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Abstract:

The use of date palm pollen as an adjutant form of medical treatment is gaining popularity all over the world. This study was performed to investigate the effects of date palm pollen on diabetic male rat's fertility. A total of twenty four rats divided into four groups were used in this study. Varying doses of the date palm pollen 5, 10 and 20% were used. Parameters used in the methodology were weight of testis, malonildiahidehyde concentration, histological study, hormonal assay and sperm analysis. Results of date palm pollen revealed no significant change in weight of testis, inhibition of testicular tissue peroxidation, improve testosterone levels, parameters of sperm analysis such as sperm concentration and motility in comparison to the control group. Obvious cellular changes were also observed in the high level 20% treated groups. The changes observed in groups treated with 10% and 5% were minimal and statistically in significant. The results showed that oral administration of date palm pollen to male rats for 65 days kept the weight of testes and seminal vesicles; improved semen quality and quantity and testosterone levels in the opposite of the results of the level 20% of the pollen. So, this study recommends that intake of 5 and 10% of date palm pollen extracts powder may be beneficial for diabetic male patients who suffer from infertility as these enhance male fertility in rats also more studies on human recommended.

Key words: date pollen- Antioxidant- Histopathology- Malonildialdehide.

Introduction:

Fertility is one of the major health problems in life, and approximately 30% of these problems are due to male factors (**Isidori *et***

***al.*, 2006**). Several factors can interfere with the process of spermatogenesis and reduce sperm quality and quantity. Some diseases and conditions such as coronary heart diseases; diabetes mellitus; chronic liver diseases; chronic smoking; insecticide contaminants; air pollutants and insufficient vitamins intake have been reported to cause deleterious effects on spermatogenesis (**Mosher and Pratt, 1991**). On the other hand, previous study reported that intake of antioxidants and vitamins A, B, C and E can increase stability of testicular blood barrier and protect sperm DNA from oxidative stress caused by active free radicals (**Jedlinska *et al.*, 2006**).

Diabetes mellitus, primary or idiopathic, is a chronic disorder of the carbohydrate, lipid and protein metabolism, characterized by insulin disorders, hyperglycemia and glycosuria. This condition may contribute to arteriosclerosis, microangiopathy, nephropathy and neuropathy (**Camilleri, 2007**).

Diabetes mellitus has been associated to sexual disfunction, both in men and women. It is believed that neuropathy, vascular insufficiency and psychological problems may be involved in the pathogenesis of some phenomena, such as impotence, ejaculation disorders and decreased libido, in addition to the reduced vaginal lubrication and orgasm disfunctions
(**De Berardis *et al.*, 2007**).

Traditional plants are used as source of treatment of diseases in different parts of the world. The use of plant extract as adjutant form of medical treatment is presently enjoying great popularity. The use of date pollen to treat disease is almost universal among non-industrialized societies (**Dasilva *et al.*, 2002**). A number of traditions came to dominate the practice of plant medicine at the end of the twentieth century. Many of the pharmaceuticals currently available to physicians have a long history of use as plant remedies, including opium, aspirin, digitalis, and quinine. The (**World Health Organization, 1999**) estimates that 80 percent of the world's population presently uses date palm for some aspect of primary health care (Traditional medicine). Earlier in the 1990's, approximately one-third of people, surveyed in the United States used at least one unconventional therapy during the previous year (**Eisenberg *et al.*, 1993**). Traditional healers are still consulted in Nigeria

as a first choice probably due to the fact that traditional medicine blends readily into the socio-cultural life of the people. **Adomi (2006)** showed that date palm pollen remedies are fast growing and might take over from over-the-counter (OTC) drugs. Some of these supplements include Aloe Vera products, Chinese balms and other herbal concoctions that are preferred by the Yoruba speaking people of Nigeria as first choice.

Numerous plants have been used historically to increase fertility and modern scientific research has confirmed fertility effects in at least some of the date palm pollen tested (**Koneri *et al.*, 2006**).

