

**EFFECT OF *PHOENIX DACTYLIFERA* L. VARIETY  
SEWI (DATE PALM) DIFFERENT EXTRACTS ON  
FERTILITY OF MALE ALBINO RATS**

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**ABSTRACT**

*The effect of different date palm extracts (pollen grain, leaves, fruits and pits) on male fertility was investigated. Eighty one adult male rats were classified into nine groups and were given 500 mg / kg b. wt. of each extract daily for 65 successive days. The body and sex organs weight, sperm motility percentage, sperm count, live sperm percentage, sperm abnormalities, serum ALT, creatinine, FSH, LH, testosterone and histopathological studies were evaluated after 65 days post-administration. The obtained results revealed that different extracts induced significant increase in body weight. Alcoholic, watery of pollen grains, watery fruits and watery pits extracts caused significant increase in sperm motility, sperm count, live percentage, while sperm abnormalities were decreased. Also significant increase in serum FSH, LH and were recorded testosterone. The alcoholic and*

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*watery extracts fruits of reduced the level of ALT. Date palm extracts could be used to enhance male fertility without adverse effect.*

## INTRODUCTION

There are many ancient records of herbal medicinal plants. The use of herbal medicine has become increasingly popular worldwide. Many reviews of using date palm in folk medicine for treatment of both male and female infertility. Many date palm organs as pollen grains, fruits, leaves and pits could be of pharmacological importance. A suspension of *Phoenix dactylifera* date palm pollen grains was widely used as a folk remedy for curing male infertility in traditional medicine (**Mirheydar, 1992 and Zargari, 1999**). The male flowers of date palm were eaten directly by peoples to enhance fertility. There are reports that date palm pollen grains possess gonad stimulating activity (**Soliman and Soliman, 1958 and El-Ridi et al., 1960**). Also improve fertility in male rats (**Bahmanpour et al., 2006**) were recorded. Date palm fruits possess gonadotrophic activity in male rats (**El-Mougy et al., 1991**). Date palm pits possess growth promotor action by increasing body weight gain and deposition of back fat in sheep (**Elgasim et al., 1995**). Date palm pits increase body weight, testicular weight, serum level of LH and testosterone in male rats (**Ali et al., 1999**). Date flesh and pits exert hepatoprotective effect against carbon tetrachloride hepatotoxicity in rats (**Al-Qarawi et al., 2004**).

Thus, the aim of the present study is to investigate the effect of different date palm extracts (pollen grains, fruit, leaves and pits) on fertility of male rats for potential use in treatment of infertility.

## MATERIALS AND METHODS

### 1- Extracts:

Pollen grains, leaves, fruits and pits of *Phoenix dactylifera L.*, were used in this study. They were collected from certain farms of Beni-Suef governorate, Egypt.

- a) Alcoholic extracts:** Alcoholic extract of each plant organ was prepared by macerating 100 g of each of powdered pollen grains, leaves, fruits or pits (date stones) in successive portions of ethanol (95%) till exhaustion. The alcoholic extract in each case was evaporated under reduced pressure to obtain a semisolid residues. Extracts of pollen grains, leaves, fruits and pits were dissolved as 20% in distilled water containing few drops of Tween 80 were prepared.
- b) Aqueous extracts:** They were prepared by boiling 100 g of each of the powdered pollen grains, leaves, fruits or pits (date stones) in distilled water. The aqueous extracts were dried by evaporation in rota vapour. The residues were freshly dissolved as 20% in distilled water before use.

## 2- Experimental animal

**a- Albino mice** of 18-20 g body weight.

**b- Adult male albino rats** of 100-125 g body weight.

### Biological study:

#### 1. Determination of acute median lethal dose in mice:

Mice, 18-20 g, were administered extracts (starting with a dose of 50 mg/kg b.wt. till 7 g/kg b.wt.). They were given orally using stomach tube. All groups of animals were observed for 24 hours for any toxic symptoms and mortality.

