A COMPARATIVE STUDY ON THE EFFECTS OF THE FENUGREEK SEEDS' POWDER AND ITS AQUEOUS AND OIL EXTRACTS ON THE MALE REPRODUCTIVE SYSTEM IN ALBINO RATS

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Trigonella foenum-graecum has long been used as a medicinal plant for the treatment of nutritional, metabolic and sexual dysfunction in both genders. Objectives: Our study aimed at evaluating the possible effects of different dosage forms of fenugreek seeds on the male reproductive system in animals. Methods: In a randomized controlled study, 40 male albino rats weighing 180 to 260 gm were equally divided into four groups, one control and the other three groups were treated by the administration of either powder (200 mg/kg), aqueous (500 mg/ml) or oily extract (200 mg/ml) forms, 3 times weekly for 8 weeks. Serum luteinizing hormone, follicle-stimulating hormone, prolactin, estrogen, progesterone, and testosterone levels were evaluated, as well as histological examination & sperm analysis. Results: Concerning the oily extract dosage form, a highly significant decrease (P< 0.01) in FSH was recorded in comparison to other groups. LH was reduced significantly (P< 0.05) in the three treated comparative groups. However, progesterone and estrogen levels were significantly increased (P<0.05) after the administration of the oily form. Testosterone level was detected higher only in the aqueous form with a significant increase (p< 0.05) in sperm count, unlike the other 2 forms. The results have revealed a significant (p< 0.05) decrease in all sperm evaluated parameters as well as destructions in testicular tissues after the administration of the oily form. Conclusion: The effect of the aqueous form on the male hormonal levels have been significantly noticed with remarkable changes in the sperm vitality as well the sperm count. The oily form showed a devastating action on all the evaluated parameters.

INTRODUCTION

Fenugreek is one of the oldest medicinal plants used for its medicinal benefits around the world4. Fenugreek or Trigonella foenum-graecum is a fragrant herb that belongs to the family Fabaceae and has been grown extensively in most of the world. However, fenugreek is specially cultivated in the Indian subcontinent, South Asia, the Middle East, some African countries, Mediterranean Europe, China, Australia, the USA, Canada and Argentina2,3. Fenugreek is a clover-like herb in which the most important part is the seeds. Its seeds smell and taste like maple syrup and have been used in industrial, cooking and medicinal products4,5.

Trigonella foenu-graecum has been widely used in folk medicine and have a wide range of pharmacological actions: as an antihypertensive, anti-hypercholesterolemic4.
gastroprotective activity, appetite stimulation, antioxidant action, improving cardiac function in patients with coronary artery diseases, as hormonal replacement therapy in postmenopausal symptoms, and in male sexual dysfunction.

Fenugreek contains a number of active constituents that were used since thousands of years ago in folk medicine. These include mucilage, the alkaloid hormone trigonelline, 4-hydroxyisoleucine, galactomannan, luteolin, sotolon, diosgenin, protodioscin, flavonoids, steroidal saponins, vitamins, minerals, and phenolic acids. Galactomannan is a soluble fiber (carbohydrate) which represents 45-60% of the fenugreek seed's active constituents.

Steroidal saponins (Diosgenin and yamogenin) have been well known for their estrogenic effects such as binding to E2 receptors and persuading of E2 expression genes. In addition, few studies have mentioned the ability of these saponins to positively influence the male sexual function.

In addition, diosgenin is a famous precursor used for more than 60% of the commercially available cortisone, pregnenolone, progesterone, and other steroids. It is mainly used as a stimulator for growth hormone from the pituitary.

Another study evaluating the administration of 600 mg/day of testofen for 12 weeks in men revealed an increase in the number of morning erections and rate of sexual activity. The study has detected an increase in total serum testosterone and free testosterone compared to placebo after 12 weeks of active treatment in healthy middle-aged and elder men.

Accordingly

This study aimed at evaluating the possible effects of fenugreek seeds' different dosage forms (powder, aqueous and oily extracts) on the male reproductive systems using albino rats as experimental animals.
MATERIALS AND METHODS

Animals

Animals used were purchased from the Animal Care House, Faculty of Medicine, Assiut University. The study was approved by the Ethics Board of Beni Suef University. This study was conducted in accordance with the ethical procedures and policies approved by the Animal Care and Use Committee of Faculty of Medicine, Assiut University, Egypt.

