. It could be concluded that the steroidal extract of fenugreek seeds causes a significant infertility effects on male reproductive system of the mice.

### دراسة تأثير المستخلص الستيرويدي لبذور نبات الحلبة (*Trigonella foenum graecum*)

#### على بعض جوانب الكفاءة التناسلية في ذكور الفئران

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### الخلاصة

هدفت هذه الدراسة إلى معرفة تأثير المستخلص الستيرويدي لبذور الحلبة على الجهاز التناسلي الذكري في الفئران والمؤثرة في نوعية السائل المنوي، مستوى هرمون الشحمون الخصوي ووزن الجسم ومقارنة هذا التأثير مع نظيره عند إعطاء مركبات الاستروجين. شملت التجربة 30 فأر ذكر بالغ بعمر 8-10 أسابيع وبمعدل وزن تراوح من 25-30 غم. قسمت الحيوانات عشوائياً إلى ثلاث مجاميع وبواقع 10 حيوانات لكل مجموعة. تضمنت التجربة 3 مجاميع جرعت المجموعة الأولى (T1) فموياً بالمستخلص الستيرويدي لبذور الحلبة وبواقع 100 ملغم/ كغم وزن الجسم يومياً وبالمقابل استخدمت مركبات الاستروجين 10 مايكروغرام/ كغم وزن الجسم للمجموعة الثانية (T2) يومياً ولمدة 38 يوم في حين جرعت مجموعة السيطرة بخلات الاثيل 20% يومياً. أوضحت نتائج الدراسة انخفاضاً معنوياً ( $P<0.05$ ) في معدل تغيرات وزن الجسم للمجموعة المعاملة بالمستخلص الستيرويدي لبذور الحلبة 100 ملغم/ كغم وزن الجسم (T1) عند مقارنتها مع مجموعة السيطرة، اما المجموعة المعاملة بمركبات الاستروجين (T2) فقد سجلت ارتفاعاً معنوياً ( $P<0.05$ ) عند مقارنتها مع مجموعة السيطرة. وقد أظهرت النتائج انخفاضاً معنوياً ( $P<0.05$ ) في نسبة وزن الخصى إلى وزن الجسم لكل

من T1 و T2 عند مقارنتهما مع مجموعة السيطرة. بينت النتائج ان النسبة المئوية للحيامن الحية قد سجلت انخفاضاً معنوياً ( $P < 0.05$ ) في كل من T1 و T2 عند مقارنتهما مع مجموعة السيطرة. سجلت المجموعتان T1 و T2 انخفاضاً معنوياً ( $P < 0.05$ ) لكل من المعايير الآتية (تركيز الحيامن، مستوى هرمون الشحمون الخصوي) بالمقارنة مع مجموعة السيطرة. وقد أستنتج من الدراسة ان المستخلص الستيرويدي لبذور الحلبة له تأثير تثبيطي واضح على الجهاز التناسلي الذكري للفئران مشابه لحد كبير إلى تأثير هرمون الاستروجين.

### Introduction

Fenugreek (*Trigonella foenum graecum* L.) seeds is an annual plant from the family of Papilionaceae–Leguminosae and is extensively cultivated in India, the Mediterranean region, North Africa and Yemen. Fenugreek seeds are well known for their pungent aromatic properties (1). Fenugreek (*Trigonella foenum-graecum*) is considered to be a rich source of steroidal sapogenins (2). It is also considered to be hypo-glycaemic (3). Saponins have considerable potential as medication and/ or nutraceutical agent in natural and synthetic forms. Saponin from a variety of sources have been shown to have hypocholesterolemic, anti-coagulant, anti-carcinogenic, hepatoprotective, hypoglycemic, immunomodulatory, neuro-protective, anti-inflammatory, antifungal, antiviral and antioxidant activities (4). According to several reports *Trigonella foenum graecum* seed extract contains saprogenic and diosgenin, which are precursor of progesterone and have anti-gonadotropine and anti-androgenic characters (5). In light of the fact that previous examination of fenugreek seeds at 30% lowered plasma levels of cholesterol (6), the precursor of steroid hormones, and that fenugreek is considered as a rich source of steroids (2). The aim of this study is to investigate the infertility activity of oral administration of steroidal extract of fenugreek seeds on male mice.