#### 2. Effect of different date palm extracts on male rat fertility:

Eighty one apparently healthy male albino rats of 90-110 gm b.wt were used. Rats were divided into nine groups each of 9 rats. Animals were kept under hygienic measures, fed a balanced ration and water *ad-libitum* and were left for two weeks for acclimatization before experiment. Rat dose was calculated from mice dose according to (**Paget and Barnes,1964**) as 5 g/kg b.wt. The extracts were used in 1/10 this dose. The first group was given 1 ml of distilled water containing few drops of Tween 80 and serves as control, while the other groups were given extracts at a dose of 500 mg /kg bwt. of each extract. Group II received alcoholic extract of the pollen grains, Group III received aqueous extract of the pollen grains, while group IV received alcoholic extract of the leaves and group V received the aqueous extract of the leaves. The group VI received alcoholic extract of the fruits, group VII received the aqueous extract of the fruits, group VIII received alcoholic extract of the pits and group IX received the aqueous extract of pits. Extracts were given orally and daily using stomach tube for 65 successive days to cover complete spermatogenic cycle.

### **Sampling:**

Blood, semen and tissue samples were collected at 65 days post-extracts administration

#### **a-Blood samples:**

Blood samples were obtained from supra-orbital venous plexus using non-heparinized microhaematocrit tube then, left to clot at room temperature, centrifuged at 3000 rpm for 10 minutes to separate clear sera and kept in clean, dry vials in deep freezer at -20°C until analysis .

#### **b-Semen samples:**

Semen was obtained by maceration of epididymis, the sperm motility, count, live percentage and total abnormalities were carried out according to *Blom (1950)* and *Bearden and Fuquay (1980)*.

### **c-Tissue samples:**

Five rats from each group (at each collection) were scarified to obtain sex organs (testes, epididymis, seminal vesicles and prostate glands). Liver and kidney were dissected out, grossly examined, accurately weighed and then fixed in 10% formalin and prepared for making paraffin sections of 5-6 u m thickness that ,stained with haematoxylin and eosin stain for histopathological examination (*Culling, 1980*).

### **Serum biochemical analysis:**

#### **1. Serum alanine aminotransferase (ALT) (unit / ml):**

The serum level of ALT was determined calorimetrically according to *Reitman and Frankel (1957)* using transaminase kit (Bio Merieux, France).

#### **2. Serum creatinine (mg %):**

Serum creatinine level was determined calorimetrically according to *Siest et al (1985)* using kinetic creatinine kit (BioMerieux, France).

#### **3. Determination of FSH, LH and testosterone hormones using radioimmunoassay.**

### **Statistical analysis:**

Calculation of mean ,standard error as well as one way ANOVA test were carried out according to *Snedecor and Cochran(1980)*.

## **RESULTS**

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### **1- Determination of LD<sub>50</sub>:**

None of the tested extracts produced any apparent behavioral changes or mortality up to 24 hour in mice at all tested doses.

### **2- Effect on body weight:**

The obtained data revealed that body weight was significantly increased in all treated group (2<sup>nd</sup> to 9<sup>th</sup>) week as compared to control group (Table, 1).

### **3- Effect on male fertility:**

The effect of different date palm extracts on fertility of male was recorded (Table, 2). There was significant increase in sperm motility, sperm count, FSH, LH and testosterone. A decrease in sperm abnormalities percentage in groups treated with alcoholic pollen (II), watery pollen (III), water fruits (VII) and watery pits (IX) extracts were recorded. These were reflected by increase in weight of testis and accessory genital glands) at most treated groups as shown in Table (3).

### **4- Effect on some liver and kidney function test:**

The obtained results revealed that watery fruits extract exerted a significant hepatoprotective and diuretic a effect. The alcoholic fruit extract exerted a hepatoprotective action, as shown in Table (2).

### **5- Histopathological findings:**

Examination of liver, kidney and male genital system showed that rats treated with alcoholic and watery pollen grains, watery fruits and watery pits extracts nearly had similar picture.