Kits

All kits used were produced by Autobio Diagnostic co., LTD No.87 Jingbie Yi Road, China. Kits were used for hormonal assay.

Fenugreek seed powder and its aqueous & oil extracts

Commercial Trigonella seeds were used.

The aqueous extract was prepared using a simple traditional decoction method. The seeds (50gm.) were soaked in 100ml distilled water and allowed to boil for 5 minutes. Then, subjected to a double filtration procedure, to recover the aqueous extract (450mg/ml), which was preserved at 4°C until use.

Fenugreek oil in a concentration of 200 mg/ml, was purchased from Cap Pharm for Extracting Natural Oils & Herbs, Cairo, Egypt.

For the preparation of fenugreek powder, 50 gm. of the seeds were washed with tap water and then air dried under shade. The seeds were then crushed and powdered by electronic grinder and kept at room temperature until used.

Experimental animals and study design

This study was conducted on 40 male albino rats weighing 180 to 260 gm. were used. Animals were housed in stainless cages and kept in suitable environmental conditions of 20 - 25 °C air-conditioned room and daily photoperiod of 12 hours. Food and water were made available throughout the day, ad libitum. Animals were equally divided into four groups, one control and other three treated ones, as follows:

- Group 1 Control group, received distilled water for 8 weeks
- Group 2 Powder treated group, received the powder form of fenugreek seeds (200 mg/Kg body weight, 3 times per week for 8 weeks) added to animals' prepared food.
- Group 3 Aqueous extract treated group, received the aqueous extract (500 mg/ml) form of fenugreek seeds (2 ml, 3 times per week for 8 weeks) orally by a stomach tube.
- Group 4 Oil extract treated group, received the oil extract (200 mg/ml) form of fenugreek seeds (2 ml, 3 times per week for 8 weeks) orally by a stomach tube.

Blood sample collection

At the end of the study period, blood samples were collected from the retro-orbital plexus. Serum was separated by centrifugation at 2500 rpm, for 15 min. at room temperature. The sera were then stored at -18 °C until time of analysis.

Hormonal assay

Estimation of serum luteinizing hormone (LH), follicle-stimulating hormone (FSH), prolactin, estrogen (E2), progesterone (P) and testosterone (T) levels were estimated by using standard assay kits.

Sperm collection and evaluation

At the end of the study, animals were sacrificed under anesthesia and the male reproductive organs were carefully dissected out

Sperm count (sperms/ ml)

The testis and epididymis excised from the rats were minced into 20 ml of 0.9% NaCl. The cell count was carried out directly by a microscope counting, using a Malassez cell and was expressed in million/ml of the sperm suspension.

Sperm Motility

The sperm progressive motility (SPM) was estimated by evaluating 4 fields of sperm droplet under a cover-slip on a warm glass slide under light microscopy (×40).

% Sperm vitality

For the assessment of live and dead sperms, Chemineau et al method was used. By mixing one drop of sperm suspension with one drop of Eosin-Nigrasin stain on a glass slide. Live sperms repel the vital stain (Eosin-Nigrasin) while the dead sperms absorb the dye.
Sperms vitality was calculated by using the following equation:
Percentage of dead sperms = (No. of live sperms / Total No. of Sperms) × 100.

**Histological studies on testis**
At the end of the study, animals were sacrificed and the male reproductive organs were carefully dissected out.

**Light microscopic examination**
Testes specimens from all groups were taken, and fixed in bouin for 16-18 hrs., washed by 70% alcohol, dehydrated by ascending grades of alcohols and embedded in paraffin, sectioned at (5μm) thickness by a microtome and stained with the following stains: general H & E stain for general histological examination.

**Statistical analysis**
The statistical package (SPSS) program, version 23 was used for data analysis. Data were expressed as Mean ± SEM. Data were analyzed by ANOVA, and confidence limit was accepted at 95% and 99% interval.

