Journal of Complementary and Alternative Medical Research

8(3): 1-8, 2019; Article no.JOCAMR.53003
ISSN: 2456-6276

Effect of Ethanolic Extract of *Trigonella foenum-graecum L.* Seeds on Reproductive System of Male Albino Rats

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Authors' contributions

This work was carried out in collaboration between both authors. Author MS designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author GNV managed the analyses of the study. Author MS managed the literature searches. Both authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JOCAMR/2019/v8i330122
Editor(s):
(1) Dr. Francisco Cruz-Sosa, Metropolitan Autonomous University Iztapalapa Campus, Av. San Rafael Atlixco 186 México City 09340 México.
Reviewers:
(1) Anwar Tawfik Amin, Assiut University, Egypt.
(2) A. Ayeshamariam, Khadir Mohideen College, India.
Complete Peer review History: http://www.sdiarticle4.com/review-history/53003

Original Research Article

Received 28 September 2019
Accepted 02 December 2019
Published 09 December 2019

ABSTRACT

Aims: The present study was undertaken to assess the antispermatogenic and antifertility efficacy of ethanolic extract of *Trigonella foenum-greacum* seeds.
Methodology: Aqueous solution of the extract (250 mg/kg b.wt/day) when administered orally for 45 days to adult male albino rat (Duckray strain).
Results: Seeds extract caused inhibition of spermatogenesis as well as inability to mate with normal untreated female rats of proven fertility. There occurred a significant decrease in absolute and relative weights of testis and seminal vesicle and increase in epididymal weight, whereas of ventral prostate and coagulating gland remained unchanged. Sperm concentration and motility in the cauda epididymis was also decreased. Increased incidence of separation of head and tail pieces of spermatozoa was seen in the epididymal smear. Histologically, testis of experimental animals showed arrest of spermatogenesis at the secondary spermatocytes stage and there was deposition of cellular debris in semineferous tubular lumen. The spermatids were not properly

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developed and interstitial cells were very sparse and degenerated. However, there was no evidence of damage to the spermatogonia. Oral treatment of extract did not affect the body weight of animals. Quantitative estimation of marker testicular enzymes, e.g., sorbitol dehydrogenase showed a significant decrease whereas lactate dehydrogenase and gamma-glutamyl transpeptidase levels were significantly increased.

In conclusion: These results suggest that *T. foenum-greacum*, may induce male infertility in rats, therefore, should be considered further as a potential male antifertility agent.

Keywords: Trigonella foenum-graecum; antifertility; antispermatogenic; testicular marker enzymes; rat testis.

1. INTRODUCTION

Plant products have attracted the attention of many scientists as a primary source of naturally occurring antifertility agents. Discovery of gossypol [1] and triptoloid [2] as a male antifertility agent by Chinese scientists provided a major lead in male herbal contraception. Since then, a number of plants have been identified and evaluated by the researches for the fertility regulation in male [3,4,5,6,7,8] (Sharma and Jacob, 2001)

Trigonella foenum-graecum Linn (commonly known as fenugreekbelongs to family-Leguminosae), a common plant cultivated throughout in India (in Hindi-Methi) and other parts of world, used as traditional medicine and natural additive food. It is one of the most widely used plants in various indigenous systems of medicine for the treatment of different ailments. The medicinal value of fenugreek seeds is mentioned in Ayurvedic medicine as well as in Greek and Latin pharmacopoeia. Since past in India it is used as medicine to ameliorate various ailment of kidneys, disperse cold and alleviate pain etc. Fenugreek seeds are considered to have aromatic, carminative, tonic galactogogue, antidiabetic, hepatoprotective, anti-inflammatory, antiulcer and hypocholesteraemic activity by many workers [9,10,11,12,13,14].