- **liver** showed normal central veins, hepatic sinusoids and hepatocytes. (fig. 1).
- **Kidney** showed normal cortical and medullary blood vessels and renal tubular epithelium. (fig. 2).
- **Testis** The seminiferous tubules were dilated with the presence of normal spermatid radial orientation.(fig. 3 a and b).
- **Epididymis** Showing excessive proliferation of the layers till stage of stratification and aggregation of sperms in lumen occurred (fig. 4a and b).
- **Prostate gland** Prostate showed excessive proliferation and projected as folds in the lumen secretions (fig. 5a and b).
- **Seminal vesicles** Proliferation of Seminal vesicular epithelium with excessive retained secretions (fig. 6 a and b).

## DISCUSSION

The present study on different date palm extracts (pollen grains, leaves, fruits and pits) revealed that all extracts exerted growth promoter action as reflected by significant increase in body weight gain beginning from the 2<sup>nd</sup> to 9<sup>th</sup> week after administration compared to control group. This provides a base for use of different date palm organs as constituent in formulation of fattening ration in animals. Pits were used as date palm seed meal as constituent in ration of chicken, sheep, fish and rats (*Kamel et al., 1981; Elgasim et al., 1995; Yousif et al., 1996 and Ali et al., 1999*).

Concerning the results of male fertility, the daily administration of 500 mg/kg b.wt of different date palm extracts for a consecutive 65 days in male rats produced an improvement in male fertility picture especially in rats treated with alcoholic and watery pollen, watery fruit and watery pits extracts.

The alcoholic and watery pollen grains treated rats showed a significant enhancement in sperm motility 76.6% and 78.2% compared to control rats 74.8%. There was a significant increase in sperm count  $235 \times 10^6$  and  $248.6 \times 10^6$ /epididymis also significant increase in percent of a live sperms 91% and 92% compared to control group(  $196.2 \times 10^6$  / epididymis and 86.8%). These results were coincident with the significant increase in serum levels of FSH, LH and testosterone also with increase weight of testes, epididymis, seminal vesicle and prostate these results were confirmed to it with the histopathological picture. These results are in agreement with report of *Soliman and Soliman (1958)*. They recorded that date palm pollen grains possess a gonad stimulating potency. Also *El-Ridi et al. (1960)* demonstrated that the presence of FSH & LH in pollen gains, cause an enlargement of seminiferous tubules containing sperms compared to control when injected into immature male rats. Similar findings were showed on using aqueous pollen suspension in rats (*Bahmanpour et al., 2006*) as it improves sperm parameters, increases weight of testes and epididymis. This could be explained on basis that date palm contains flavonoids components (*Bennet and Heftmann, 1966 and Mahran et al., 1976*) that have positive effect on sperm quality (*Kostyuk et al., 2004 and Vayalil, 2002*). Also pollen posses gonadotrophin action ,which might be due to presence of gonadotrophin like substance or steroid component present in date palm pollen (*Miura et al., 2003 and Nayernia et al.,, 2004*).



Concerning the effect of fruits extracts, the watery extract was more effective than alcoholic extract resulted in significant improvement in sperm motility (76.6%, 74.8), sperm count (230.6,  $6.2 \times 10^6$ /epididymis) as well as significant increase in live percent (91.8, 86.8%) for watery fruits and control groups, respectively. These results coincident with significant increase in weight of testes, accessory genital glands and increase in serum level of FSH, LH and testosterone. These results were agreed with finding of gonadotrophic activity of watery extract date fruits palm in mature male rats that produce significant increase in FSH, LH and testosterone serum level as well as significant in weight of sex organs (*El-Mougy et al., 1991*). Also, the present results of inducing an increase in sperm count and motility agreed with finding that date extract increase, G. pig sperm count and motility (*Omar and Shanawany, 1986*).

The present results showed that alcoholic and watery fruits extract caused significant lowering in ALT. This may serve as hepatoprotective effect. This consistent with finding of ameliorative activity of aqueous date extract on carbon tetrachloride that induce a hepatotoxicity in rat (*Al-Qarawi et al., 2004*) which could be explained on basis of date flavonoids and vitamine c content through inhibition P450 aromatase enzyme (*Kowalska et al., 1990*). Also it may play a role as hepatoprotective. Liver cytochrome P450 was significantly reduced in ascorbic acid deficient G. pigs (*Rikans et al., 1978*).