**RESULTS AND DISCUSSION**

**Results**
**Effects of fenugreek seeds different dosage forms (Powder, aqueous and oil extracts) on hormonal levels**
Comparison regarding hormonal levels in the three different dosage forms of fenugreek namely; powder, aqueous and oily forms has revealed a significant decrease of FSH hormone by 34.5% in the oily dosage form, followed by a slightly comparable reduction in both the aqueous and powder forms in comparison with the control group by 24.2% and 16.2%; respectively. LH hormonal levels were reduced significantly in the three groups. Progesterone level was remarkably elevated (p < 0.001) in the oily form by 222%, followed by the powder form with a 140% elevation and finally, a 129% elevation in the aqueous form compared to the control group. Similarly, E2 was elevated in the oily treated group when compared to the control group with high significant increase (p < 0.001). On the contrary, the powder form has reduced the release of E2 by 10% in comparison to the control. Testosterone levels were detected higher solely in the aqueous form by 34.5% (p < 0.001) unlike the other 2 forms. In addition, no significant change was detected in prolactin level in all groups compared to the control group as shown in table (1).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>Fenugreek powder</th>
<th>Fenugreek aqueous extract</th>
<th>Fenugreek oil extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>FSH (ng/ml)</td>
<td>2.9 ± 0.1</td>
<td>2.4 ± 0.08</td>
<td>2.6 ± 0.15</td>
<td>1.9±0.14**</td>
</tr>
<tr>
<td>% of change</td>
<td></td>
<td>-16.2%</td>
<td>-13.8%</td>
<td>-34.5%</td>
</tr>
<tr>
<td>LH (ng/ml)</td>
<td>1.7 ± 0.1</td>
<td>1.45 ± 0.1*</td>
<td>1.3 ± 0.12*</td>
<td>1.4±0.12*</td>
</tr>
<tr>
<td>% of change</td>
<td></td>
<td>-14.7%</td>
<td>-23.5%</td>
<td>-18%</td>
</tr>
<tr>
<td>Progesterone (ng/ml)</td>
<td>2.7± 0.4</td>
<td>3.8 ± 0.18*</td>
<td>3.5 ± 0.3*</td>
<td>6±0.5**</td>
</tr>
<tr>
<td>% of change</td>
<td></td>
<td>+140%</td>
<td>+129%</td>
<td>+222%</td>
</tr>
<tr>
<td>E2 (pg/ml)</td>
<td>20 ± 1.1</td>
<td>18 ± 0.67</td>
<td>20.8 ± 1.47</td>
<td>30 ± 2.5**</td>
</tr>
<tr>
<td>% of change</td>
<td></td>
<td>- 10%</td>
<td>+ 4%</td>
<td>+ 50%</td>
</tr>
<tr>
<td>Prolactin (μIU/ml)</td>
<td>2.1 ± 0.15</td>
<td>1.9 ± 0.1</td>
<td>1.9 ± 0.05</td>
<td>2.0 ± 0.1</td>
</tr>
<tr>
<td>% of change</td>
<td></td>
<td>-9.5%</td>
<td>-9.5%</td>
<td>-4.7%</td>
</tr>
<tr>
<td>Testosterone (μu /dl)</td>
<td>3.2 ± 0.18</td>
<td>2.3 ± 0.2*</td>
<td>4.3 ± 0.3**</td>
<td>2.1 ± 0.2**</td>
</tr>
<tr>
<td>% of change</td>
<td></td>
<td>-29.1%</td>
<td>+ 34.5%</td>
<td>-34.4%</td>
</tr>
</tbody>
</table>

Values represent mean ± SE (standard error). (p**< 0.01, p*< 0.05, as compared to control group). E2: Estogen, FSH: Follicle Stimulating hormone, LH: Luteinizing hormone.
Histopathological changes in testicles regarding the 3 comparative groups

Group I: Control one

![Fig. 1](image1.jpg)

Fig. 1: Histological appearance of normal seminiferous tubules in the control group

Light microscopic examination demonstrated that the control albino rats' testes formed of groups of seminiferous tubules having regular rounded or oval shape, and surrounded by regular basement membrane. Each tubule was lined by germinal epithelium at different stages of development and supporting Sertoli cells, (Fig. 1.a)

All the cells of the spermatogenic series such as spermatogonia, spermatocyte, spermatids and spermatozoa, and sertoli cells could be identified in the tubules. The lumen could easily be delineated in almost all the tubules and majority of them was occupied by mature spermatozoa, (Fig 1.b).

In between the tubules, the interstitial tissue contains blood vessels and interstitial cells of Leydig. Either arranged single or in clumps, the cells appear polyhedral in shape with large rounded or oval vesicular nuclei and pale finely vacuolated cytoplasm (Fig. 1.c).

In group (I), examination of Masson trichrome stained sections revealed collagen fibers with normal distribution in the capsule. Few collagen fiber depositions in the seminiferous tubules basement membranes and around blood vessels walls, (Fig. 1.d).

