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# Evaluation of the potential antifertility effect of fenugreek seeds in male and female rabbits

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**Abstract**

**Objective:** The objective of this study was to evaluate the potential antifertility activity of feeding diets containing 30% fenugreek seeds to male and female white New Zealand rabbits.

**Results:** The data presented in this study clearly demonstrate an antifertility effect of fenugreek seeds in the female rabbits and more of a toxicity effect in the male rabbits. In males, testis weight was reduced, with evident damage to the seminiferous tubules and interstitial tissues as shown by the histopathology of testis tissue sections. In addition, the plasma concentration of the androgen hormone and sperm concentrations were halved in the treated animals. In the case of the females, there was evidence of a significant reduction of developing fetuses as observed by reductions of both fetal and placental weights at 20 days of gestation and of the litter size. This was further supported histopathologically by the observed proliferative changes of the endometrial glands. The circulating plasma progesterone concentrations at 10 and 20 days of gestation significantly increased with no significant effect on the prebreeding estrogen concentrations in the treated animals.



*Keywords:* Fenugreek seeds; Antifertility; Toxicity

## 1. Introduction

Fenugreek (*Trigonella foenum graecum* L.) 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] as a spice and are most commonly used for seasoning. They are used in India as a condiment and in Egypt as a supplement to wheat and maize flour for bread making; in Yemen, they are considered to be one of the essential dietary components of the general population. For centuries, fenugreek has been used in folk medicine to heal a range of ailments [2] and has been credited with many medicinal properties (reviewed in Refs. [3,4]). In particular, antidiabetic and hypocholesterolemic effects have been demonstrated in diabetic animals and both insulin-dependent and non-insulin-dependent (Type I and Type II) diabetes mellitus [5–9]. Fenugreek seeds have also been reported to have hypocholesterolemic activity in rabbits [10], rats [11–15], dogs [16] and humans [17–19]. The antidiabetic and hypocholesterolemic activity of fenugreek is primarily associated with the defatted fraction of its seeds [16,20,21] and can largely be attributed to their saponin and high fiber content but not to the major alkaloid trigonelline [3–6,10,22,23].

In light of the fact that previous examination of fenugreek seeds at 30% lowered plasma levels of cholesterol [10], the precursor of steroid hormones, and that fenugreek is considered as a rich source of steroids [24], the aim of this study was to investigate further the potential antifertility activity of feeding diets containing 30% fenugreek seeds to both female and male rabbits.

## 2. Materials and methods

Fenugreek seeds were purchased from the local market, and a voucher specimen was deposited in the pharmacognosy department. Fenugreek seeds were ground in a Maig grinder to pass through a 0.8-mm mesh sieve.

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### 2.1. Experimental design

Thirty-two randomly selected 3-month-old New Zealand white rabbits (8 males and 24 females) were caged individually and given water and unpelleted food ad libitum. All animals were weighed and then allocated to one of two groups (4 males and 12 females per group) that were given either a control diet or a fenugreek seed powder-containing diet (30%). All diets (Table 1) were formulated according to the guidelines set by Cheeke et al. [25]. Animals were fed these diets for 3 months, during which individual body weights were recorded and the total body gain was then calculated.

Three types of mating were conducted: (1) control male×control female; (2) control male×treated female; (3) and treated male×control female. Mating was carried out at random between does and bucks. Pregnancy was diagnosed by palpation on the 10th day after mating. Does failing to conceive were immediately remated. Upon confirming pregnancies in both the control and treated groups, we sacrificed eight control and eight treated females on the 10th day and on the 20th day of gestation. Day 10 examinations revealed the effect of fenugreek seeds on the implanted embryo, since normal implantations in rabbits start on the 7th–8th day after fertilization [26]. Day 20, on the other hand, examined the effect of fenugreek seeds on developmental growth rate and resorption. The remaining females (four control and four treated) were left until parturition.

