Antifertility effects of Trigonella foenum-graecum (fenugreek) ethanolic extract in male rats & cocks *1Mohaned M. Mohammed, 2Abd Elkhalig Mudathir, 3Sania A. I. Shaddad, 4Elshari...

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Antifertility effects of *Trigonella foenum-graecum* (fenugreek) ethanolic extract in male rats & cocks

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Abstract:

Introduction: *Trigonella foenum-graecum* (Fenugreek) is a member of the Fabaceae family. Fenugreek is native to the area from the eastern Mediterranean to Central Asia and Ethiopia, and much cultivated in Pakistan, India and China. Its dried ripe seeds are variously referred to as Trigonella seeds or as Fenugreek. It is well known for its pungent aromatic properties, and is often used to add flavor in Malaysian homes. *Trigonella foenum-graecum* (Fenugreek) seeds are used in India as condiment as a green, leafy vegetable and are a rich source of calcium, iron, β-carotene and other vitamins. In folk medicine fenugreek is used to treat cold pain in the lower abdomen, impotence, and hernia. Also in Indian medicine fenugreek is used for fever, vomiting, anorexia, coughs, bronchitis, and colitis. It also demonstrates an antifertility effect in the female rabbits and more of a toxicity effect in the male rabbits. It produces antinociceptive effects through central and peripheral mechanism. It has significant chemopreventive effects against breast cancer. It also showed a stimulatory effect on immune functions in mice.

Materials and Methods: Extraction of *Trigonella foenum-graecum* seeds was carried out according to standard method. Two groups of adult male rats and other two groups of cocks were used. Group 1 rats and cocks served as control, while group two received *Trigonella foenum-graecum* (F) crude ethanolic extract 1g/kg/day orally. Total serum testosterone was measured in rats before and after two and four weeks of treatment. Cocks semen was evaluated before and after two and four weeks of treatment.

Results: Preliminary phytochemical screening of *Trigonella foenum-graecum* seeds ethanolic extract showed the presence of high concentrations of coumarins, flavonoids tannins and saponins, various concentrations of alkaloids, sterols and triterpenses, and the absence of anthraquinone glycoside compounds.

*Trigonella foenum-graecum* seeds ethanolic extract decreased insignificantly (p>0.05) serum testosterone concentration in the treated rats and both mass and individual motility of the sperms in the treated cocks. Histopathological examination of rat’s testes treated with *Trigonella foenum-graecum* seeds ethanolic extract showed presence of inactive seminiferous tubules and oedema formation between the seminiferous tubules. Conclusion: *Trigonella foenum-graecum* tends to reduce the male fertility by reducing testosterone concentration, sperms concentration and inhibiting mass and individual motility of the sperms.

Key Words: *Trigonella foenum-graecum*; Antifertility; Rats; Cocks.
Introduction

Trigonella foenum-graecum (Fenugreek) is a member of the Fabaceae family. Fenugreek is native to the area from the eastern Mediterranean to Central Asia and Ethiopia, and much cultivated in Pakistan, India and China (Morton)\(^\text{13}\). Its dried ripe seeds are variously referred to as Trigonella seeds or as Fenugreek. It is well known for its pungent aromatic properties\(^\text{10}\), and is often used to add flavor in Indian dishes. Trigonella foenum-graecum (Fenugreek) seeds are used in India as condiment as a green, leafy vegetable and are a rich source of calcium, iron, \(\beta\)-carotene and other vitamins\(^\text{17}\), in Egypt as a supplement to wheat and maize flour for bread-making and in Yemen it is one of the main constituents of the normal daily diet of the general population. In folk medicine fenugreek is used to treat cold pain in the lower abdomen, impotence, and hernia. Also in Indian medicine fenugreek is used for fever, vomiting, anorexia, coughs, bronchitis, and colitis\(^\text{10}\).

Chronic oral administration of an ethanolic extract of Trigonella foenum-graecum (Fenugreek) significantly increased food take and the motivation to eat in rats\(^\text{15}\).

Fenugreek seeds demonstrate an antifertility effect in the female rabbits and more of a toxicity effect in the male rabbits\(^\text{1}\). It produces antinociceptive effects through central and peripheral mechanism\(^\text{13}\). It have significant chemopreventive effects against breast cancer\(^\text{2}\). It also showed a stimulatory effect on immune functions in mice\(^\text{5}\).