Effect of date palm pollen on sperm parameters and reproductive system of adult male rats was studied and the results indicated that the consumption of Phoenix dactylifera pollen suspensions improved the sperm count, motility, morphology and DNA quality with a concomitant increase in the weights of testis and epididymis (**Bahmanpour *et al.*, 2006**). Date extract caused a significant increase in sperm cell concentration (total count) and motility in male guinea pigs and adult male rats (**Hassan *et al.*, 2012**). Also, investigators showed statistically significant increase in serum testosterone levels in rats they received DPP (**Iftikhar *et al.*, 2011**). Date extracts have been shown to increase in sperm concentration and motility with a increased normal morphology and a significant increase in testosterone level in rats (**Adaay and Mattar, 2012**). They revealed that the aqueous extract of Phoenix dactylifera pollen can be used as a sex enhancer and seems to cure male infertility in rats. Also, their findings support the traditional use of this plant for the treatment of pre-ejaculation and impotency (**Abedi *et al.*, 2012**). Two studies have shown protective effect on cisplatin and cadmium induced male infertility in rats (**Hassan *et al.*, 2012**).

There are a few studies for effect of DPP on male fertility in human. (**Saad *et al.*, 2012**) showed that adding the 20% Phoenix dactylifera pollen extract to the culture medium of the in vitro sperm activation leads to an improvement in the sperm motility. In another studies, a combined therapy includes DPP and zinc sulfate capsules (Al-Sanafi et al., 2006) and DPP capsule alone (Marbeen et al., 2005) used for treatment of 25 infertile men. The treatment was significantly increased serum LH, FSH and testosterone levels. It was also, increased significantly sperm count and motility. Sexual desire was also significantly increased. So, the

present study was designed to investigate the effects of date palm pollen on diabetic male fertility.

Materials and Methods:

Materials:

This study was carried out using date pollen as powder which obtained from Ministry of Climate Change and Environment (MOCCAE).UAE.

Thirty (30) mature male albino rats each weighing 150 ± 5gm were and 14-16 weeks old of Sprague Dawley strain.Rats were obtaied from the Laboratory Animal Colony, Helwan, Egypt.

The basel diet consisted of casein as a source of protein, corn oil as a source of fat, choline chloride, vitamin mixture, cellulose as a source of fiber, salt mixture and corn starch were obtained from El-Gomhoria Co., Dokki, Giza. Basal diet was prepared according to (AIN, 1993). It was consisted of 14% casein, 10% sucrose, 10% corn oil, 0.2% choline chloride, 1% vitamin mixture (Campbell, 1963), 4 % salt mixture (**Hegsted *et al*., 1941**) and 5% fibers (cellulose). The remainder was corn starch.

Alloxan:

Was purchased from El-Gomhoryia Company for Chemicals; Cairo, Egypt.

Glucose enzymatic kits of BioMeriuex were purchase from Alkan Company, Dokki, Egypt for determination of serum glucose. Radioimmunoassay kits were obtained from Gamma Trade Company, Egypt for estimating of the Malondialdehyde (MDA) level and testosterone hormones in treated rats.

Methods:

Experiment and Grouping of rats:

The rats will divide into 5 groups (6 animals) of each one. The 1st group fed on the basal diet and served as normal control, while the other five groups were given alloxan by intraperitoneal injection of a single daily dose of 120 mg/Kg. for 3 days to induce moderate stable diabetes as described by Ashok et al. (2007). The 2nd group of rats was left as

diabetic control. Diabetic rats of the 3rd,4th and 5th groups were given date palm pollen at the levels 5, 10 and 20% respectively, for 65 days to cover the period of spermatogenesis in the rat. Doses have been used by researchers to achieve fertility effects (Rao, 1987 and Sinha, 1990).

