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Study The Effect of Tribulus terrestris and Phoenix sylvestris powder on Infertility in Male Rats

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Abstract:

This study aimed to investigate the effect of diets enriched with *Tribulus terrestris and Phoenix sylvestris* powder on subfertility rats. Thirty adult male albino rats, weighting 150 - 160 g used in this study and assigned to two main groups. The first is negative control group (6 rats) was received basal diet throughout the experiment .The second group (24 rats) was injected intrapretonial by cadmium chloride ($CdCl_2$, 0.1%) at 0.1 ml/100g body weight to induced subfertility, and divided into 4 subgroups as following: one kept as positive control group, and the others were received *Tribulus terrestris* , *Phoenix Sylvestris* powder and their mixture by 5% from the basal diet. Body weight gain (BWG), feed intake (F.I), feed efficiency ratio (FER) and Relative weight of tests were calculated. Serum follicle stimulating hormone (FSH) and serum luteinizing hormone (LH) were determined. Also, tests was examined histopathologicaly. BWG, FER, and Testes weight were significantly ($p \leq 0.05$) decreased comparing with positive control, while FI was nonsignificant increase. For FSH and LH hormone there were significantly ($p \leq 0.05$) increases comparing with positive control. Histopathological examinations showed that feeding on diet containing the tested plant parts for 4 weeks induced improvement on the histological structure of the testes.

Key words: Infertility, plant parts, LH, FSH, and histopathological changes .

Introduction

Infertility is defined by the failure to conceive a child after one year or more of regular unprotected sexual intercourse. Infertility affects society at large and has a negative impact on the social and emotional aspects of the patient. Infertility is an important problem, which is also so interested for psychosocial issues. Men are responsible in almost 50% of infertility cases **(Christopher *et al.*, 2017)**. Different causes, such as congenital or acquired urogenital disorders, urinary tract infection, endocrine disorders, systemic diseases, genetic causes, and immunologic and environmental factors are effective in male infertility. Complementary therapies for infertility have received growing attention during the recent years and various antioxidants, nutritional approaches, and medicinal plants have been proposed for the treatment of fertility problems in infertile and subfertile couples **(Kolahdooz *et al.*, 2014).** A prospective cohort study in the US estimated the prevalence of using alternative and herbal medicines by couples with fertility problems at 29 and 17%, respectively. Due to increasing interest in the health benefits of medicinal plants, a wide range of herbal products has been produced and distributed in the global health market. The benefits of medicinal plants in the treatment of sperm abnormalities have been attributed to their antioxidant, anti-inflammatory, and venotonic activity along with their contents that promote sperm production and increase blood testosterone levels **(Taleb *et al.*, 2016).** In this context, **Tahvilzadeh *et al.*,(2016**) reported that date palm pollen (DPP) is one of the herbal monographs used in the treatment of male infertility. As DPP can increase male fertility, it is regarded as a strong sexual performance enhancer. Also, **Wenyi *et al.*, (2017)** mentioned that *Tribulus terrestris L.* (TT) is an annual plant of the family *Zygophyllaceae* that has been used for generations to energize, vitalize, and improve sexual function and physical performance in men. The fruits and roots of TT have been used as a folk medicine for thousands of years in China, India, Sudan, and Pakistan. Numerous bioactive phytochemicals, such as saponins and flavonoids, have been isolated and identified from TT that are responsible alone or in combination for various pharmacological activities. It has been certified for its pharmaceutical activities for improving sexual function and cardiac protection and providing anti-

urolithic, antidiabetic, anti-inflammatory, antitumour and antioxidants effects **(Gamal El Din ,2018).**

Tribulus terrestris is an annual herb of the *Zygophyllaceae* family and is commonly known as Gokshur, Gokharu, or Puncturevine. Flavonoids, alkaloids, saponins, lignin, amides, and glycosides are the main active phytoconstituents of this plant. Therefore, The present search aimed to study the effect of *Tribulus terrestris* and *Phoenix sylvestris* powder on Infertility in male rats.

Materials and Methods

Materials:

Tribulus terrestris and *Phoenix sylvestris* powder were obtained from a spices shop, Shebin El-kom. Egypt then kept in dusky stoppard glass bottles after miling until use.

