

Original research article

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.

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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;

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±0.07 ^a	0.90±0.09 ^b
Sperm concentration (×10 ⁶)	70.80±4.50 ^a	37.50±1.60 ^b
Testosterone (ng/mL)	7.60±0.30 ^a	2.60±0.33 ^b

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

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.

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

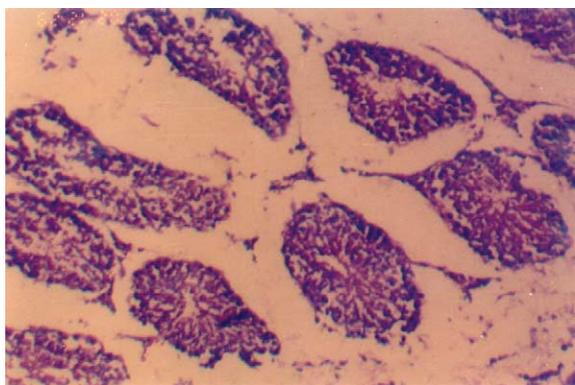


Fig. 1. Histoarchitecture of the testis of a fenugreek (30% per day for 3 months)-treated rabbit (H&E, 5- μ m sections): there is a decrease in the number of seminiferous tubules with mild spermatogenesis hypoplasia (4 \times 10).

(400 μ L per well). The wells were then dried by firmly tapping the plate on absorbent paper to remove residual droplets. Two hundred microliters of a substrate solution was added to each well, at timed interval, prior to its incubation for 15 min at room temperature. The enzymatic reaction was stopped by adding 100 μ L of a stop solution to each well at the same timed intervals as in the previous step; absorbency was read at 450 nm within 10 min of stopping the reaction. The inter- and intra-assay variations for testosterone were 3.28–4.16 and 4.73–9.94 ng/mL, respectively, and those for progesterone were 4.6–8.3 and 4.8–9.9 ng/mL, respectively.

Estradiol [1,3,5 (10)-estratrien-3, 17 β -diol] (Abbott IMx, No. 2215, Abbott Park, IL, USA) was assayed by an Abbott IMx automated system, which is based on a microparticle enzyme immunoassay — the required materials and procedure to perform an IMx estradiol assay can be found in Section 5 of the IMx system operation manual. The inter- and intra-assay variations for estradiol were 3.8–10.4 and 4.3–16 μ g/mL, respectively.

Table 3

Effect of fenugreek seeds-containing diet (30%) for 3 months on female rabbit fetal number, corpus luteum number, litter size and newborn weight

Parameter	Control group (n=4)	Treated group (n=4)
At 10 days of gestation		
Fetal number	5.25 \pm 0.25	5.63 \pm 0.80
Corpus luteum number	6.25 \pm 0.50	8.00 \pm 1.00
Fetal implantations	5.25 \pm 0.25	5.63 \pm 0.80
At 20 days of gestation		
Fetal number	4.50 \pm 0.90	5.38 \pm 0.70
Corpus luteum number	5.25 \pm 0.90	6.25 \pm 0.60
Fetal implantations	4.50 \pm 0.90	5.38 \pm 0.70
Fetal+placenta weight (g)	11.02 \pm 0.90 ^a	2.30 \pm 1.10 ^b
At birth		
Litter size	5.50 \pm 0.30 ^a	1.38 \pm 0.40 ^b
Newborn weight (g)	36.59 \pm 0.64 ^b	46.09 \pm 4.40 ^a

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

Table 4

Effect of fenugreek seeds-containing diet (30%) for 3 months on female rabbits prebreeding progesterone (ng/mL) and estrogen (μ g/mL) and postbreeding progesterone (ng/mL) levels

Parameter	Estrogen estradiol [1,3,5 (10)-estratrien-3, 17 β -diol] (μ g/mL)	Progesterone (ng/mL)
Prebreeding		
Control (n=12)	8.70 \pm 0.42 ^a	0.56 \pm 0.01 ^a
Treated (n=12)	7.10 \pm 0.10 ^b	0.48 \pm 0.004 ^b
Postbreeding (10 days)		
Control (n=12)	–	6.83 \pm 0.13 ^b
Treated (n=12)	–	12.15 \pm 0.52 ^a
Postbreeding (20 days)		
Control (n=8)	–	6.83 \pm 0.13 ^b
Treated (n=8)	–	14.44 \pm 0.50 ^a

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

2.3. Histopathological analysis

Fresh reproductive tissues (i.e., uteri, ovaries and testes) were fixed in 10% neutral formalin for histopathological studies. Tissues were processed by conventional technique and paraffin-embedded sections of 5 μ m thickness were prepared and stained with hematoxylin and eosin for microscopic examination.