Retrieval of tissue:

At the end of experiment, rats were anaesthetized with ketamin 1mg/kg [intramuscularly (i.m.)], the chest was opened and blood samples collected by heart puncture. Serum samples were directly frozen at –10 Ċ till biochemical analyses. Testicular pieces were collected in Bouin's fluid for histology and rapidly frozen for malonildialdehide (MDA) estimation.

Organ weights:

The testes, seminal vesicle and prostate gland of rats were dissected out and weighed. The testes were preserved in 10% neutral formalin solution till processed for histopathological examination.

Quantitative:

Semen analysis:

Epididymal contents of the treated rats were obtained after cutting the tail of epididymis, squeezing it gently on clean slide and the sperm progressive motility and cell count were determined according to the method described by **Bearden and Fluquary (1980)**. Microscopic examinations of the seminal smears stained with Eosin Nigrosin stain were carried out to determine the percentages of sperm viability (ratio of alive/ dead) and sperm cell abnormality according to **Amman (1982)**.

Sperm Concentration Measurements:

Haemocytometer chambers were prepared for counting according to the **WHO (1999)** criteria of semen analysis. Spermatozoa are viewed and counted under a light microscope, the haemocytometer is divided into nine fields, but spermatozoa are counted and recorded for just five random fields and the value is recorded in (106) millions (**Tomlinson *et al.*, 2001**).

Sperm Motility:

Sperm motility was assessed using the **WHO (1999)** classification system, with only the three grades (a, b and c) reported; rapid forward

progression, medium forward progression and slow forward progression. Each sample was assessed twice. For consistency, all readings were carried out at 37ºC (**WHO, 1999**).

Hormonal assay:

Testosterone assay procedure was carried out using immunometric direct human serum testosterone enzyme based immunoassay (EIA) the assay was carried out in five steps as previously described by **Wilke and Utley (1987)**.

Determination of Malonidiadehide Concentration:

Malondialdehyde (MDA) level was determined in the supernatant of the testicular homogenates by the modified method of **Buege and Aust (1978)**. The concentration was calculated using the molar absorptivity of malondialdehyde which is 1.56×100000 M. Percentage oxidation was calculated by: 1- means value of treated group/mean value of control x**.**

Histopathological examination:

Testes of rats were taken and fixed in 10% neutral formalin solution. The fixed specimens were then trimmed, washed and dehydrated in ascending grades of alcohol. These specimens were cleared in xylene, embedded in paraffin, sectioned at 4-6 microns thickness and stained with Hematoxylen and Eosin (H and E) then examined microscopically according to the method described by **Luna (1968)**.

Statistical Analysis:

Data were expressed as means ± S.E. and statistical analysis was carried using computerized SPSS program. Significance was performed using the least significant difference and paired Student "t" test according to **Snedecor and Cochran (1986)**.

Results and Discussion :

1. **Effect of administration of the date palm pollen for 65 days on the absolute weight of sexual organs of normal and diabetic male rats (n= 6 animals):**

Administration of date palm pollen at 5, 10 and 20% to adult rats for 65 days significantly increased the weight of testes and seminal vesicle and did not affect the weight of prostate glands as compared to the

control group. The positive control group when given the basal diet without any additive and for the same period significantly decreased the weight of the above mentioned sexual organs as shown in Table (1).

Table (1): Effect of administration of the date palm pollen for 65 days on the absolute weight of sexual organs of normal and diabetic male rats (n= 6 animals).

Groups and Treatment	Sexual organs weight (g) Mean ± SE		
	Testes	Seminal vesicles	Prostate glands
***Negative control* group (G1)**	2.56 ±0.63	1.65 ± 0.22	0.62 ± 0.01
Positive control group (G2)	1.89±0.04	0.87 ± 0.15	0.52 ± 0.04[a]
Diabetic rats treated with 5% DPP for 65 days (G3)	2.58 ±0.12 [b]	1.69 ± 0.24[b]	0.63 ± 0.01
Diabetic rats treated with 10% DPP for 65 days (G4)	2.61 ±0.63 [b]	1.73 ± 0.16 [a]	0.63 ± 0.03
Diabetic rats treated with 20% DPP for 65 days (G5)	2.69 ±0.28 [a]	1.85 ± 0.18 [a]	0.64 ± 0.12

The treated groups were compared to control group using Student't' test.