Group II & III: powder and aqueous treated groups : (Fig. 2 & 3)

In these groups, the majority of seminiferous tubules appear more or less regular in outline. They showed nearly normal architecture with the appearance of the germinal cell population including different types of germ cell (Fig. 2.a) & (Fig. 3.a). But some tubules showed some degenerative effects in the form of irregularity of the basement membrane, multiple empty spaces, residual cytoplasmic droplets and few degenerated cells [Fig. 2.(b & c)] & [Fig. 3.(b & c)].

Regarding the interstitial tissue, it appeared nearly similar to the control group except for the presence of some Leydig cells that declare some abnormalities in the form of irregular nucleus with some areas of chromatin condensation, [Fig. 2.(b & c)] & [Fig. 3.(b & c)].

In Masson trichrome stain, group (II) & (III) showed thick collagen fiber distributions in the capsule. Also, the distribution of collagen fibers around the blood vessels and in the basement membrane of seminiferous tubules were slightly thickened than normal (Fig. 2.d) & (Fig. 3.d).
Group IV: Oil extract treated group (Fig. 1)

In comparison to the control group, light microscopic examination of the testes revealed severe degeneration and disruption of the integrity of the tubules. Many tubules revealed multiple vacuolation and their germinal epithelium appeared dissociated from each other. The basement membrane is severely interrupted at multiple areas. Sertoli cells are completely destructed and replaced by swollen degenerated apoptotic cells. There are no noticeable mature sperms within many tubules. Some tubules appear severely atrophied and only occupied by degenerated destructed cells (Fig. 4.a).

The interstitial tissue examination exhibited obvious changes, it showed thickened dilated blood vessel. Most of the Leydig cells are destructed leaving multiple vacuoles clearly seen within most of the sections, (Fig. 4.b&c).

In group 4, the sections stained with Masson trichrome stain revealed dense collagen fibers deposition in the capsule and around blood vessels, as well as the basement membrane of most seminiferous tubules (Fig. 4.d).
Fig. 4: Histological changes in seminiferous tubules in fenugreek seeds oil extract treated group

Effects of fenugreek seeds different dosage forms on sperm parameters

Sperm count

As shown in table (2), the results obtained, revealed a significant (p< 0.05) decrease in the sperm count in both the oil extract and powder treated groups by (41.7% & 33.3% respectively), while a significant increase (p< 0.05) by 25% was detected in the aqueous treated group as compared to the control one.

Sperms viability (%)

The effects of fenugreek different dosage forms (powder, aqueous and oil extracts) on the percentage of live sperms have been pointed in Table (3) & Fig. 5. There was a significant decrease (p< 0.05) in the percentages of live sperms in both, the oil extract and powder treated groups as compared to the control one. Whereas, no significant change was detected with the aqueous extract treated group as compared to the control group.

Table 2: Sperms count in the control and treated groups (N = 10)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>Fenugreek powder</th>
<th>Fenugreek aqueous extract</th>
<th>Fenugreek oil</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sperm count*10^6/ml</td>
<td>60</td>
<td>40</td>
<td>75</td>
<td>35</td>
</tr>
<tr>
<td>% of change</td>
<td></td>
<td>-33.3%</td>
<td>+25%</td>
<td>-41.7%</td>
</tr>
</tbody>
</table>

Values represent (sperm/ml)

Table (3): Sperms vitality in the control and treated groups (N=10)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>Fenugreek powder</th>
<th>Fenugreek aqueous extract</th>
<th>Fenugreek oil</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sperm vitality</td>
<td>75.3±2.1</td>
<td>67.1±2.4*</td>
<td>76.4±1.7</td>
<td>43.7±1.4**</td>
</tr>
<tr>
<td>(No. of live sperm)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values represent mean ±SE (stander error). (p**<0.01, p*<0.05, as compared to control group).
Fig. 5: Percentage of Live spermatozoa after administration of fenugreek seeds different forms (powder, aqueous & oil extracts) after 60 days treatment.

**Sperm motility**

As shown in Fig. 6: administration of fenugreek seeds aqueous extract and powder did not affect sperm motility in treated animals compared to the control group. While the percentage of spermatozoa motility was significantly decreased (p < 0.05) in the oil extract treated animals.

Fig. 6: Effects of the (powder, aqueous, and oil extracts) of fenugreek seeds on the mobility of spermatozoa after a 60 days treatment.