All pregnant does were sacrificed by cervical dislocation and longitudinally opened. The uteri and ovaries were partitioned by cesarean section. Each uterus was divided into its right and left horn and the two parts were longitudinally opened; the fetuses with placenta were removed. Fetal number (viable and nonviable fetuses) and resorption were determined, and implantation was calculated according to the method of Tariq et al. [27].

At the end of prebreeding and on Days 10 and 20 of pregnancy, food was withheld for 16 h to provide fasting blood samples (feeding was resumed afterwards). Blood was withdrawn from the marginal ear vein of each animal; samples were centrifuged for 5 min at 2500×*g* and the separated plasma was stored at −20°C. In addition, tissue sections from the ovaries and uteri were removed for histopathological analysis.

Table 1
Composition of experimental diets

| | Control diet | Diet containing fenugreek seeds |
|---|---|---|
| Ingredients (%) | | |
| Corn | 30 | 20 |
| Wheat | 30 | 20 |
| Sorghum | 20 | 20 |
| Fenugreek | – | 30 |
| Soya bean meal | 10 | – |
| Concentrate | 10 | 10 |
| Nutrients (% dry matter) | | |
| Crud protein | 16.99 | 17.46 |
| Crud fibers | 11.21 | 11.92 |
| kcal/kg | 2799.7 | 2765.9 |
| Calcium | 0.95 | 0.96 |
| Phosphorous | 0.64 | 0.65 |

Table 2
Effect of fenugreek seeds-containing diet (30%) for 3 months on male rabbit testicular weight, sperm concentration and plasma androgen (testosterone) levels

| Parameter | Control group ($n=4$) | Treated group ($n=4$) |
|---|---|---|
| Testicular weight (g) | $1.20\pm0.07^{a}$ | $0.90\pm0.09^{b}$ |
| Sperm concentration ($\times10^{6}$) | $70.80\pm4.50^{a}$ | $37.50\pm1.60^{b}$ |
| Testosterone (ng/mL) | $7.60\pm0.30^{a}$ | $2.60\pm0.33^{b}$ |

Mean values (±SEM) within the same row bearing different superscript letters are significantly different ($p<.05$).

At the end of the breeding period, food was withheld for 16 h to provide fasting blood samples (feeding was resumed afterwards). Blood was withdrawn and centrifuged and the separated plasma was stored in the same manner as discussed previously.

Eight males (four control and four treated) were then sacrificed and their gonads (testes) were removed and weighed. Spermatozoal studies were performed immediately. Spermatozoa were obtained by making small cuts into the cauda epididymis; semen was drawn to the 0.5 mark halfway up the stem using a white blood cell pipette and the spermicidal solution (physiological saline 0.9% NaCl+0.001% mercury chloride) was subsequently drawn to the 101 mark at the top of the bubble chamber. The preparation was thoroughly mixed for 5 min and the first four to five drops were omitted so as to ensure sperm homogeneity. One drop was then added to both sides of an improved Neubaur blood cell hemocytometer following the method of Anderson et al. [28]. Spermatozoa were allowed to settle for 10 min. The spermatozoa in the appropriate squares of the hemocytometer were counted under light microscopy with the use of a manual counter. Tissue sections from the testes were removed for histopathological analysis.

### 2.2. Biochemical analysis

Plasma was assayed for hormones (progesterone, estrogen and testosterone) using enzyme-linked immunoassay (ELISA) kits. Testosterone ELISA (DRG EIA-1559) and Progesterone ELISA (DRG EIA-1561) (Mountainside, NJ, USA) were assayed as follows: test components and specimens were first brought to room temperature and 25 μL of each standard, control and sample was dispensed into the designated wells, followed by the addition of 200 μL of enzyme conjugate into each well. The wells were gently tapped and rocked for 10 s and then the wells were incubated for 60 min at room temperature. The incubation mixture was then thoroughly removed by flicking into a container containing a disinfectant. The microwells were rinsed and washed three times with a diluted washing buffer