[a] Significant at P < 0. 05 [b] Significant at P < 0. 01.

2. Effect of administration of the date palm pollen for 65 days on parameters of sperm analysis of normal and diabetic male rats (n= 6 animals):

Result in diabetic group as positive control showed a significant reduction in both sperm concentration and motility. While, administration of different levels of DPP prevented a reduction in the sperm analysis parameters to a reasonable degree. These parameters were improved by adding the high levels of DPP when compared with normal group as seen (Table 2).

Table (2): Effect of administration of the date palm pollen for 65 days on parameters of sperm analysis of normal and diabetic male rats (n= 6 animals).

Groups and Treatment	AVE. sperm concentration ×106	Motility (%)	Abnormality (%)	Description value of sperm
***Negative control* group (G1)**	147.34±0.1	91.00±2.11	3.57 ± 0.08	EXCELLENT
Positive control group (G2)	101.24 ±0.28[c]	50.00 ±1.03[c]	9.67±0.18[c]	FAIR
Diabetic rats treated with 5% DPP for 65 days (G3).	150.28 ±0.24[c]	92.00 ±1.83[c]	3.25±0.88 [c]	EXCELLENT
Diabetic rats treated with 10% DPP for 65 days (G4).	168.76 ±0.18[c]	94.00 ±2.76[c]	2.33 ±0.08[c]	EXCELLENT
Diabetic rats treated with 20% DPP for 65 days (G5).	179.92 ±0.19[c]	98.0 ±5.22[c]	1.76 ±0.08[c]	EXCELLENT

The treated groups were compared to the control group using Student't test

[c] Significant at $P < 0.001$

3. Effect of administration of the date palm pollen for 65 days on testosterone concentrations of normal and diabetic male rats (n= 6 animals):

Plasma testosterone concentrations were significantly increased in treated groups compared to the positive control group ($p < 0.05$). The changes were more prominent in group 4 and 5, which received DPP at the levels 15 and 20% as shown table (3). The study also revealed a marked decreased of plasma testosterone levels after 65 days in case of positive control group when compared with normal control group.

Malonildiadehide (MDA) concentrations were nearly stable in case DPP treated groups. Diabetic group which was fed on basal diet as positive control group had high level of MDA in comparison to the DPP treated and the negative control group. This proved that the rate of

oxidation is significantly lower in the treated groups (3, 4 and 5) in comparison to the diabetic untreated group (2).

Table (3): Effect of administration of the date palm pollen for 65 days on serum testosterone and testicular tissue malonildialdehide concentrations of normal and diabetic male rats (n = 6 animals).

Groups and Treatment	Testosterone concentration (nmol ml-1)	Malonildeldehide concentration (umol mg-1)
Negative control **group (G1)**	4.40±0.18	0.53±0.013
Positive control group (G2)	1.15±0.07 [a]	0.75±0.21[a]
Diabetic rats treated with 5% DPP for 65 days (G3).	4.45±0.36	0.53±0.16
Diabetic rats treated with 10% DPP for 65 days (G4).	6.62±0.44[a]	0.53±0.16
Diabetic rats treated with 20% DPP for 65 days (G5).	7.15±0.23[a]	0.52±0.02

[a] Significant difference ($p<0.05$)

Histopathological Examinations:

Control group tests (Photo 1) showed that normal histological structure of active mature functioning seminiferous tubules associated with complete spermatogenic series. The histological study in the liquorice roots treated (Group 1) showed normal chromatin clumping, nuclear vacuolation, cytoplasmic macrovesicles and isolated small collection of apoptotic cells. In group (2), there are more germ cells in some of the seminiferous tubules of this group. The interstitium is narrowed and infiltrated with inflammatory cells. Also nuclear changes and swelling that was observed in this group. The interstitial spaces were seen to be widened. However, shows a lot of inflammatory cells infiltration, interstitium is not very remarkable. Also, degeneration of most seminiferous tubules with absence of spermatogenic series in tubular lumen was observed.