### Materials and Methods

- **Experimental animals:** Thirty male mice were used in this study, (aged 8-10 weeks and weighted 25-35 gram). The animals were kept under controlled environmental conditions (20-25 °C in an air conditioned room and photoperiod of 12 hours). The animals were housed in plastic cages of dimensions 12×15×29 cm. Pellets of freshly prepared ration was given. A great care was taken to avoid unnecessary stress and the cages were cleaned once weekly. The animals were kept for 2 weeks for adaptation before beginning of the experiment.
- **Preparation of steroidal extract from fenugreek seeds:** Fenugreek seeds were grounded by electrical mill and dried on the sun light for 2.5 hrs. The powder of fenugreek seeds were hydrolyzed with 2N HCl for 4h on water bath, then cooled and filtered. The filtrate was air dried for 48 hrs and then defatted in a soxhlet apparatus for 16 hrs with chloroform using double-thickness cellulose extraction thimbles. The extract was dried by incubator at 45°C (7, 8). The result about of 500 gm of crushed Fenugreek seeds produced 12.5 gm of steroidal extract (2.5%). Steroidal extract of Fenugreek solution was prepared by dissolving steroidal extract in ethyl acetate 20% (20 ml of ethyl acetate completed by distilled water to 100 ml). to be given to mice orally via stomach tube at a dose of 100 mg/kg B.W. The amount of the given solution was adjusted individually according to body weight (7, 8).
- **Preparation of estrogen solution:** Estrogen stock solution was prepared by dissolving estrogen product (purity of 0.625 mg in tablet) to be given to mice orally via stomach tube at a dose of 10 µg/ kg B.W. The amount of the given solution was adjusted individually according to body weight.
- **Preparation of epididymal tail suspension:** The mice were at the time of sacrifice first weighted and then numb by placing them in a close jar containing cotton sucked with diethyl ether. The abdominal cavity was opened up through amid line abdominal incision to expose the reproductive organs, the testes were excised and trimmed of all fat, the tail of the left epididymus was removed and embedded in one ml of normal saline at 37 °C in a watch glass, then the tail was cut into at least 200 sections by microsurgical scissor, to perform the microscopical examination of sperm characters.

The experiment was extended for 38 days. Final body weights were recorded. Animals were anesthetized for obtaining blood and organ samples to study the following parameters:

- Body weight changes.
- Testis weight (gm/100 gm body weight).
- Viability of sperms.
- Concentration of sperms.
- Estimation of plasma testosterone concentration.

- **Parameters of the Study**

- **Body weight changes (gm):** The body weight of males were checked at the beginning (day one) of treatment and at end point before sacrificing. The animals were weighted. The body weight changes were calculated as follow:

$$\text{Body weight changes (g)} = \text{Final body weight} - \text{Initial body weight (9).}$$

- **Testicular weight (gram/100 gram body weight):** After treating period, animals were weighted, anesthetized by exposing to high amount of diethyl ether, and testis were obtained and weighted by sensitive balance. Testicular weight to body weight ratio was calculated using the following equation: (9).

$$\text{Testicular W. (gram/100g.B.W.)} = \frac{\text{Weight of testis (gm)}}{\text{Weight of animal (gm)}} \times 100$$

- **Serum testosterone concentration (ng/ml):** Serum testosterone concentration was measured by Automatic Gamma Counter kit after treating the samples with  $I^{125}$  (Labeled testosterone tracer), then by Gamma Counter the connection between  $I^{125}$  with testosterone hormone were measured in ng/ml unit. The analysis was achieved at Dr. Munzr Mustafa Laboratory at Saadun's Street-Baghdad.

- **Sperms viability (%):** The assessment of life and dead sperms was estimated by method of Chemineau *et al.* (10). By putting one drop of sperm suspension on a slide and a drop of Eosin-Nigrosin stain was added and mixed. Viable sperms repel the vital stain (Eosin-Nigrosin), where as dead sperms had lost the structural integrity of their plasma membrane and therefore absorb the dye. Two smears of each sample were made, 200 sperms were examined in each smear.