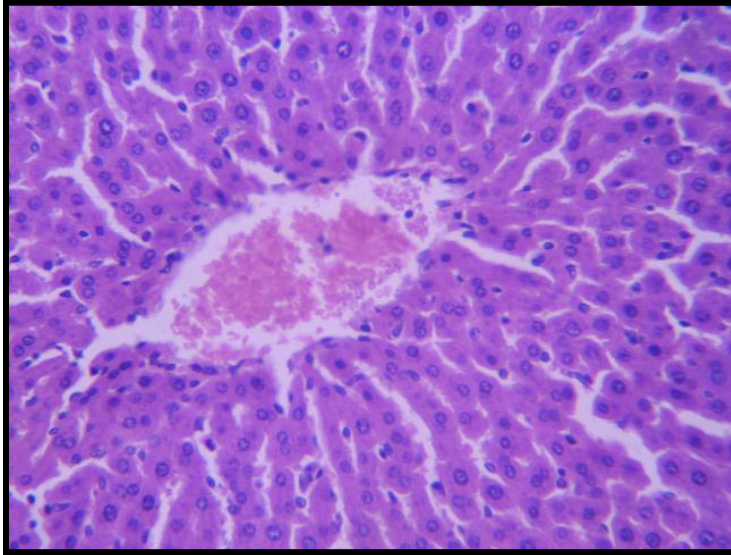
Concerning our the present results showed that watery pits extract induced a significant increase in sperm count (238,  $196.2 \times 10^6$ /epididymis) with increase in weight of sex organs as well as increase in serum level of FSH, LH and testosterone as compared to control group

.These findings were agreed with the report recorded that pits significantly increase level of LH and testosterone. Also increase weight of testes (*Ali et al., 1999*) and sperm count may be due to stimulatory effect of testosterone on testis.

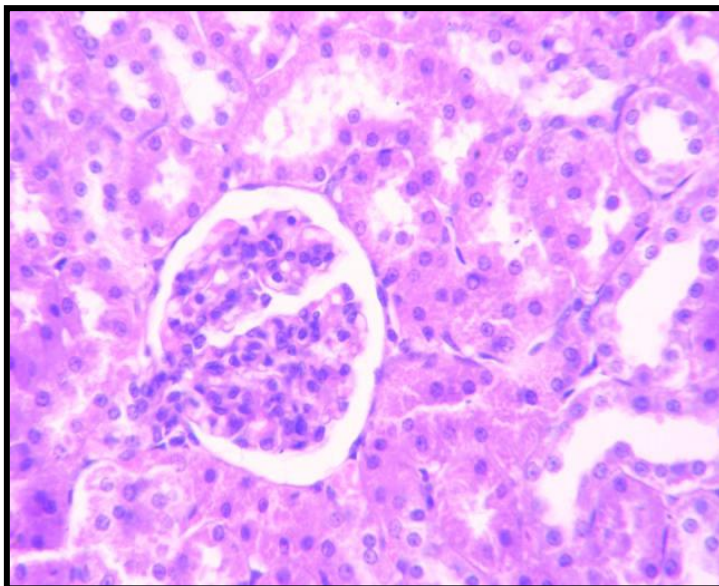
We could conclude that date palm pollen grains, fruits and pits may be beneficial in treatment of infertility in albino rats.