There was no significant inflammatory cell infiltration and the interstitium not remarkable (Photo 3). Groups 4 and 5 which were treated

with DPP at the levels 10 and 20% revealed normal histological structure of most seminiferous tubules with normal spermatogenic series, the nuclear changes and swelling are absent in the slides of these groups, (Photos 4 and 5). So also cell death is significantly not present and the interstitial spaces were seen to be widened.

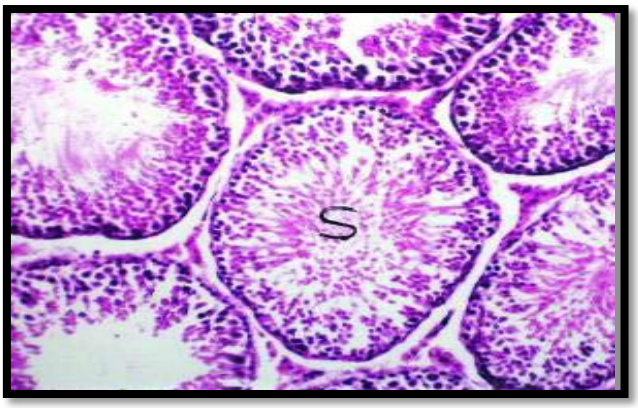

Photo (1): A photomicrograph of rat's tests from negative control group (Mag.x 100).

Photo (2): A photomicrograph of rat's tests from group 2 (Mag.x 40).

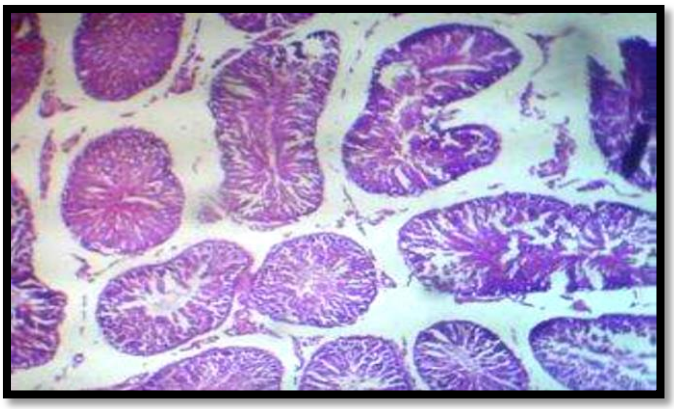

Photo (3): A photomicrograph of rat's tests from group 3 (Mag x 100).

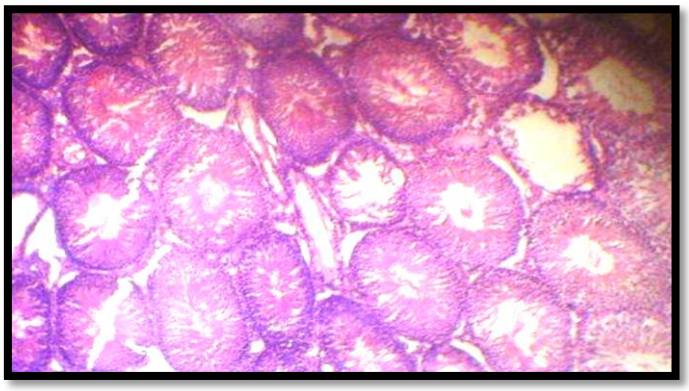

Photo (4): A photomicrograph of rat's tests from group 4 (Mag.x 100).