Repeated topical application of fenugreek can induce hypersensitivity reactions. High levels of specific IgE to both peanut and fenugreek were seen in most sera. Fenugreek sensitization is believed to be a consequence of cross-reactivity in patients with peanut allergy\(^\text{6}\).

Objectives

Evaluation of the phytochemical screening of Trigonella foenum-graecum extract. And study the effects of the extract on serum testosterone levels and semen quality.

Materials and methods

Plant: Trigonella foenum-graecum (family Fabaceae) (Fenugreek) seeds were purchased from the local market in Omdurman. The plant was identified and authenticated by the Medicinal and Aromatic Plants Research Institute, Khartoum, Sudan.

Animals:
18 male wistar rats (weight: 110 – 190 g, age: 16 – 17 weeks) were obtained from the animal house of the pharmacology department faculty of Pharmacy University of Khartoum,Sudan.
18 cocks (weight: 3.5 – 5 Kg, age: 26 – 27 weeks) were obtained from Inmaa Poultry Company Omdurman Sudan.

Method

Total serum testosterone was measured at zero time (before treatment), after two and four weeks of treatment using ST AIA – PACK Testosterone TOSOH AIA system analyzers.Cocks semen was collected by dorso-abdominal massage (Burrows and Quinn)\(^\text{5}\), and evaluated as described by Rao\(^\text{16}\).

The collections were conducted in the morning before feeding to prevent semen contamination. The appearance of semen was estimated directly from the collecting glass tube for contaminants such as dirt or faeces.

The volume of the ejaculate was measured and the colour and consistency of semen were assessed by visual observation. Semen mass activity was estimated by observing the degree of wave motion and general activity of spermatozoa and is graded from zero to 5 scores according to intensity of wave motion as described by Evans and Maxwell\(^\text{8}\).

Individual motility of sperm was estimated by examining a drop of diluted semen with physiological saline solution (0.9% NaCl) on a pre-warmed slide (37°C). Only sperm that have forward or progressive movements are included, but sperm with backward, vibrating or circling movements are not included\(^\text{8}\).

The concentration of spermatozoa was counted by the aid of a haemocytometer according to the method described by Evans and Maxwell\(^\text{8}\).

Sperm head and acrosome abnormalities were evaluated on air-dried eosin-nigrosin stained slide and was expressed as a percentage for abnormalities in each ejaculate (Evans and Maxwell, 1987)\(^\text{8}\).

Eosin-nigrosin was used for routine examination of live-dead spermatozoa. The result was expressed as a percentage for live-dead sperms in each ejaculate\(^\text{16}\). Testes of the rats (two groups) were collected immediately after slaughtering in
neutral formalin, histological sections were made. Statistical analysis was performed using SPSS version 12 (ANOVA).

**Results:**

Phytochemical screening of *Trigonella foenum-graecum* seeds were shown in table 1. The plant constituents was high in coumarins flavonoids, tannins and saponins and to lower extend in alkaloids, sterols and triterpenes, anthraquinone glycosides were absent.

<table>
<thead>
<tr>
<th>Alkaloids</th>
<th>++</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anthraquinones glycosides</td>
<td>—</td>
</tr>
<tr>
<td>Coumarins</td>
<td>+++</td>
</tr>
<tr>
<td>Sterols</td>
<td>++</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>+++</td>
</tr>
<tr>
<td>Tannins</td>
<td>++</td>
</tr>
<tr>
<td>Triterpenes</td>
<td>++</td>
</tr>
<tr>
<td>Saponins</td>
<td>++</td>
</tr>
</tbody>
</table>

Results of testosterone serum concentration in control showed significant ($P < 0.05$) increased by 11.2% and 42.0% after 2 and 4 weeks respectively. In rats treated with *Trigonella foenum-graecum* seeds extract the serum testosterone decrease by 12.6% after 2 weeks of treatment and by 7.9% after 4 week of treatment (table 2).