Rats. Thirty adult male albino rats, weighting 150 - 160 g obtained from Medical Insects Research Institute, Doki, Cairo, Egypt were used. Rats were housed in wire cages under the normal laboratory condition and were fed on standard diet for a week as an adaptation period. Diet was offered to rats in special feed cups to avoid loses of feed, water was provided to the rats by glass tubes supported to one side of the cage, feed and water provided ad-libitum and checked daily**.**

Chemicals

- Cadmium Chloride Hydrate ($CdCl_2.2.5H_2O$) was purchased from Merck Chemical Company , Germany.
- Solvents , buffers, vitamin and salt mixtures were purchased from Elgomhoria Company for Trading Drugs,Chemicals and Medical Instructors , Cairo, Egypt.
- Casein was obtained from Morgan Chemical Co., Cairo, Egypt.

Methods

-Induced infertility in rats:

Rats were injected twice interapretonial by cadmium chloride ($CdCl_2$, 0.1%) at 0.1ml/100g body weight to induce male subfertility using insulin injector **(Rekha *et al.*, 2009).**

Biological experiments:

Basal diet:

Basal diet was prepared according to the formula mentioned by **AIN, (1993*)*** as follows: Protein (10%), corn oil (10%), vitamins mixture (1%), mineral mixture (4%), choline chloride (0.2%), methionine

(0.3%), cellulose (5%), and the remained is corn starch (69.5%). The used vitamins mixture component was that recommended by **Campbell, (1963*)*** while the salts mixture used was formulated according to **Hegsted *et al*., (1941*).***

Experimental design:

Biological experiments were done at the Biology Lab, Nutrition and Food Science Department., Faculty of Home Economics, Menoufia University, Shebin El-Kom, Egypt. Rats (n=30 rats) were housed individually in wire cages in a room maintained at 25 ± 2 ^{0}C and kept under normal healthy conditions. Rats were fed on basal diet for one-week before starting the experiment for acclimatization. Then, the rats were divided into two main groups as follow:

- **Main group (1) 6 rats:** Fed on basal diet and tab water only as a control negative group.
- **Main group (2) 24 rats:** Subfertility main group was divided into four groups 6 rats per each and fed on the experimental diets for four weeks as the following:

First group (1): Positive control group (untreated group, basal diet only)

Second group (2): Fed on basal diet with 5% *Tribulus terrestris* powder.

Third group (3): Fed on basal diet with 5% *Phoenix Sylvestris* powder.

Fourth group (4): Fed on basal diet with 5% mixture of (*Tribulus terrestris* + *Phoenix Sylvestris*) by (1:1).

Blood sampling

At the end of the experimental period, 28 days, blood samples were collected after 12 hours fasting using the abdominal aorta. Then, rats were scarified under ether anesthesia. Blood samples were received into clean dry centrifuge tubes and left to clot at room temperature, then centrifuged for 10 minutes at 3000 rpm to separate the serum according to **Drury and Wallington ,(1980*).*** Serum was carefully aspirated, transferred into clean cuvette tubes and stored frozen at -20^{o}C untill analysis.

Biological analysis:

Body weight gain (BWG), feed intake (F.I), feed efficiency ratio (FER) and relative weight of tests were calculated according to **Chapman *et al*., (1959).**

Hematological analysis:

Serum follicle stimulating hormone (FSH) and serum luteinizing hormone (LH) were determined according to the method of **(Fahim *et al.*, 1982)**.

Histopathological examination

Testis were removed, washed in saline solution, wiped by filter paper, weighted, and stored frozen in formalin solution 10% for histopathological examination according to method of **(Drury and Wallington, 1980).**

Statistical analyses

Statistical analyses were made by using statistical methods using the ANOVA test for comparison of data in the control group and the experimental groups. The results were expressed as mean ± SD. Significant difference between treatments calculated at ($P \leq 0.05$) **(Sendcor and Cochran, 1979).**

Results and Discution

Effect of *Tribulus terrestris* and *Phoenix sylvestris powder* on body weight gain (BWG) of subfertillity rats:

Table (1) illustrate the mean value of BWG (g) of control negative group and the other treated groups. It could be noticed that BWG of all treated groups were significantly decreased ($p \leq 0.05$) when compared with control (+ve) group. All treated groups didn't show any significantly difference between them.