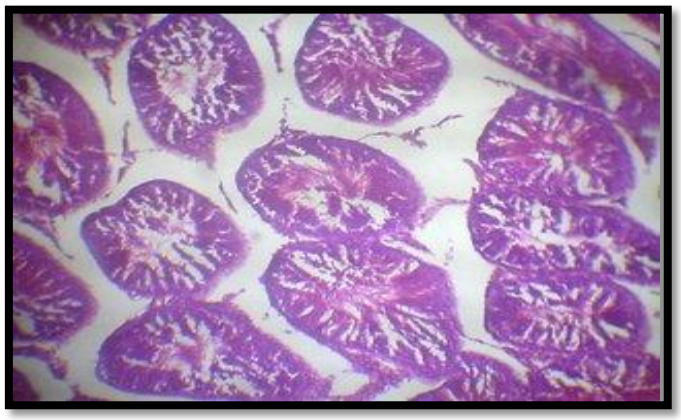

Photo (5): A photomicrograph from rat's tests in group 5 (Mag.X 100).

Discussion:

Researchers have expressed their concerns about the rising cases of male spermatozoa abnormalities (**Kuku and Osegbe, 1989**). Sperm factor constitutes about forty percent (40%) of infertility and this is due to abnormalities of the male reproductive system (**Ashiru *et al.*, 1993**). Infertility among couples in African societies especially Nigeria, is causing increasing concern (**Uriah *et al.*, 2001**). The infertility in Africa at large could be due to the fact that traditional healers are still consulted as a first choice because traditional medicine blends easily into the socio-cultural and economic life of the people (**Kela and Kufeji, 1995**).

Defective sperm function in cases of male infertility is being caused majorly by free radical activities (**Sharma and Agarwal, 1996**). There is growing evidence that spermatozoa are protected from the detrimental Reactive Oxygen Species (ROS) effect by the powerful antioxidants in seminal plasma since disturbances of sperm functions by ROS has been demonstrated in the absence of seminal plasma (**Raji *et al.*, 2007**). In light of the above, it is not surprising that testes of rats administered with liquorice showed significant evidence of inhibition of lipid peroxidation. Liquorice roots are a known powerful antioxidant with the ability to scavenge Reactive Oxygen Species (ROS). It also prevents the oxidation of essential cellular components because it is an excellent vitamin fat soluble source as vitamin E, chain breaking antioxidant (**Raji *et al.*, 2007**).

The obtained results showed that DPP administration at 10 and 20% for 56 days to male rats caused increases in the weight of testes and seminal vesicle and prostate glands when compared to control group. Whereas, diabetic rats as positive control group which fed on basal diet led to decrease the weight of the above organs. So, the preventive functions of DPP on testicular tissue oxidation and function were improved. These findings agree with those reported by **Polentseva *et al.* (2009)** who concluded that liquorice roots may be promising in enhancing sperm healthy parameters. Also, our study supports that of **Adedapo *et al.* (2007)** who found that high blood sugar caused testicular degeneration, lowered semen quality and quantity. With regard to DPP, the present data revealed that its oral administration at the large dose (20%) increased sperm motility and quantity as well as alleviation of testicular degenerative changes that seen in the testis of rats which administrated DPP at the same level. These findings are partially similar to those reported by **Verma and Kanwar (1999)** who concluded that the use of DPP in vitro has been also documented to improve sperm motility and viability. Also, **Hughes *et al*. (1998)** results are in consonance with our findings which show is a protective effect in all the parameters used. This due to DPP contained vitamins C, E, and urate separately has protective effects on sperm DNA integrity on irradiation.

