Effect of Trigonella Foenum-Graecum (Foenugreek) oil extract on some parameters of sperms and in vitro fertilization in mice

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تاثير المستخلص الزيتي لبذور نبات الحلبة على بعض معايير النطف والخصاب خارج الجسم في الفئران

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الخلاصة:

هدف من هذا البحث ، دراسة تأثير المستخلص الزيتي لنبات الحلبة على وزن الجسم وزن الخص وبعض معايير النطف في ذكور الفئران ، وعلى الاخصاب الخارجي ومرافئ تطور الجنين في الأثاث. استخدم للدراسة 15 ذكر واثنتي بالغة باعمر (8-9 أسابيع) من الفئران قسمت إلى ثلاثة مجموعات ، كل مجموعة تتضمن 5 فئران ، عُموْلت مجموعتان بتركيز 2 و 4 ملغم من المستخلص الزيتي لنبات الحلبة /كمغم من وزن الجسم عن طريق الحقن داخل الخيل البروتوني. عُموْلت المجموعة الثالثة بالمحلول المائي الفسيولوجي كمجموعة سيطرة. قُتلت الحيوانات (الذكور والاناث) بعد 35 يوم من المعاملة عن طريق فصل الرقبة. عُزلت النطف من ذيل البرخ وتم إجراء الفحوصات التالية، حركة النطف، نسبة النطف الحية والميتة وتشوهات النطف. وجمع المصلى لإجراء تحليل هرمون الشحمون الخصوي وكذلك تم عزل الليمفوس من قناة البويضة لاثاث الفئران بعد سحقها في ماء و㎡كلواقع من الوسط الزرعوي النسيجي المحور 119 وجمع المصلى لإجراء تحليل الهرمون المحفز لنمو الجريبات والهرمون اللوتيني. حضنت النطف والبويضات الناضجة في الوسط الزرعوي النسيجي المحور 119 لغرض التلقيح. وتمت متابعة تطورات الجنين بعد 24 ساعة من التلقيح. أظهرت النتائج وجود زيادة معنوية في وزن الجسم الخصوصي لمجموعة الحيوانات المعالمة بالمستخلص وزيادة معنوية في حركة النطف وانخفاض في نسبة النطف.
Abstract:
This project, aims to study the effect of *Trigonella* seeds oil extract on body weight, testes weight and some parameter of reproductive system in male mice, and on *In vitro* fertilization (IVF), cleavage stages of embryos in females. Fifteen adult (8-9 weeks) males and females mice were divided into three groups, each group containing 5 mice, first and second groups were treated with (2 and 4 mg/kg body weight) of *Trigonella* oil extract (intraperitonally) while the third group treated with normal saline as control group. After 35 days of treatment with *Trigonella* the animals (male and female) were sacrificed by dislocation of cervical vertebral. Isolated sperms from tail of epidydymis were used for the following tests. Sperm motility, dead / live sperm, sperm abnormalities and serum ws collected to assay testosterone. Isolatation of ova from oviduct after micing in 500µl of TCM-199 media serum blood to assay FSH and LH. Sperm and matured ova were incubated in TCM-199 media for insemination. The results showed increase in body weight and testes weight in groups treated with *Trigonella*. significant increase in sperm activity and decrease in percentage of dead sperm and abnormalities in groups treated with *Trigonella* oil. The male testosterone and female FSH, LH increased significantly in groups treated with *Trigonella*. IVF study showed significant increase in percentage of maturation, fertilization and cleavage (2-4 cells).
Key words: *Trigonella*, oil, sperms, *in vitro* fertilization.

Introduction
The name of the plant fenugreek comes from *Foenum-graecum*, meaning Greek Hay, the plant being used to scent inferior hay. The name of the genus, *Trigonella*, is derived from the old Greek name, denoting three-angled, and the form of its corolla. The seeds of Fenugreek have been used medicinally all through the ages and were held in high repute among the Egyptians, Greeks and Romans for medicinal and culinary purposes [1]. Fenugreek is an erect annual herb, growing about 2
feet high, seeds are brownish, about 1/8 inch long, oblong, rhomboidal, with a deep furrow dividing them into two unequal lobes. They are contained, ten to twenty together, in long, narrow, sickle-like pods [2].

Leaves and seeds of the plants have been used for centuries to prepare extracts and powders for medicinal use. In ancient Rome, fenugreek was thought to be used to aid labor and delivery. It was used as incense and in the embalming of mummies in ancient Egypt. In traditional Chinese medicine, fenugreek seeds were used to treat weakness and oedema of the legs[3]. In India, fenugreek is used as a lactation stimulant. Many other medicinal uses of the plant (e.g., the treatment of baldness and indigestion) have been reported. The preliminary results of human and animal studies suggest that oral fenugreek seeds have hypoglycemic and antihyperlipidemic properties [3]. Fenugreek has very important roles in improving fertility due to the presence of diosgenin that considered precursor for synthesis of sex hormone [4 , 5, 6]. *In vitro* fertilization (IVF) has been frequently used for treatment of sterility in humans', this project, aimed to study the effects of *Trigonella* oil extract on some reproductive parameters and on *In vitro* fertilization IVF in mice.