Table (1): Effect of *Tribulus terrestris* and *Phoenix sylvestris* powder on body weight gain (BWG) g of subfertillity rats

Groups	BWG (g)	LSD ($p \leq 0.05$)
	Mean±SD	
Control (-)	18.67 ± 2.31 ^{b}	5.34
Control (+)	26 ± 5.65 ^{a}	
Tribulus terrestris (5%)	5 ± 2 ^{c}	
Phoenix sylvestris (5%)	8.67 ± 5.01 ^{c}	
Mixture (5%)	6.33 ± 2.31 ^{c}	

Means in the same column with different letters are significantly different at ($p \leq 0.05$)

Effect of *Tribulus terrestris* (TT) and *Phoenix sylvestris (PS) powder* on feed intake (FI) of subfertillity rats

Table (2) Results indicate the mean value of FI (g/day/rat) of control negative group and the other treated groups. It could be noticed that FI of all treated groups were increased comparing with control (+ve) group, but this increases didn't show any significant differences.

These results agree with **(Tatar and Akdevelioğlu , 2018)** they found that pollen, pit powder, and gemmule extract of the date palm created positive changes in hormone levels that have a role in male fertility and increased sperm motility and quality, spermatogenesis, and weights of testes and epididymis. Date palm pollen, pit powder, and gemmule extract have a high antioxidant capacity because of phenolic formations in the composition. Such characteristics have a positive effect on prevention and treatment of male infertility. Furthermore, gonadotropic and steroidal compounds within date palm pollen play a role in treatment of male infertility.

Table (2): Effect of *Tribulus terrestris* and *Phoenix sylvestris* powder on feed intake (FI) of subfertillity rats

Groups	FI (g/day/rat)	LSD ($p \leq 0.05$)
	Mean±SD	
Control (-)	17± 1 [a]	
Control (+)	15.33 ± 2.5 [a]	
Tribulus terrestris (5%)	18 ± 1 [a]	4.45
Phoenix sylvestris (5%)	15.89 ± 2.5 [a]	
Mixture (5%)	15.78 ± 3 [a]	

Means in the same column with same letters are non significantly different.

Effect of *Tribulus terrestris* and *Phoenix sylvestris powder* on feed efficiency ratio (FER) of subfertillity rats

Table (3) Results showed the mean value of FER of control negative group and the other treated groups. It could be noticed that FER of all groups were decreased significantly ($p \leq 0.05$) when compared with control positive group.

El-Neweshy *et al.*, (2013) regarded that treatment with date palm pollen can ameliorate the deleterious effects of cadmium, probably by activating testicular endocrine and antioxidant systems.Date palm pollen seems to cure male infertility by improving the quality of sperm parameters.

Table (3): Effect of Tribulus terrestris and Phoenix sylvestris powder on feed efficiency ratio (FER) of subfertillity rats

Groups	FER	LSD ($p \leq 0.05$)
	Mean±SD	
Control (-)	**1.09± 0.08 [b]**	**0.551**
Control (+)	**1.7 ± 0.52 [a]**	
***Tribulus terrestris* (5%)**	**0.23 ± 0.17 [c]**	
***Phoenix sylvestris* (5%)**	**0.54 ± 0.23 [bc]**	
Mixture (5%)	**0.41 ± 0.08 [bc]**	

Means in the same column with different letters are significantly different at ($p \leq 0.05$)

Effect of *Tribulus terrestris* and *Phoenix sylvestris powder* on testis weight (g) of subfertillity rats

Table (4) Results illustrate the mean value of testes weight (g) of control negative group and the other treated groups. It could be noticed that testes weight of all groups were pronounced decreased when compared with control positive group. In the same study, The consumption of *T terrestris* L., in general, resulted in the improvement of sperm parameters **(Sima *et al.*,2018)**. **Mehrdad *et al.*, (2018)** indicate that date palm pollen (DPP) may be operative in improving semen parameters and the impressive improvement in the quality of semen parameters. Within six months, the initial values of normal morphology (1%), total motility (3%), progressive motility (0.0), and sperm

concentration (0.1 million) increased to 20%, 60%, 10%, and 10 million, respectively.

Kazim *et al*., (2016) demonstrated that for the first time that *Mucuna, Tribulus* and *Ashwagandha* supplementation improves sexual function in male rats via activating Nrf2/ HO-1 pathway while inhibiting the NF-κB levels. Moreover, *Tribulus terrestris* extract was found to be more bioavailable than Ashwagandha extract followed by Mucuna extract.