Sperms viability was calculated as in the following equation:

$$\text{Percentage of dead sperms} = \frac{\text{No. of live sperms}}{\text{Total No. of Sperms}} \times 100$$

- **Sperms concentration (sperm/ ml):** Sperm count was done by using Hemocytometer (Neubauer Type). The Hemocytometer sides were filled with  $5\mu\text{l}$  of a sperm suspension and covered by cover slide, the sperms of the chamber were counted (11).

Estimation of sperms was made according to the following formula:

$$\text{Total sperms No.} = \frac{\text{No. of sperms in five squares}}{80} \times 4000 \times 1000$$

**80 = Number of small squares in five large squares.**

**4000 = Number of sperms in  $1\text{ mm}^3$ .**

**1000 = Number of sperms in 1 ml of solution.**

- **Statistical analysis:** Results were expressed as Mean  $\pm$  Standard Error (M $\pm$ SE). Analysis of Variance (ANOVA one-way) was used to analyze data obtained in the current study (12) and LSD was calculated to determine the significant differences between the experimental groups. Differences between mean data of groups were considered significant at (P<0.05).

- Note: All the percentages were converted into  $\arcsin \sqrt{\%}$ . ANOVA was undertaken on these values. The real percentages were presented in the tables.

## Result and Discussion

- **Body weight changes (gm):** The results explained in (table 1) illustrated the effect of steroidal extract of fenugreek seeds at dose 100mg/kg (T1) and estrogen (T2) on body weight. There was a significant increase ( $P<0.05$ ) in a group treated with estrogen (T2) as compared with group treated with steroidal extract of fenugreek seeds (T1) and control group. The anabolic effects of testosterone act by stimulation of anabolic metabolism and stimulation of proteins production and decrease their destruction all that leading to general body growth and promote metabolism by stimulation of body tissues to utilize glucose (13). Estrogen is anabolic hormone and increases the metabolic rate and growth hormone (13), and thus lead to increase body weight in estrogen group. In the male, the physiological role of estrogens involves multiple actions, from masculinization of brain areas related to reproductive function and sexual behavior to regulation of testicular development and function, as well as direct effects on bone and the cardiovascular system and muscles (14).
- **Testicular weight (gram/100gram):** The results showed in (Table 1). There was a significant decrease ( $P<0.05$ ) in both treated groups (T1 and T2) as compared with control group. The decrement of testicular weight principally may be due to the decrement of testosterone level in treated mice (Table 3) in comparison with control group, at which testosterone has an important role in increasing the weight of the reproductive organs including the testes and seminal vesicles (15). The results of the present study agreed with AL-Kassim and Qasab-Bashi (16) who found that high doses of estrogen administered to male rats lead to significant decrease ( $P<0.05$ ) in male reproductivity represented by decrease testicular weight and decrease weight of both prostate and seminal vesicle glands, eventually lead to decrease in testosterone secretion.
- **Sperms concentration (sperm  $\times 10^4$ / ml) and Sperms viability (%):** The data referring to sperms concentration in epididymal suspension of control and treated groups have been shown in Table 2. After the end of the experiment, sperms concentration tend to be decreased sharply and significantly ( $P<0.05$ ) in the T1 and T2 groups as compared to the values in the control.
- **Sperms viability (%):** The effects of the steroidal extract of fenugreek (T1) and estrogen (T2) on the percentage of live sperms has been pointed in Table 2. The percentages of live sperms have been decreased significantly ( $P<0.05$ ) in T1 group and estrogen T2 in comparison with the control groups. Fenugreek contains steroidal saponins including yamogenin and diosgenin (17). Many studies have also shown that fenugreek contains phytonutrients or more specifically phytoestrogens. Phytoestrogens are a group of substances found in plants that have a weak estrogenic properties. The phytoestrogens compete with the true estrogen on the same cells receptor (18).