2.4. Statistical analysis

Data are presented as means \pm SEM and analyzed as a complete randomized design using one-way analysis of variance [29]. Significant differences among individual treatment means were determined using Duncan's multiple range test [30].

3. Results

Table 2 shows the effect of feeding a diet of 30% fenugreek seeds for 3 months on the testicular weight, sperm concentration and plasma androgen levels of male rabbits. All three parameters were found to be significantly ($p < .05$)

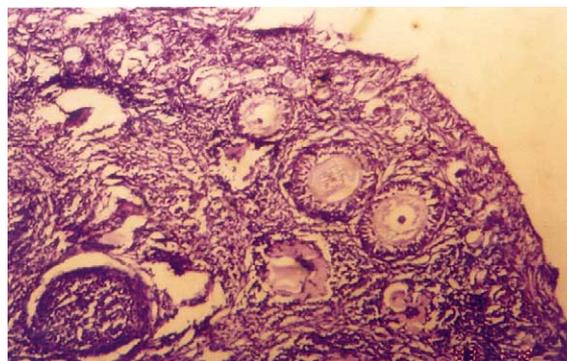


Fig. 2. Histoarchitecture of the ovaries of a fenugreek (30% per day for 3 months)-treated rabbit (H&E, 5- μ m sections): there is a higher follicular development in the secondary and tertiary follicles in the treated cortex area (40 \times 10).

lower in the fenugreek-fed animals with respect to the control animals. The testicular weight and sperm concentration were lower by 25% and 47%, respectively. Along the same line, the plasma androgen (testosterone) levels of the fenugreek-fed animals were lower by 65.8% when compared with those of the control animals. Tissue sections from the testes showed a decrease in the number of seminiferous tubules with mild thickening of the basement membrane and mild spermatogenesis hypoplasia (Fig. 1) when compared with those of the control animals.

Table 3 shows the fetal number at 10 and 20 days of gestation to be nonsignificantly higher in the fenugreek-treated female rabbits by 7.1% and 19.4%, respectively, as compared with that at 10 and 20 days of gestation in the control animals. Similarly, the corpus luteum number at 10 and 20 days of gestation was observed to be nonsignificantly higher in the treated group by 28% and 19.1%, respectively, with respect to that in the control group. On the other hand, the number of implanted embryos was significantly higher in the fenugreek-fed animals at both 10 and 20 days of gestation by 7.2% and 19.6%, respectively. In contrast, litter size, newborn weights and fetal and placental weight were significantly lower in the treated females by 75%, 20.6% and 79.1%, respectively, when compared with those in the control animals.

Table 4 shows the effect of feeding 30% fenugreek seeds on the serum levels of both progesterone and estrogen {estradiol [1,3,5 (10)-estratrien-3, 17 β -diol]} in the prebreeding state and that of progesterone at 10 and 20 days of gestation as well as at parturition. In the prebreeding state, both progesterone and estrogen levels were nonsignificantly lower in the fenugreek-fed females by 14.3% and 18.4%, respectively, as compared with those in the control animals. In contrast, the level of progesterone was significantly ($p < .05$) higher in the treated group than in the control group by 77.9% at 10 days and by 111.4% at 20 days.

Histopathological investigation of tissue sections from the ovaries of the treated group showed higher follicular

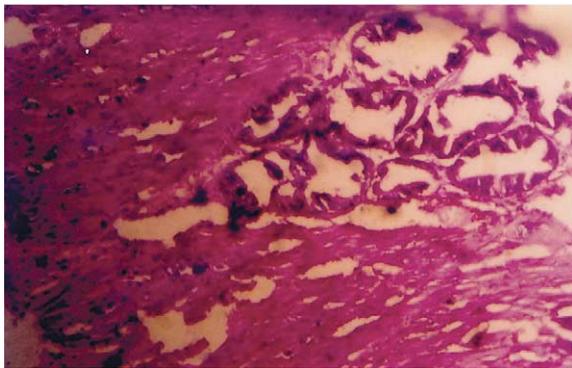


Fig. 3. Histoarchitecture of the uterus of a fenugreek (30% per day for 3 months)-treated rabbit (H&E, 5- μ m sections): there is an increased proliferation of the endometrial glands with hyperplastic changes and some of the glands show mild dilatation (40 \times 10).