**Discussion**

Trigonella foenu-graecum has been widely used in folk medicine as an antihypertensive, anti-hypercholesterolemic, improving cardiac function in patients with coronary artery diseases, as hormonal replacement therapy in postmenopausal symptoms, and in male sexual dysfunction (4,11,18).

In the first part of our study, we have examined the effect of fenugreek seeds different dosage forms (powder, aqueous and oil extracts) on sex hormonal levels in male rats.

Changes in the hormonal levels, showed a highly significant reduction of FSH level with the oily form treated group compared to the other comparative groups. While a significant reduction in LH hormone was clearly observed with all treated groups compared to the control one. This significant reduction in both FSH & LH levels was accompanied with observed significant reduction in serum testosterone levels in rats administered either fenugreek seeds' powder or oily form compared to the control group.

In the year 2005, Grover et al. 23 revealed the importance of both FSH and LH hormones in the maturation of spermatozoa and testosterone production. LH stimulates testosterone production from the interstitial cells of the testes (Leydig cells). While, FSH stimulates testicular growth and enhances the production of an androgen-binding protein by the Sertoli cells, which are a component of the testicular tubule necessary for sustaining the maturation of sperm cells. This protein causes high local concentrations of testosterone near the sperm, an essential factor in the development of normal spermatogenesis. Also, Sertoli cells secrete inhibin, a polypeptide, under the influence of androgens, which may help to locally regulate spermatogenesis 23. Testosterone is the principle male hormone; it is synthesized by Leydig cells of testes from cholesterol 24.

This decrement of testosterone level may be due to the effects of saponin on serum cholesterol level, which is essential for testosterone synthesis by its action on the Leydig cells. Numerous studies reported that saponin lowers serum cholesterol level and hence affect testosterone production & spermatogenesis 25.

These results agreed with our study findings where the significant reduction in both FSH & LH levels was accompanied with a significant reduction in testosterone level in both powder and oily forms treated groups. In 2006, Kassem and his colleagues 26 revealed
that Trigonella foenum-graecum has decreased the plasma androgen concentration & decreased the sperm count. These results agree with that of the present study that fenugreek decreased the sperm count and this may be due to the presence of steroidal oestrogen-like saponins. In contrast, some animal and human studies introduced Trigonella foenum-graecum extract as a food supplement to boost testosterone.

Although, aqueous extract of fenugreek exert significant reduction in LH level as well as the other forms, but testosterone level & sperm count were significantly increased after the administration of the aqueous form. Therefore more investigations are needed in this field.

Considering the results, the amount of progesterone hormone was increased significantly in all experimental groups with a highly marked increase with the oily form compared to the other experimental groups. Progesterone increase can be ascribed to dioxygenin compounds of fenugreek which act as progesterone precursor.

On the other hand, estrogen level was significantly increased in oily form treated group with insignificant decrease in the aqueous form treated group compared to the control group. Moreover, the present study showed insignificant decrease in prolactin levels in all comparative groups compared to control one. Sperm motility and vitality were both decreased significantly following the administration of the powder & oily forms.

Histological examination in both powder and aqueous dosage forms showed nearly normal appearance of the germinal cell population and tubules. Whereas, the oil form showed severe degeneration and disruption of the integrity of the tubules. Many tubules revealed multiple vacuolation and their germinal epithelium appeared dissociated from each other.

Moreover, sertoli cells were found completely destructed and replaced by swollen degenerated apoptotic cells in the oil dosage form. These results contradict another study that assessed the protective effect of fenugreek seeds extract after the administration of the anticancer drug cyclophosphamide caused by cyclophosphamide.

On the other hand, a study was conducted to assess feeding 30% of fenugreek powder for three months to New Zealand rabbits showed a significant reduction in testicular weight, sperm concentration and plasma androgen levels. The testicular weight and sperm concentration were both found lesser than the control by 25% and 47%, respectively. Moreover, testosterone was lower by 65.8% in comparison to the control animals. Histopathological examination of the testes revealed a decrease in the number of seminiferous tubules along with mild thickening of the basement membrane and mild spermatogenesis hypoplasia in comparison to the control group.

However, the elevation in sperm count in the aqueous extract treated group in the present study is in accordance with another study which was conducted to assess a new fenugreek extract. The study revealed an elevated level of free testosterone in blood up to 46% in 90% of the samples. Sperm count was improved in 85.4% of the study population as well as, sperm morphology with 14.6%.