Table (4): Effect of *Tribulus terrestris* and *Phoenix sylvestris* powder on tests weight (g) of subfertillity rats

Groups	Testis weight (g)	LSD ($p \leq 0.05$)
	Mean±SD	
Control (-)	1.75± 0.22 [ab]	
Control (+)	2.18 ± 0.07 [a]	
Tribulus terrestris (5%)	1.78 ± 0.07 [ab]	0.461
Phoenix sylvestris (5%)	1.46 ± 0.43 [b]	
Mixture (5%)	1.98 ± 0.12 [ab]	

Means in the same column with different letters are significantly different at ($p \leq 0.05$).

3.5 Effect of *Tribulus terrestris* and *Phoenix sylvestris powder* on serum follicle stimulating hormone (FSH) of subfertillity rats

Table (5) Results show the mean value of FSH (mlu/ml) of control negative group and the other treated groups. It could be noticed that FSH of all treated groups were increased ($p \leq 0.05$) when compared with control positive group.

Gamal El Din , (2018) reported that the exact role of *Tribulus terrestris* in male infertility is still controversial and needs future double-blind placebo-controlled studies that deploy larger cohorts. **Tahvilzadeh *et al*., (2016)** consistented with findings from previous study conducted on the effects of the medication on improving semen parameters. An in vitro study demonstrated increased motility as an effect of this drug. Elevated sperm count and motility have also been reported in several animal studies, whereas normal morphology improvement has been

reported only in some studies. **Fouad *et al.,* (2014)** reported that date palm pollen (DPP) doses significantly raised the ratio of testis or epididymis to body weight, sperm count, sperm motility , and estradiol level compared to the control group ($p \leq 0.05$). LH and testosterone levels noticeably increased with DPP at ($p \leq 0.01$) and ($p \leq 0.001$) respectively. Therefore results indicated that DPP could improve fertility factors in rats.

Table (5): Effect of *Tribulus terrestris* and *Phoenix sylvestris* powder on serum follicle stimulating hormone (FSH) of subfertillity rats

Groups	FSH (mlu/ml)	LSD ($p \leq 0.05$)
	Mean±SD	
Control (-)	1.27± 0.25 [a]	
Control (+)	0.43 ± 0.15 [b]	
Tribulus terrestris (5%)	0.73 ± 0.21 [ab]	0.52
Phoenix sylvestris (5%)	1 ± 0.2 [ab]	
Mixture (5%)	1.06 ± 0.37 [ab]	

Means in the same column with different letters are significantly different at ($p \leq 0.05$)

Effect of *Tribulus terrestris* and *Phoenix sylvestris powder* on serum luteinizing hormone (LH) of subfertillity rats

Results for table (5) show the mean value of LH (mlu/ml) of control negative group and the other treated groups. It could be noticed that FSH of all treated groups were increased pronouncedly when compared with control positive group.

Mohamed *et al.,* (2017) observed that no statistically significant difference was recorded in the levels of testosterone (total and free) , LH and semen parameters (sperm concentration or motility, or abnormal forms) before and after the treatment. In addition, no statistically significant correlations were observed between testosterone (free and total) , LH and semen parameters before and after the treatment. *Tribulus terrestris* was ineffective in the treatment of idiopathic infertility. **Natasha *et al.,* (2018)** suggested in their study that protodioscin present in *T.terrestris* extract could be the main responsible for the beneficial effects visualized in investigation the efficacy of dry extract of *Tribulus*

terrestris to protect against testicular damage induced by cyclophosphamide. CP is the most commonly used anticancer and immunosuppressant drug, and patients who need to use CP therapy exhibit reduced fertility or infertility. However, more studies are needed in order to understand the mechanism of TT dry extract in relation to its beneficial effects and possible interaction with anticancer drugs. **El-Sayed *et al.*, (2017)** mentioned that there was a significant improvement in all studed parameters with DPP (date palm pollen) & DPS (date palm seed extract) administration so consumption of DPP or DPS might be considered as a functional treatment for retarding risks of infertility associated with cadmium exposure.