Despite the vast use of fenugreek seeds in everyday lives, little attention has been paid to examine the action of fenugreek on the male reproductive system. Although the saponin isolated from seeds of fenugreek has been shown to possess *in-vitro* spermicidal properties against human and rat spermatozoa [15]. Diet containing 30% fenugreek seeds was able to reduce testis weight, with evident damage to the seminiferous tubules and interstitial tissues in male rabbits [16]. In addition, the plasma concentration of the androgen hormone and sperm concentrations were reduced to half in the treated animals. One more histological study revealed that morphological changes in seminiferous tubules and atrophy of Leydig cells and epididymis by seed extract of fenugreek [17]. The present investigation was undertaken to examine the effects of ethanolic extract of fenugreek seeds on male reproductive system, to explain the antifertility activity and the possibility of developing it as an oral male contraceptive of plant origin.

2. MATERIALS AND METHODS

2.1 Method of Extraction

Fresh seeds of fenugreekobtained locally were air-dried under shad, and powered. The powdered seeds were extracted with 90% ethanol at 65ºC for 24-48 hrs using a soxhlet extractor. The total extract (14.08% w/v) thus obtained was concentrated in vacuum at reduced pressure and temperature. On testing, it was found to be freely soluble in water. The required doses were freshly prepared by dissolving it in the requisite quantity of water.

2.2 Animals Treatment

Colony-bred adult male albino rats of proven fertility weighing between 200-220 grams were used for present investigation. Feed and water were provided *ad libitum*, and the animals were maintained in steel cages under standard laboratory conditions. They were divided into two groups, each consisting of 6 animals.

Group I: Rates were given vehicle (i.e., Distilled water), 1ml/animal by oral route.

Group II: % Rats were given Fenugreek seeds extract 250 mg/kg body weight per day for 45 days by oral route. This dose was selected on the basis of pilot experiments involving histological examination of testis of limited numbers of rats (un-published data) by us using 150, 200, 250 and 300 mg/kg oral doses of the same test material for periods varying from 30 to 60 days.

2.3 Body and Reproductive Organs Weight

Body weight of control and experimental animals were recorded before and after the treatment. They were sacrificed by exsanguination under ether anesthesia 24 h after the termination of each experimental schedule. Testes and accessory sex organs were removed, fat and connective tissue cleared off and organ weights were recorded.

2.4 Fertility Test

Fertility test of individual male animal was done after completion of the treatment period. Four males from each group were caged separately with regularly cycling females in the ratio of 1:2. Next morning after the mating exposure, the presence of spermatozoa in the vaginal smear of the female animal was taken as day 'o' (zero) of pregnancy. Mated females were laprotomized on day 15th post coitum, the number of corpora lutea and the number of implantation and/or resorption sites, it any, were recorded.

2.5 Histological Preparations

Tissues were fixed in Bouin's fluid. Paraffin sections were made and stained with hematoloxylin and eosin [18].

2.6 Sperm Count and % Sperm Motility

Motility and number of cauda epidiymal spermatozoa in control and treated rats were assessed using a haemocytometer (Neubaur's Chamber) by the method described by Freund and Carol [19]. Motile spermatozoa were calculated per unit area and expressed as % sperm motility. Sperm counts were recorded as million/ml of suspension [20].

2.7 Enzymology

A portion of the testis was homogenized (1:9) in 0.2 mTris/HClBuffer (pH 7.0), having 0.1% cetyltrimethyl-ammonium bromide using Potter Elvegham homogenizer for the estimation of sorbilol dehydrogenase (SDH) lactate dehydrogenase (LDH) according to the method described by Gerlach [21] and Vassault [22], respectively.

Another portion of the testis was homogenized (1:9) in 0.05 M Tris/HCL Buffer, pH-7.4, for the assay of $\gamma - glutamyl\ transpeptidase$ $(\gamma - GT)$ by the method described by Roomi and Goldberg [23]. Protein content of the sample was estimated by the method of Lowry et al. [24].

2.8 Statistical Analysis

Where applicable, data obtained were analyzed statistically by student' t-test. Values were expressed as Mean $\pm$ SE. P≤0.05 considered as significant.