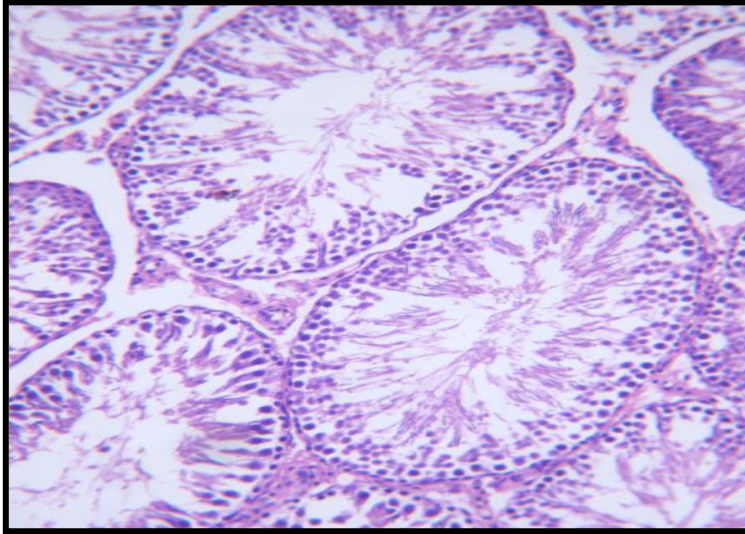




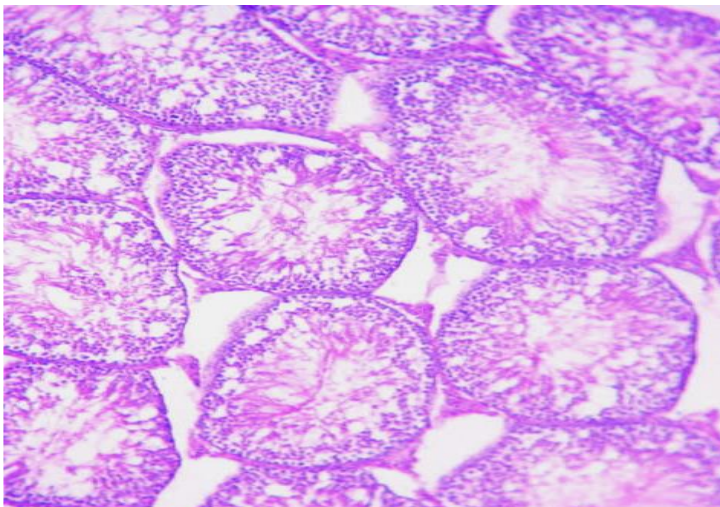
**Fig.(1):** Rat liver after 9 weeks Post-administration of different extracts showing no changes(H&E x100)



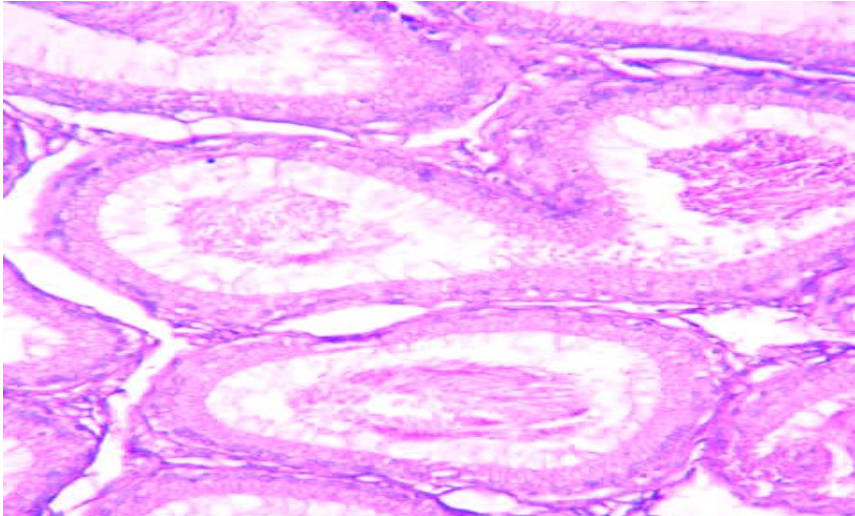
**Fig.(2):** Rat kidney after 9 weeks post- administration of different extracts showing no changes (H&E x100)