Table (6): Effect of *Tribulus terrestris* and *Phoenix sylvestris* powder on serum luteinizing hormone (LH) of subfertillity rats

Groups	LH (mlu/ml)	LSD ($p \leq 0.05$)
	Mean±SD	
Control (-)	2.4± 0.46 [a]	0.526
Control (+)	1.4 ± 0.2 [b]	
Tribulus terrestris (5)	1.56 ± 0.31 [b]	
Phoenix sylvestris (5%)	1.8 ± 0.43 [ab]	
Mixture (5%)	2.26 ± 0.25 [a]	

Means in the same column with different letters are significantly different at ($p \leq 0.05$)

Histopathological examination

Microscopically, testes of rats from group 1(control "-") showed the normal histological structure of seminiferous tubule with normal spermatogoneal cells and complete spermatogenesis (Photos 1a and 1 b). In contrary, testes of rats from group 2(control "+") revealed small diameter seminiferous tubules with degeneration and interstitial oedema (Photo. 1-c). However, testes of rats from group 3 (*T.terrestris* diet) revealed no histopathological changes with complete spermatogenesis and sperm production (Photos 1- d and 1- e). Some examined sections from group 4 (*P.sylvestris* diet) showed necrosis of spermatogoneal cells lining seminiferous tubules (Photo 1- f), whereas, other sections from this group as well as testes of rats from group 5 (Mixture diet) showed no histopathological changes with complete spermatogenesis and sperm production (Photos 1-g, 1- h and 1- i).