3. RESULTS

3.1 Fertility Test

Daily oral administration of crude ethanol extract of fenugreek seed (dose 250mg/kg b.wt.) for 45 days duration caused complete inhibition of male fertility.

3.2 Body and Reproductive Organ Weight

The effect of treatment on weights of body and reproductive organs is shown in Table. 1. Data reveal that daily ingestion of the test substance did not change body weight gain of treated males as compare to control. Statistically significant decrease in the weights of testis, seminal vesicles and coagulating glands were found while prostate gland and epididymis weights exhibited statistically insignificant change (P >0.05).

3.3 Sperm Count and Motility

Along with progressive inhibition of fertility, there also occurred statistically significant depression in the cauda epidiymal sperms motility (P<0.01) and sperms counts (P<0.01) (Table 2). Alteration in the structure of spermatozoa was also noticed. Tails of most of the sperms in cauda epididymis were separated from head region.

3.4 Histopathology of Testis

All the seminiferous tubules of the control animals showed normal spermatogenesis (Fig.1) and those of the treated animals exhibited suppressed spermatogenesis (Figs. 2 and 3) with a moderate reduction in tubular diameter and decreased number and size of all cellular elements including the spermatocytes. Most of the tubules showed arrest of spermatogenesis at the spermatocyte stage. Tubular lumina were filled with either necrosied sperms or were empty. Sertoli cells and spermatogonia were not affected by the extract treatment. At many places vacuolated, fluid-filled interstitial space indicated the disappearance of Leydig cells. Leydig cells where present, were normal in appearance but in reduced numbers.

Table 1. Effect *T. foenum-graecum* seeds extract on body weight and male reproductive organs weight

Group	Body weight (in grams)		Organs weight/100 gm body weight (in grams)				
	Initial	Final	Testis	Epididymis	Accessory Reproductive Organ		
					Seminal vesicle	Ventral Prostate	Coagulating gland
I	214.17±3.8	235±3.6	0.696±0.03	0.236±0.01	0.234±0.02	0.113±0.01	0.065±0.01
II	238.33±8.9	247.80±5.0^{NS}	0.548±0.01** (21.23%)	0.225±0.01^{NS} (4.82%)	0.132±0.01** (43.35%)	0.110±0.01^{NS} (2.3)	0.052±0.01 (19.07%)

Data represent mean± S.E. of 6 rats in each group.
*$P \leq 0.05$, **$P \leq 0.01$ considered as significant. $^{NS}p \geq 0.05$ considered as not significant*
Group I- Control, Group II- Rats treated with T. foenum-graecum seeds extract (250mg/kg b.wt.)

Table 2. Effect of *T. foenum-graecum* seeds extract on % sperm motility and total Epididymal sperm count

Group	% Sperm motility (range of median)	Total epididymal sperm count (per epididymis) $\times 10^6$
I	87.17±1.68 (80-92)	224.00±14.73
II	74.00±1.65** (70-80) (14.15%)	106.66±9.94** (52.67%)

Data represent mean± S.E. of 6 rats in each group
$^{**}P \leq 0.01$, considered as not significant. $^{NS}p \geq 0.05$ considered as not significant
Group I- Control, Group II- Rats treated with 250mg of T. foenum-graecum seeds extract/kg b.wt

Table 3. Effect T. foenum-graecum seeds extract on marker testicular enzymes

Enzymes	Group I	Group II
Sorbitol Dehydrogenase	4.4142±0.1659	1.4135±0.28**(67.98%)
Lactate dehydrogenase	250.60± 1.50	516.02±15.50**(105.9%)
$\gamma - Glutamyl$ transpeptidase	39.56±5.616	76.15±3.30**(92.49%)

Enzymes activities are expressed as specific activities (n moles of substrate oxidized or product formed/min/mg protein)
$P \leq 0.01$, considered to be statistically significant.
Group I- Control, Group II- Rats treated with 250mg of T. foenum-graecum seeds extract/kg b.wt