**Materials and Methods:**

**Preparing of *Trigonella* seeds oil extract**

Dry seeds of *Trigonella* were purchased from local markets and identified by the Iraqi national herbarium, the seeds were cleaned, dried and finally powdered. The *Trigonella* oil was extracted by using petroleum ether, dosage of *Trigonella* oil used was 2 and 4 gm/kg B.W[7].

**Laboratory animals and experimental design**

Adult male mice (30-36 gm) were purchased from Biotechnology Research Center and maintained on a 14:10-hour light dark cycle in the animal house control and treated mice were provided with feed and water ad libitum; there were no differences in feed intake. Males were randomly divided into 3 groups, each composed of 5 mice. First and second groups were treated with (2, 4 gm/kg B.W) respectively of *Trigonella* oil intraperitoneally while administered for 35 days and the third group was given normal saline as a control group. The animals in each group were sacrificed by dislocation of cervical vertebrate. Sperms were obtained from the two tails of epididymides by mincing in 500 µl
TCM-199, and maintained at 37°C in 5% CO2 incubator. Sperms maintained prior treatment to capacitation. (sperms motility, percentage of dead/live sperms and abnormalities of sperm were recorded).
As well as, in females the ovaries and oviduct were removed and placed in a sterile disposable petridish containing 1ml TCM-199 medium, then the oviducts were isolated, ova were obtained from the two oviduct by mincing in 500 µl of TCM-199 media, and maintained at 37°C in 5% CO2 incubator.

**Reproductive hormones assay**
Serum hormones (Testosterone, FSH, LH) concentrations were evaluated with a Bio merieux Italia S.P. a vidia campigliano, 58 50015-point A EMA (F1) Italia miniVIDAS. following the manufacturer's recommendations.

**Microscopic examination**
Sperms parameters were assessed according to WHO Laboratory manual [8] for Motility, percentage of dead/live and abnormalities sperms. Ova were examined to isolate matured ova by obtained first polar body.

*In vitro fertilization (IVF):*

Sperm and matured ova were co-incubated in TCM-media-199 having (20mg/ml B.S.A. and heparin) for insemination. Ova were observed for cleavage after 24h.of inseminate under phase contrast microscope [9].

**Statistical Evaluation**
Data were analyzed by one-way analysis of variance (ANOVA- test). Data are presented as means ± SE. The level of significance was $P < .05$. [10].

**Results and Discussion**
Body and testes weights were increased in mice treated with *Trigonella* oil extract as compared with control group (table 1). The results indicated that treatment with *Trigonella* oil extract may caused increase the appetite to feed, which lead to increase body weight [11,12].
Table (1): Effect of Trigonella oil extract on body weight and testes weight

<table>
<thead>
<tr>
<th>Treatment groups</th>
<th>Final Body Weight (gm±SE)</th>
<th>Testes weight (mg/100gm±SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>33.98±1.91</td>
<td>0.20±0.006</td>
</tr>
<tr>
<td>Trigonella (2gm/kg B.W)</td>
<td>39.81±1.06</td>
<td>0.36±0.098</td>
</tr>
<tr>
<td>Trigonella (4gm/kg B.W)</td>
<td>38.44±0.89</td>
<td>0.34±1.020</td>
</tr>
</tbody>
</table>

n=(5) numbers of animals in each group

Several studies suggested that Trigonella extract can affect the metabolism of animals treated with it due to the principle materials necessary in maintaining metabolism, such as amino acids and protein which are found in Trigonella [13,14,15,16]. Significant differences in mean testes weight in mice treated with Trigonella extract was observed when compared with control mice. This may be due to water accumulation in testes tissue, coumarine found in Trigonella is one of the compounds considered to be responsible [17]. Table (1).

The results obtained pointed significant increase in percentage of sperm activity and significant decrease in percentage of dead sperm and abnormalities of sperm (77.71±1.99, 80.6±1.05) (23.21±2.02, 21.11±2.06) (25.55±0.98, 23.87±2.91) respectively in treated mice as compared with the control mice (65.87±2.05, 35.6±1.43, 33.3±3.2) respectively (Table 2 and Fig1).

Table (2): Effect of Trigonella oil extract on sperm motility, dead sperm and abnormalities of sperm %

<table>
<thead>
<tr>
<th>Treatment groups</th>
<th>Sperm motility % (µ+SE)</th>
<th>Dead sperm % (µ+SE)</th>
<th>Abnormalities sperm (µ+SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>A 65.87±2.05</td>
<td>A 35.6±1.43</td>
<td>A 33.34±3.2</td>
</tr>
<tr>
<td>Trigonella 2gm/kg B.W</td>
<td>B 77.71±1.99</td>
<td>B 23.21±2.02</td>
<td>B 25.55±0.98</td>
</tr>
<tr>
<td>Trigonella 4gm/kg B.W</td>
<td>B 80.6±1.05</td>
<td>B 21.11±2.06</td>
<td>B 23.87±2.91</td>
</tr>
</tbody>
</table>

Differences A, B, C are significant (P<0.05) to compared rows
Figure (1): Sperm morphology, showing: normal sperm (A), sperm hummer head (B), sperm curve tail (C) (40X)

Clemont, [18] demonstrated that spermatogenesis is regulated by the accurately coordinated expression of many genes. Disruption of this process can be brought about by mutations of many genes leading to abnormalities.