The study also revealed a marked decreased of plasma testosterone levels after 56 days in case of positive control group while, plasma

testosterone levels in case of using treatment with DPP especially at high doses 20% led to increase plasma testosterone levels. For, Malonildiadehide (MDA) concentrations of rats administrated with DPP were decreased with increasing the level of DPP whereas the rats as a positive control group, the Malonildiadehide (MDA) concentrations increased and due to reduce volume of contents in the testes and reduced testosterone producing leydic interstitial cells level seen in the treated groups as clearly shown in the histological study, which indicates that this extract suppresses leydic cell steroidogenesis and this tallies with work done by **Amr (2008)**. The effect of DPP on sperm function may therefore be caused by an increase in the lipid peroxidation as DPP leads to a significant decrease in the MDA levels, increased spermatogenesis and the near normal level of the histological studies. The antifertility potentials of high blood sugar due to its oxidative properties might be due to presence of some known constituents such as Abrin (**Bhaskar *et al*., 2008**) and lectin (**Myung-Sunny Kim *et al*., 2003**).

Earlier study carried by **Sinha (1990)** revealed a normal histological appearance with DPP which matched with our histological findings in this study and this might be due to the higher dose used in this study.

Conclusion:

The results of this study indicated that high blood sugar caused a harmful effect on the fertility potentials in male rats due to probably an imbalance between levels of Reactive Oxygen Species (ROS) production and the natural antioxidant defense system. While, the administration of DPP at the same levels 10 and 20% can be used to protect spermatozoa against the free radical-induced injury such as diet which forms an important component of the antioxidant protection system; it supplies the major antioxidants sources such vitamin C, vitamin E, and carotenoids and for those patients who are suspected to have high levels of ROS, DPP at the level 20% supplement can be considered.

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تأثير طلع النخل علي خصوبة ذكور الفئران المصابة بداء السكري

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الملخص:

إن استخدام حبوب لقاح النخيل كمعاون فى العلاج الطبي يكتسب شعبية في جميع أنحاء العالم. وقد أجريت هذه الدراسة لمعرفة تأثير حبوب لقاح النخيل على خصوبة ذكور الفئران المصابة بالسكري.قد تم تقسيم مجموعة الفئران المستخدمة فى البحث (٣٠ فأر) إلى خمس مجموعات كل مجموعة ٦ فئران واستخدمت المجموعة الأولى كمجموعة ضابطة سلبية و المجموعة الثانية كمجموعة ضابطة موجبة وأعطيت المجموعات الثالثة والرابعة و الخامسة مستويات ١٠.٥ و ٢٠% على التوالي من حبوب لقاح النخيل بالنسبة لكل ١٠٠جم غذاء، واشتملت المعايير المستخدمة في منهجية البحث ما يلي: وزن الخصية، تركيز المانولدهيد، دراسة نسيجية، الفحص الهرموني وتحليل الحيوانات المنوية، وأظهرت نتائج استخدام لقاح النخيل عدم حدوث أي تغيرات في وزن الخصيتين، وتثبيط بيروكسيد أنسجة الخصية، وبينما حدث تحسن فى مستويات هرمون التستوستيرون، معالم تحليل الحيوانات المنوية مثل تركيز الحيوانات المنوية وتركيز المالونالدهيد والحركة الحيوانات المنوية بالمقارنة مع المجموعة الضابطة. وقد لوحظت تغييرات خلوية في مجموعة الفئران التى تناولت الحبوب بتركيز٢٠%. كانت التغيرات غير ملحوظة في المجموعات المعاملة ب ٥و١٠% حبوب لقاح منخفضة وغير معنوية احصائيا، وأظهرت النتائج أن تناول حبوب اللقاح عن طريق الفم لذكور الفئران لمدة ٦٥ يوما حافظت على وزن الخصيتين والحويصلات المنوية، كما أدت إلى تحسن المني في جودته و كمية هرمون التستوستيرون عكس النتائج التي لوحظت عند استخدام حبوب اللقاح بتركيز ٢٠%، لذلك، توصي هذه الدراسة بأن تناول ٥ و١٠% من مسحوق حبوب اللقاح النخيل قد تكون مفيدة لمرضى السكري الذين يعانون نقص فى الخصوبة.

الكلمات الاسترشادية: حبوب لقاح النخيل– مضادات الأكسدة – التشريح – المالونالدهيد.