development in the secondary and tertiary follicles in the cortex area as compared with those of the control group (Fig. 2). An increase in ovulation activity was reflected by the higher number of corpus luteum in the cortex of the treated animals as compared with that of the control animals. Tissue sections from the uteri of the treated group showed endometrial tissue with a few endometrial glands that had undergone proliferative changes, with the stroma being cellular (Fig. 3), as well as increased proliferation of the endometrial glands with hyperplastic changes, with some of the glands showing mild dilatation when compared with those of the control group.

4. Discussion

Feeding fenugreek seeds-containing diets at 30% for 3 months showed no significant effect on liver function tests as well as body weight and body gain of both male and female rabbits (data not shown). This is in agreement with previous reports [13,31,32].

The results reported in this study show that diets containing 30% fenugreek seeds significantly reduced male testis weight (~25%) as well as sperm concentration (~43%), indicating a toxic effect of fenugreek seeds on seminiferous tubules and the interstitial tissue (Leydig cells). Furthermore, feeding fenugreek seeds at 30% to male rabbits lowered circulating androgen (testosterone) by 65.8%. The negative impact of fenugreek seeds on the male structural and functional integrity of testicular tissues was evidenced by the histopathological data highlighting the damage of interstitial tissue, showing a decrease in the number of seminiferous tubules with mild spermatogenesis hypoplasia when compared with that in the control animals. The latter finding may support the hypothesis that a component of fenugreek seeds might have a direct toxic effect on the cells responsible for synthesis of androgens [31]. This is further supported by the lack of differences in the number of litter size (data not shown) when treated males were mated with control females, despite the decrease in androgen levels and sperm concentration, suggesting a toxicity effect rather than an antifertility effect in the male rabbits.

In the case of female rabbits, diets containing 30% fenugreek seeds resulted in an abnormal development of fetuses, as evidenced by the smaller fetal plus placenta size and by the significantly diminutive embryo plus placenta weight reaching ~80% at 20 days of gestation, resulting in a reduction of the litter size (number of newborns) of treated females by ~75%. This reduction in the number of the young in the litter was reflected in the significant increase of the weight of newborns as a result of nutritional competency. These abnormal fetal development and fetal resorption might be the consequences of fenugreek seeds containing an estrogenic activity that disturbs the endometrial lining system and interferes with fetal development [33–35].

Alternatively, abnormal fetal development could be explained by transplacental passage of a toxic substance to a fetus in addition to modification of the uterine lining function as suggested by Elbetieha et al. [35]. The latter finding is confirmed by our histopathology of tissue sections from the uteri showing the endometrial tissue to have fewer endometrial glands that had undergone proliferative changes, with the stroma being cellular. Also, there was increased proliferation of the endometrial glands with hyperplastic changes, with some of the glands showing mild dilatation in the treated group when compared with those in the control group.

Interestingly, however, diets containing 30% fenugreek seeds showed initial enhanced ovulation prior to the abnormal development of fetuses. This was evidenced by the lack of a significant effect on both fetal and corpus luteum numbers at 10 and 20 days of gestation in fenugreek-fed females, indicating no impaired effect on follicular development, ovulation rate, fertilization and number of embryos implanted in the uteri. This is confirmed by our histopathological finding of higher follicular development in the primary, secondary and tertiary follicles in the treated cortex area, indicating increased ovulation activity as evidenced by the higher number of corpus luteum in the cortex of the treated animals with respect to the control animals. Moreover, feeding fenugreek seeds at 30% was associated with a significant increase in plasma progesterone levels at postbreeding in the treated female rabbits by 50% as compared with those of the control animals. This increase in progesterone will maintain the endometrium for the continuation of pregnancy and maintain the development of the uterine glands. This might be the result of an effect of fenugreek seeds on the stimulation of FSH, which improves maturation and ovulation rates of oocytes, resulting in increased corpus luteum number and progesterone levels in the circulation.

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