<table>
<thead>
<tr>
<th>Duration</th>
<th>Group</th>
<th>Testosterone (ng\dL) Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zero time</td>
<td>Control</td>
<td>130.49 ± 68.52</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>215.45 ± 117.28</td>
</tr>
<tr>
<td>2 weeks</td>
<td>Control</td>
<td>145.15 ± 93.10</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>188.32 ± 156.26</td>
</tr>
<tr>
<td>4 weeks</td>
<td>Control</td>
<td>185.52 ± 135.01</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>198.34 ± 99.53</td>
</tr>
</tbody>
</table>

The cocks semen analysis results show that the colour of semen in both control and treated cocks were white and the consistency were varied from creamy to creamy – milky in control and in treated. There is slight change in semen volume in treated compared with control cocks, semen volume in the control decreased by 47.7% after 2 weeks and by 54.5% after 4 weeks. In the cocks treated with *Trigonella foenum-graecum* seeds extract the semen volume decrease by 10.7% after 2 weeks and by 35.7% after 4 weeks of treatment as shown in table 3.

<table>
<thead>
<tr>
<th>Duration</th>
<th>Group</th>
<th>Colour</th>
<th>Consistency</th>
<th>Volume (Mean ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zero time</td>
<td>Control</td>
<td>White</td>
<td>Creamy</td>
<td>0.44 ± 0.44</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>White</td>
<td>Creamy</td>
<td>0.28 ± 0.10</td>
</tr>
<tr>
<td>2 weeks</td>
<td>Control</td>
<td>White</td>
<td>Creamy – Milky</td>
<td>0.23 ± 0.14</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>White</td>
<td>Creamy – Milky</td>
<td>0.25 ± 0.08</td>
</tr>
<tr>
<td>4 weeks</td>
<td>Control</td>
<td>White</td>
<td>Creamy – Milky</td>
<td>0.20 ± 0.15</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>White</td>
<td>Creamy</td>
<td>0.18 ± 0.17</td>
</tr>
</tbody>
</table>

Results show that in the control the sperm concentration decreased by 4.5% after 2 weeks and by 1.2% after 4 weeks. In cocks treated with *Trigonella foenum-graecum* seeds extract the sperm concentration increased by 8.3% after 2 weeks and decreased by 12.9% after 4 weeks of treatment. Results show that in the treated cocks *Trigonella foenum-graecum* seeds extract decreased the mass motility of the sperm by 18.7% after 4 weeks and the individual motility of the sperms by 4.3% after 2 weeks of treatment and by 9.7% after 4 weeks of treatment. In treated cocks *Trigonella foenum-graecum* seeds extract increase the abnormal sperm percentage by 10.4% after 2 weeks and by 31.5% after 4 weeks of treatment as shown in table 4.
Table 4. Sperm evaluation in control cocks and treated with *Trigonella foenum-graecum* seeds ethanolic extract (F) (1g/kg/day orally)

<table>
<thead>
<tr>
<th>Duration</th>
<th>Group</th>
<th>Mass motility 1–5</th>
<th>Individual motility %</th>
<th>Dead and abnormal %</th>
<th>Sperm concentration×10^6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zero time</td>
<td>Control</td>
<td>2.50±0.55</td>
<td>78.33±11.26</td>
<td>3.33±0.52</td>
<td>9.05±1.40</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>2.67±0.82</td>
<td>77.50±9.35</td>
<td>3.17±0.98</td>
<td>6.77±1.96</td>
</tr>
<tr>
<td>2 weeks</td>
<td>Control</td>
<td>2.50±1.23</td>
<td>76.67±18.35</td>
<td>3.17±0.75</td>
<td>8.64±1.81</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>2.67±0.52</td>
<td>74.17±8.61</td>
<td>3.50±1.38</td>
<td>7.33±4.91</td>
</tr>
<tr>
<td>4 weeks</td>
<td>Control</td>
<td>2.50±0.84</td>
<td>79.17±12.01</td>
<td>3.00±0.63</td>
<td>8.94±1.78</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>2.17±1.17</td>
<td>70.00±20.74</td>
<td>4.17±1.33</td>
<td>5.90±1.14</td>
</tr>
</tbody>
</table>

Values are expressed as Mean ± SD.

* *P* < 0.05.

Histopathologically the seminiferous tubules of control rat’s testes active spermatogenesis (Figure 1) compared to those of treated rats which were inactive with presence of oedema between the tubules (Figure 2).