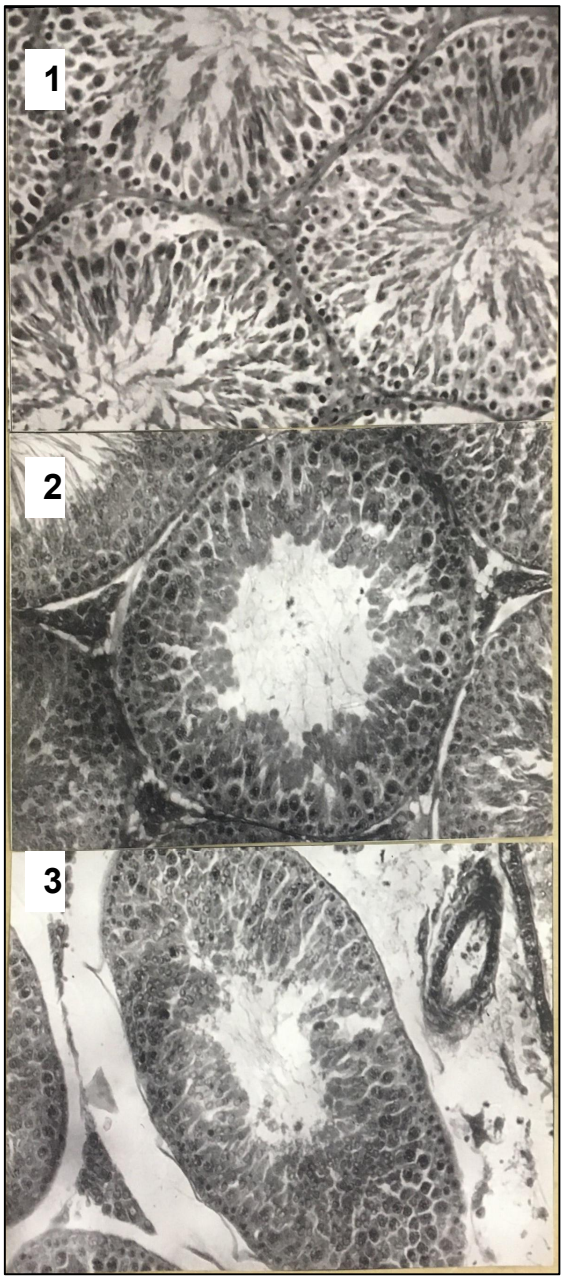

Fig. 1. (H&E, X200): Representative testicular histomorphology of controls showing normal spermatogenesis, normal tubular diameter and normal cellularity of the seminiferous tubules. Note the intertubular distances and compact intertubular connective tissue

Figs. 2 & 3. (H&E, X200): Representative testicular histomorphology of *T. foenum- graecum* (250 mg/kg b.wt./day for 45 days) treated animals showing suppressed spermatogenesis in majority of seminiferous tubules. Note that the tubular diameter and cellularity are decreased. Arrest of spermatogenesis was noted at the spermatocyte stage. Tubular lumina are filled with either disintegrated spermatozoa or were empty. Sertoli cells and spermatogonia were not affected by the treatment. Interstitium was filled with fluid and intertubular distances were increased are several places. Leydig cells are reduced in numbers

3.5 Enzymes

Significant decrease in the activity of SDH and an increase in the activity of $\gamma - GT$ and LDH were found after 45 days of fenugreek extract treatment (P <0.01; Table 3).