Estrogen receptors are present in the testis, efferent ductules and epididymis of most species. Its primary function is to regulate expression of proteins involved in fluid reabsorption. This phytoestrogen possesses anti-estrogen activity and causes dilution of cauda epididymal sperm, disruption of sperm morphology and decline count of sperms (19). Excess exposure to estrogen decreases sperm counts by suppressing the development of sertoli cells in the testicles and testicular growth. Suppression of sertoli cells is most significant to males during growth (20). The role of endogenous estrogen in testes and epididymis in normal conditions is necessary for maturation of spermatozoa whereas high doses of estrogen causes antiestrogen results in dilution of cauda epididymal sperm, disruption of sperm morphology, inhibition of sodium transport and subsequent water reabsorption, increased secretion of  $Cl^-$ , and eventual decreased fertility (21). Inhibition of the hypothalamo-pituitary axis as the results of elevated estrogens levels, will suppresses the secretion of both ICSH and FSH. Deficiency of ICSH and FSH and will produce an inhibition in testosterone biosynthesis (22).

- **Testosterone concentrations (ng/ml):** The results of the present study revealed the effect of steroidal extract of fenugreek seeds and estrogen on testosterone concentration table 3. There was a significant noticeable decrease ( $P < 0.05$ ) in both treated groups (T1) and (T2) as compared with control group. Testosterone is the principle male hormone; it is synthesized by Leydig cells of testes from cholesterol (13) and also formed from androstenedione secreted by the adrenal cortex in small quantities about 5% (15). This decrement of testosterone level may be due to the effects of saponin on serum cholesterol level, which is essential for testosterone synthesis by its action on the Leydig cells. Numerous studies reported that saponin lowers serum cholesterol level (14). Cholesterol is the precursor of sex hormones and is utilized during steroidogenesis. During the investigation of Kamal *et al.* (23), the cholesterol concentration of testes increased after fenugreek treatment, indicating non-utilization of cholesterol by the system. Hence, reduced level of circulating testosterone contributes to altered physiology of reproductive system. Thus, the present investigation suggests that steroidal fraction of fenugreek seeds extract exerts infertility and antiandrogenic activity in male mice.

**Table (1) Effect of the steroidal extract of fenugreek seeds at dose 100mg/kg (T1) and estrogen at dose 10 µg/kg (T2) on body weight changes (gm) and testicular ratio (%) in male mice after 38 days**

Groups	Parameters	Body weight changes (gm)	Testicular weight( g/100g)
Control		+1.6±0.2 B	0.354±0.01 A
T1 (100 mg/kg) B.W.		-1±0.3 C	0.274±0.01 B
T2 (10 µg/kg) B.W.		+2.6±0.6 A	0.220±0.02 C

Values are presented as Means ± SE (n = 6 mice/ group).

Capital letters denote significant differences between different groups ( $P < 0.05$ ).

**Table (2) Effect of the steroidal extract of fenugreek seeds at dose 100mg/kg (T1) and estrogen at dose 10 µg/kg (T2) on sperms concentration (sperm × 10<sup>4</sup>/ ml) and viability (%) in male mice after 38 days**

Groups	Parameters	Sperm concentration (sperm × 10 <sup>4</sup> / ml)	Viability (%)
Control		300±35.45 A	72.2±1.02 A
T1 (200 mg/kg) B.W.		190±17.00 B	40.2±1.16 C
T2 (10 µg/kg) B.W.		134±13.02 B	42.4±1.15 B

Values are presented as Means ± SE (n = 6 mice/ group).

Capital letters denote significant differences between different groups ( $P < 0.05$ ).

**Table (3) Effect of the steroidal extract of fenugreek seeds at dose 100 mg/kg (T1) and estrogen at dose 10 µg/kg (T2) on serum testosterone level (ng/ml) in male mice after 38 days**

Groups	Parameters	Testosterone assay (ng/ml)
Control		1.738±0.54 A
T1 (100 mg/kg) B.W.		0.372±0.31 B
T2 (10 µg/kg) B.W.		0.324±0.02 B

Values are presented as Means ± SE (n = 6 mice/ group).

Capital letters denote significant differences between groups ( $P < 0.05$ ).

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