The increase observed in mitotic index MI of mouse testis may be returned to the Trigonella extract which may have mitogenic effects through the presence of active compounds that act as or stimulate the division process without addition of any mitogenic agents [19]. These plants extracts may improve animal fertility and it can cross testis barriers. Decreasing in the abnormalities of sperms (head and tail) especially after 35 days of treatment with trigonella extracts occur when the sperms are in the spermatogonia stage and before mitotic division which represent a source of sperms, and this will be in agreement with AL-Rubia, [20] and AL-Rubia, [21] who clarified that Trigonella extract does not contain any mutagenic agents. As we know, these mutagenic agents are responsible for abnormalities in sperms head and tail [22] compounds found in Trigonella extract may play a role in antioxidant protection against sperm damage [23]. This test represents a more sensitive test for detection of the mutagenic compounds. On the other hands trigonella extracts has protective effectiveness in germ stem cells (spermatogonia) which act as a source of all sperms [24].

Significant increase in levels of hormones (male testosterone and female FSH, LH) in treated mice also was noticed as compared with control mice (table 3).
Table (3): Effect of *Trigonella* extract on reproduction hormones (male testosterone, female FSH and LH) after 35 days treatment in mice

<table>
<thead>
<tr>
<th>Treatment groups</th>
<th>Male testosterone ng/ml (µ+SE)</th>
<th>Female FSH IU/ml(µ+SE)</th>
<th>Female LH IU/ml(µ+SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>A 0.97±0.29</td>
<td>A 0.34±0.87</td>
<td>A 0.28±0.06</td>
</tr>
<tr>
<td>Trigonella 2gm/kg B.W</td>
<td>B 1.88±1.03</td>
<td>B 0.51±0.92</td>
<td>B 0.43±0.95</td>
</tr>
<tr>
<td>Trigonella 4gm/kg B.W</td>
<td>C 2.02±1.21</td>
<td>C 0.82±1.02</td>
<td>C 0.94±0.93</td>
</tr>
</tbody>
</table>

Differences A, B, C are significant (P<0.05) to compared rows

This increase may be due to diosgenin found in *trigonella* extract which have important role in synthesis sex hormones which have very important role in reproduction [25,26].

The male reproductive function is under hormonal control, spermatogonic process is under control of follicular stimulating hormone (FSH) and testosterone [27], while the formation of type A spermatogonia and conversion of primary spermatocyte into secondary spermatocyte (Meiosis I) are testosterone dependant and the final step of maturation of spermatids is dependant on FSH [28].

Table 4 shows significant increase in mean number of mature ova, fertilized ova and ova cleavage (2-4 cells). Fig.2

Table (4): Effect of *Trigonella* extract on *in vitro* fertilization (% incubated ova, maturated ova, fertilized ova cleavage ova (2-4 cells) after 35 days treatment in mice

<table>
<thead>
<tr>
<th>Treatment groups</th>
<th>% of incubated ova</th>
<th>% of maturated ova</th>
<th>% of fertilized ova</th>
<th>% ova cleavage (2-4 cells)</th>
<th>% ova cleavage (4 cells)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>38</td>
<td>57.84</td>
<td>54.54</td>
<td>58.31</td>
<td>42.84</td>
</tr>
<tr>
<td>Trigonella 2gm/kg B.W</td>
<td>29</td>
<td>72.41</td>
<td>61.91</td>
<td>69.2</td>
<td>55.54</td>
</tr>
<tr>
<td>Trigonella 4gm/kg B.W</td>
<td>50</td>
<td>76.00</td>
<td>63.14</td>
<td>70.81</td>
<td>58.8</td>
</tr>
</tbody>
</table>
The seed of Fenugreek contains alkaloids, Colin, bitter material, fatty acid, minerals, protein and vitamin C, which are excellent remedies for dysorexia and weakness resulting from emaciation. It improves the appetite, it is a general health-restorer, it has a fattening effect, increases the number of red blood cells and restores the strength of a physically strained body. It is rich in phosphorus, organic iron and carbohydrates [29, 30]. Whole fenugreek seeds contain 48% total fibre, which includes 20% gum and 28% neutral detergent fibre (NDF) and about 4% of saponins [31]. Further, fenugreek seeds are reported to contain high levels of flavonoids (>100 mg/100 g) [32]. It could be possible that saponins and flavonoids present in fenugreek seeds enhance the
antioxidant potential and thus are responsible for the reduction in lipid peroxidation in tissues[32].

As we know herbs active compounds including flavonoids, minerals and vit.C exhibit strong healing effects on reproductive organs by increasing vitality and circulation and supporting the hormonal system by nourishing the endocrine gland, steroied compound found trigonella can help in regulating estrogen / progesterone ratio.[29]

References


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