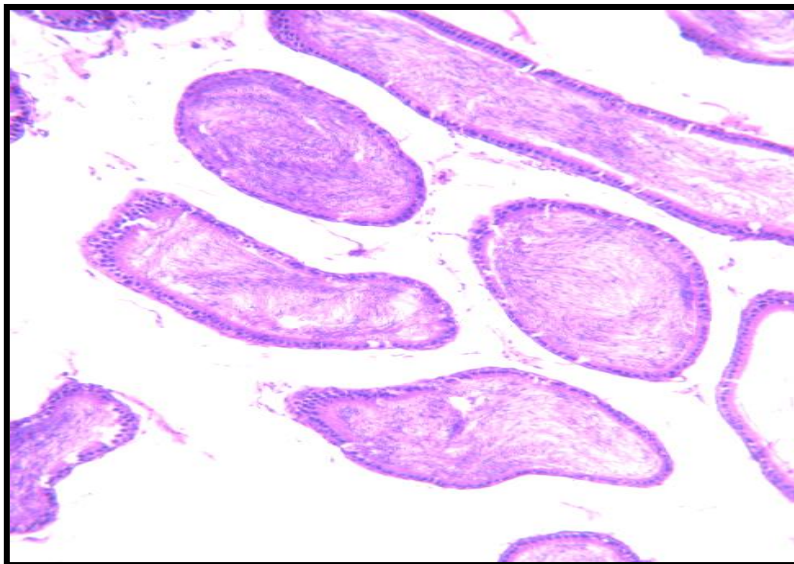
**Fig. (3a):** Rat testes of the control group showing the different layers of germinal epithelium (H&E x 200)



**Fig. (3b):** Testes of treated rat showing dialated seminiferous tubules with radial oriantation of spermatid x200

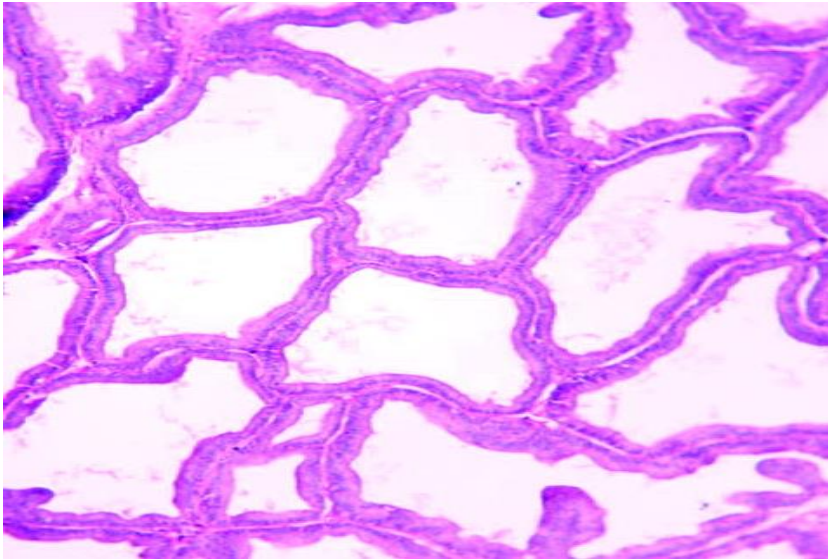


**Fig. (4a):** Normal epididymis of the control rat (H&E x 200)

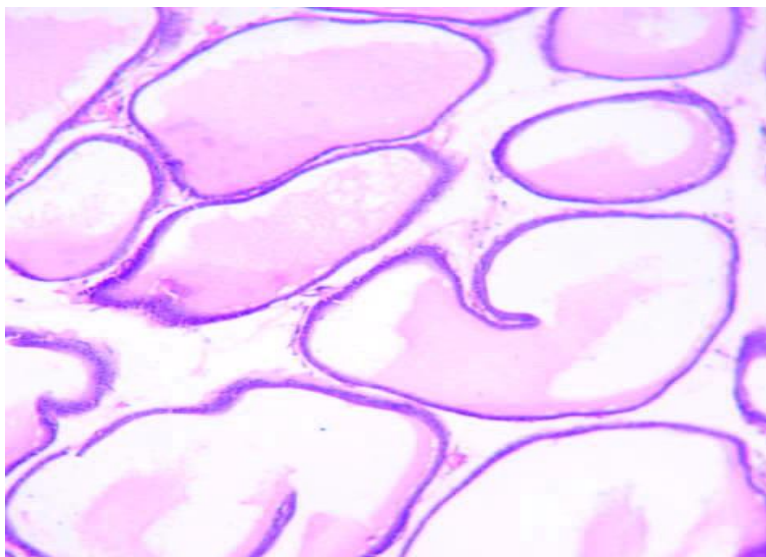




**Fig. (4b):** Epididymis of treated rats showing normal structure with the presence of large number of sperms in the lumen of the duct (H&E x 200).