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**Discussion**

Phytochemical screening of the plants is extremely valuable in giving us information about the nature of constituents found in each plant sample. It was felt necessary to correlate between the nature of chemical constituents and the biological activity. The studies of Sharma RD\(^1\) showed that chemical analysis of *Trigonella foenum-graecum* seeds identify the presence of steroidal oestrogen like saponins, this may explain the negative effect of *Trigonella foenum-graecum* seeds ethanolic extract on serum testosteron and semen quality.
Steroids are modified triterpenes, so that the triterpenes in the extract may be involved in the biological activity of the extract in this study. The primary function of testosterone is the maintenance of spermatogenesis and hence fertility also testosterone is important in the maturation of spermatozoa as they pass through the epididymes and vas deferens. A low testosterone level is one of the best indicators of hypogonadism of hypothalamic or pituitary origin, although very high levels of testosterone suppress spermatogenesis\cite{12}. Esko Veraja’nkovaa and co-workers\cite{7} had reported that 12.6\% of male infertile patients have hypotestosteronaemia.

The results in the study indicate that Trigonella foenum-graecum seeds extract decreased insignificantly (p>0.05) the serum testosterone concentration in the treated rats. Amira et al.\cite{1} study revealed that seeds of Trigonella foenum-graecum decrease plasma androgen concentration. This is agreed with our present study. Semen analysis represents the initial test for evaluating male fertility Andea M Isidori, et al\cite{3}. Semen colour can be white or yellow (normal). Yellow semen may be a result of a diet rich in sulfur (these include garlic, onions, legumes, grains, nuts, and asparagus). Yellow semen is also common in older males. Brown semen probably indicates an infection or old blood in the urethra, while red semen indicates infection, trauma, or another medical complaint. All of the semen samples that collected during this study showed a white colour.

Semen consistency can be either creamy or milky (normal), watery semen occur in case of retrograde ejaculation. There is no incidence of watery semen during semen collection in this study. Semen volume must be taken into consideration because low volumes may be associated with incomplete collection, retrograde ejaculation, ejaculatory duct obstruction, or androgen deficiency (Stephen et al\cite{9}). Compared with control there is a mild decrease in semen volume in cocks treated with Trigonella foenum-graecum seeds ethanolic extract.

In the present study we found that Trigonella foenum-graecum seeds extract decreased the sperm concentration in cocks (insignificant) this may be due to the decrease in testosterone that produced by the extract, or may be due to the fact that Trigonella foenum-graecum increases the prolactin levels and higher levels of prolactin tends to inhibit production of GnRH leading to decrease in both FSH and LH and thereby decrease spermatogenesis and decrease testosterone level. This agreed with Amira et al\cite{1} study which reported that feeding with Trigonella foenum-graecum seeds significantly decrease the sperm concentration.

Sperm motility is the most important measure of semen quality and can be a compensatory factor in male with low sperm counts. It is usually rated in two ways: the number of motile sperm as a percentage of the total, and the quality of forward progressive sperm movement i.e., how fast and how straight the sperm swims. Trigonella foenum-graecum seeds extract decreased the mass and individual motility of the sperm, although both statistically insignificant (p>0.05) this effect may be due to concurrent decrease in testosterone and sperm concentration that produced by this extract. These results confirm the potential antifertility effects (in male) that mentioned by previous study of Amira et al.\cite{3}, which reported that high concentrations of fenugreek in the diet significantly reduces fertility of both male and female rabbits and reduced testis weight, with evident damage to the seminiferous tubules and interstitial tissues.

The results of this study showed that Trigonella foenum-graecum seeds extract increased the abnormal sperm percentage, although this effect statistically insignificant but may contribute to the potential antifertility effects of this extract. Histological examination of the rat’s testes treated with Trigonella foenum-graecum seeds ethanolic extract (Figure 2) showed inactivity of some tubules and the oedema between them. This effect may also contribute to the potential antifertility effects of this extract.

**Conclusions**

Trigonella foenum-graecum tend to reduce the male fertility by reducing testosterone concentration, sperms concentration and inhibiting mass and individual motility of the sperms.

**References**

4. Bilal Bin-Hafiez et al. Immunomodulatory effects of fenugreek (Trigonella foenum graecum L.) extract in mice...

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