4. DISCUSSION AND CONCLUSION

The present study with ehtanolic extract of fenugreek seeds shows its inhibitory effect on sex organs and fertility of the male rats. Energy needs of the developing spermatozoa for metabolic reactions catalyzed by the reversible oxidation/reduction are emphasized, and met by dehydrogenases. The role of LDH is defined in the catalysis and regulation of cell metabolism. Interconversion of lactate and pyruvate with cofactor NAD is also a part of LDH activity. It has been shown that high LDH activity is present in the testis of prepeubertal rats and it declines in adult rats during maturation process [25]. High levels of LDH have also been reported from spermatogenic cells undergoing degenerative process in the testis [25]. Therefore, increased LDH activity seen in the present study could be taken as a chemical marker of degree of degeneration of the seminiferous epithelium.

The specific activity of some testicular enzymes has been utilized as a functional indicator of spermatogenesis. Role of the dehydrogenases (especially SDH) has been implicated in the changes in the metabolic activity of developing spermatozoa and as such its activity may be used as marker of specific cell type i.e. in the formation of pachytene spermatocytes during the maturation of the germinal epithelium (Bishop, 1968; [26]. We found a decrease in SDH activity in testis after treatment with fenugreek extract. Germinal epithelia of seminiferous tubules of treated animals have been found to be degenerated. This was confirmed in the testicular histology of the treated rats (Figs. 2 and 3).

The alteration caused by any agent in Sertoli cell function may also affect the process of spermatogenesis, where they are believed to play a key role. $\gamma - GT$ is considered as specific maker of Sertoli cells function and the process of sperm maturation. The specific activity of $\gamma - GT$ is reported to increase parallel to the level of spermatogenic maturation [25,27,28,29]. The enzyme activity becomes detectable during the pubertal development, increases markedly between the 15thand 20th days of age and becomes constant with completion of maturation of Sertoli cells. The increased activity of $\gamma - GT$ following fenugreek extract treatment may have due to the rapid activity of Sertoli cell to replenish the loss of germ cells during the testicular atrophy. The above pattern of activity of marker testicular enzymes following the doses of extract viz. decreased SDH and increased LDH and $\gamma - \mathrm{GT}$ has been reported in literature during testicular atrophy and degeneration [25]. In the present study, similar altered responses in the activities of SDH (decreased), LDH (increased) and $\gamma - GT$ (increased) were observed in the rats given oral doses of fenugreek (seeds) extract. The alteration in enzymatic activities associated with specific cell types in testis is supported by the finding of the testicular histology, which also showed suppressed spermatogenesis.

Extract of fenugreek seeds given to the male rats showed a direct effect on the histology of the testis. Significant degenerative changes of spermatocytes, spermatids and spermatozoa were seen (Figs. 2 and 3). Sperms present in the tubules were broken and necrosed. Leydig cells have a direct bearing on spermatogenesis. A reduction is number of Leydig cells was noticed which could have caused low androgen production affecting fertility of treated animals [30]. On other hand according to Mokhtari et al. [17] fenugreek seeds extract contains sapogenin and diosgenin, which are precursor of progesterone and have antigonadoterpine and antiandrogeni potential. Hence, they have the capability to reduce the concentration of testosterone. Statistically significant reduction in weights of testis and accessory sex organs of the treated rats could have been due to low level of androgens, which were not enough to maintain the weight of the gonads and accessories [31, 32].

The sperm motility and count in cauda epididymis may, therefore, have been adversely affected by fenugreek treatment. Androgen deprivation, not only suppresses weight of genital organ but reportedly alters the epididydymal milieu also, which renders it hostile for maturation and survival of spermatozoa [33,34, 35]. Findings of the present study are consistent with the suggestion given by others also [36,37, 32].

To conclude, the present study shows that fenugreek seeds extract has a direct action on spermatogenesis (as revealed by marker enzymes and histology) and also on Leyding cells (as indicated by histology as well as by decreased sex organs weight and sperm count

and motility). Interference with the androgen production altering structure, function, viability and number of sperms could be the cause of diminished fertility seen in fenugreek extract treated animals.

CONSENT

It is not applicable.

ETHICAL APPROVAL

Animal ethic Committee approval has been collected and preserved by the author.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sdiarticle4.com/review-history/53003