**Fig. (5a):** Prostate gland of control rat showing normal structure of acini (H&E x 200).

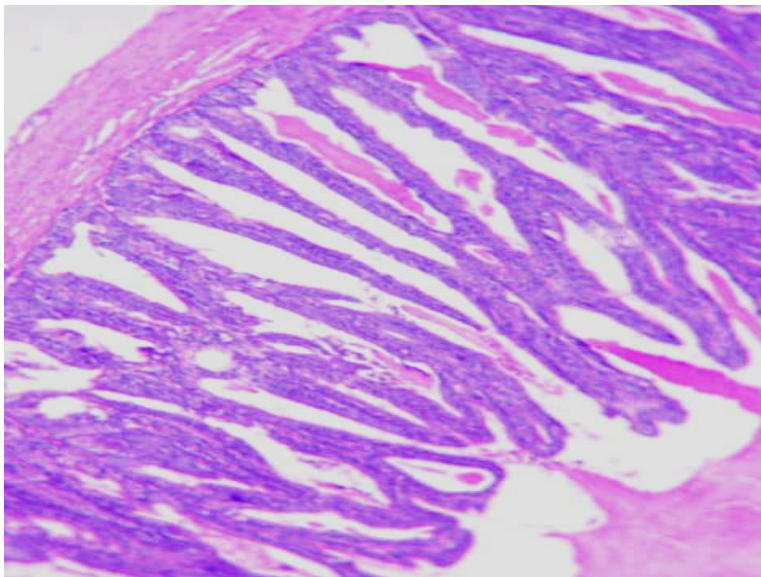




**Fig. (5b):** Prostate gland of treated rat showing normal structure of acini with excessive proliferation and excessive secretion (H&E x 200).



**Fig. (6a):** Seminal vesicles of control rat showing the normal structure of glandular tissue (H&E x 200).



**Fig. (6b):** Seminal vesicles of treated rat showing the normal structure of glandular tissue and proliferation epithelium with excessive secretion retained (H&E x 200).

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تأثير الخلاصات المختلفة لنبات فونكس داكتيليفيرا (نخل البلح)  
على الخصوبة في ذكور الفئران البيضاء

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وقد أجريت هذه الدراسة لتبحث تأثير الخلاصات المختلفة لنبات نخل البلح (طلع النخل ، الأوراق ، البلح ، والنوي) على الخصوبة في واحد وثمانون فأراً حيث تم تقسيمهما الي تسع مجاميع بكل منها 9 فئران. ثم تم تجريع الفئران بجرعة 500 مجم / كجم من وزن الجسم عن طريق الأنبوب المعدي يومياً لمدة 65 يوماً متتابعة و بعد نهاية الفترة المحددة للتجريع ( 65 يوماً ). تم تقييم وزن

الجسم و وزن الأعضاء التناسلية ونسبة حركة الحيوانات المنوية وعددها والأشكال الغير طبيعية منها وكذلك قياس , LH,FSH الكرياتينين و ALT, والتستوستيرون في المصل. وكذلك دراسة قطاعات من أنسجة الكبد والكلي والأعضاء التناسلية للفئران المعالجة .

وقد أظهرت الدراسة زيادة معنوية في وزن الجسم مع معظم الخلاصات مقارنة بالمجموعه الضابطة كما زادت معنوياً حركة الحيوانات المنوية وكذلك عددها وقلت الأشكال الغير طبيعية منها وزادت هرمونات LH,FSH والتستوستيرون وذلك في الفئران المعالجة بالخلاصة الكحولية والمائية لطلع النخل و الخلاصة المائية لكل من البلح والنوي .

كما سببت الخلاصة المائية والكحولية لثمار البلح انخفاض في مستوي ALT مقارنة بالمجموعة الضابطة مما سبق يتضح أن خلاصات نبات نخل البلح يمكن أن تستخدم في زيادة الخصوبة بدون آثار جانبية.