On the other hand, a trial was conducted to assess the safety and efficacy of the glycoside portion of Trigonella foenum-graecum seeds on muscle anabolism, androgenic hormones, and body fat in healthy male after 8 weeks of treatment, with a resistance training program. Sixty healthy males were randomized for either the administration of a capsule of 300 mg, twice per day or the matching placebo. Serum testosterone (total and free) levels, muscle strength and repetitions to failure, metabolic markers for anabolic activity (serum creatinine and blood urea nitrogen), and % body fat were assessed. The study has concluded a significant anabolic and androgenic activity as compared to the control.

These study results agree with the present study, where sperm count was enhanced with fenugreek extract, particularly, the aqueous extract. In addition, the improvement in testosterone levels in male rats were also
exhibited in the aqueous form only. All these findings, might be due to that the concentration of the steoidal saponins are less in the aqueous medium than that in the oily medium. In such a way, that also might explain that the oil form exhibited a devastating action on sperm motility, vitality, along with the testicular tissues compared to the control and the two other forms.

**Conclusion**

*Trigonella foenum-graecum* seeds showed a significant enhancement in sperm count and male hormonal function, particularly, the aqueous form. While the oil form was much more intense in the damaging effect on both the sperm count, vitality and histological changes in the testicles. Further studies are recommended for assessing the main components responsible for such effects with the proper doses.

**Limitations**

The study was limited to assess the main ingredient involved in such an effect as well as the possible mechanisms justifying the fluctuation in hormonal levels and histological changes between the three different forms.

**REFERENCES**

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Azza Badry, et al.

نشرة العلوم الصيدلية
جامعة أسيوط

دراسة مقارنة عن تأثير بذور نبات الحلبة ومستخلصيها المائي والزيتي على الجهاز التناسلي لذكور الجرذان البيضاء

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المقدمة: يعتبر نبات الحلبة أو Trigonella foenum-graecum المعروف في الطب الشعبي في علاج المضغ نبات جذري نحيل، ومن المعروف أيضاً أن نبات الحلبة غنية بال العديد من المكونات النشطة مثل الصمع، وهرمون فلويد، والجالاكتومان، والديوكسيجين، والبروتوديوسين، والفلوندوتين، والصابونين الستيرويدي. الحلبة عشب شائع في دول البحر الأبيض المتوسط مثل مصر وجنوب أفريقيا.

ولقد تم استخدامه في علاج السرطان، وصناعات الأغذية وكذلك كمضاد للالتهابات، ومضاد للسرطان، ومضاد لمرض السكر ومضاد لفقر الدم.

الأهداف: نفذت هذه الدراسة لقياس التأثيرات المحتملة لخلايا الحلبة المختلفة (مسحوق الحلبة، مستخلصات مائية وزيتية).

الطريقة: تم استخدام 60 ذكر جرد ألينو يتراوح أعمارها بين 180 و 260 جم. تم تقسيم الجرر إلى أربع مجموعات (10 في كل جروبو)، واحدة كمجموعة ضابطة وثلاث مجموعات أخرى تمت معالجتها لإعطائها إماسحوق أو مستخلص مائي أو زيت. تم تقييم هرمونات الدم، البرولاكتين، البروجسترون، الاستروجين، التستوستيرون، الماد هلليستوستيرون، والهرمونات اللوبينية، لتحديد التأثيرات المحتملة لخلايا الحلبة المختلفة على الجهاز التناسلي للذكور باستخدام قطر جوفان البيضاء.

النتائج: انخفض مستويات هرمون FSH في الجرر لخلايا الحلبة مقارنة بالخلايا الأخرى. كما تم ملاحظة

Axes Badry, et al.
عند فحص حيوية الحيوانات المنوية بتقص النشاط بصورة ملحوظة في الفئران التي تناولت المستخلص الزيت، ويليها التي تناولت المسحوق عن تلك التي تناولت الشكل المائي.

الخلاصة: لقد لوحظ تأثير زيت الحلبة على مستويات هرمون الذكورة بشكل ملحوظ وكذلك عدد الحيوانات المنوية بعد تناول الفئران لها. كما أن زيت الحلبة أثبت تقص في حيوية الحيوانات المنوية وزيادة في مدى الضرر الذي لحق بالخصية بعد تناوله. بينما تحسنت أغلب الوظائف بعد تناول المستخلص المائي للحلبة.