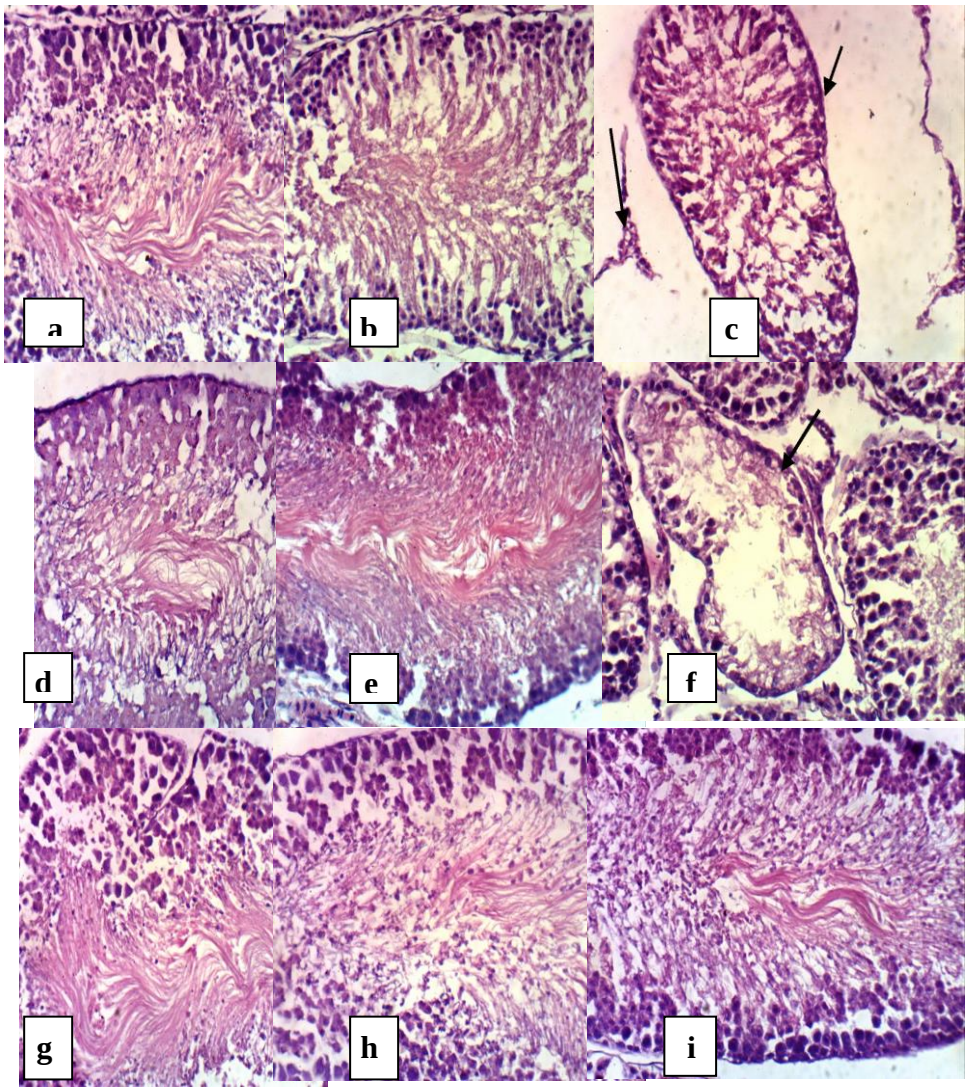

Photo 1: The effect of tested plant parts on histopathological changes of testes in rats. (a and b) normal (control negative (-ve)); (c) sub fertility group (control positive (+ve)) ; (d and e) sub fertility rats fed on diet containing 5% *Tribulus terrestris* for 4 weeks; (f) sub fertility rats fed on diet containing 5% *phoenix sylvestris* for 4 weeks; (g, h and i) sub fertility rats fed on diet containing 5% mixture of the above mentioned plants for 4 weeks (H & E X 400).

Conclusion

In conclusion, it can be said that treatment with the tested plant parts in the present study didn't cause any histopathological changes. These results supported our hypothesis that tested plant parts contain several compounds that are able to protect testis from any histopathological changes. Therefore, *T.terrestris* and *p. sylvestris* may be recommended at moderate amounts in human daily diets or drinks.

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دراسة تأثير مسحوق نبات الحسك وطلع النخيل على الفئران المصابة بانخفاض معدل الخصوبه

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قسم الاقتصاد المنزلى ,كلية التربية النوعية ,جامعة المنوفيه , أشمون. منوفيه[٢].

الملخص العربى:

تهدف هذه الدراسه الى معرفة تأثير الوجبة المدعمه بمسحوق نبات الحسك وطلع النخيل ومخلوطهما بنسبة (١:١) على الفئران المصابة بانخفاض معدل الخصوبه .لذلك تم استخدام ٣٠ من ذكور الفئران متوسط أوزانهم ١٥٠ -١٦٠ جم اوتم تقسيمهم الى ٥ مجموعات بالتساوى .تناولت المجموعة الأولى (الضابطه السالبة) الوجبة الاساسية خلال التجربة . وتضمنت المجموعه الثانيه (٢٥) فأر قد تم حقنهم بمادة كلوريد الكاديوم ($CdCl_2$, 0.1%) بمعدل ١.٠ مللى / ١٠٠جم من وزن الفأر وذللك لاصابتهم بانخفاض فى مستويات هرمونات الخصوبه. تم تقسيمها الى المجموعات الفرعيه التاليه وهى: مجموعه مصابه وتتغذى على الوجبه الأساسيه طوال التجربه (٢٨) يوما, أما الثلاث الأخرى تم تغذيتم على الوجبه الأساسيه مضافا اليها مسحوق نبات الحسكه وطلع النخيل ومخلوطهما بنسبة ٥% على التوالى . وفى نهاية التجربه تم حساب كل من الوزن المكتسب والمأخوذ الغذائى ومعدل كفاءة الغذاء والوزن النسبى للخصيه , كما تم اجراء تحاليل الدم لهرمون الملوتن (LH) و الهرمون المنشط للحوصله المنويه (FSH), كما تم أيضا عمل فحص هيستوباثولوجى للخصيه. وقد أوضحت النتائج وجود انخفاض معنوى فى كل من الوزن المكتسب ومعدل كفاءة الغذاء والوزن النسبى للخصيه مقارنة بالمجموعه الضابطه الموجبه, بينما وجد ارتفاع غير معنوى فى المأخوذ الغذائى. وبالنسبه لهرمونى LH و FSH فقد اظهرت النتائج وجود ارتفاع معنوى فى مستوى هذه الهرمونات مقارنة بالمجموعه الضابطع الموجبه. وقد أثبت الفحص الهيستوباثولوجى للخصيه التحاليل الكيميائيه حيث وجود تحسن فى نسيج الخصيه فى المجموعات المدعمه بالنباتات محل الدراسه مقارنة بالمجموعه الضابطه الموجبه.

الكلمات المفتاحية : ضعف الخصوبه , الأجزاء النباتيه, هرمون الملوتن ,الهرمون المنشط للحوصله المنويه , الخصيه, التغيرات الهيستوباثولوجيه.