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The Therapeutic Potential Of Date Palm Pollen For Testicular Disorders Induced By Diabetes In Male Diabetic Rats

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ABSTRACT

Diabetes mellitus (DM) is the main cause of large-scale morbidity and mortality. This syndrome has adverse effects on all physiological systems, including the male reproductive system. It affects a large number of men of reproductive age and causes serious reproductive disorders. This study was aimed to examine the protective effect of date palm pollen (DPP) on diabetes-induced testicular disorders in male rats. Rats were divided into 4 groups. The first group represents the negative control, while the second group is the positive control. In contrast, the following two groups represent the treated groups of DPP suspension with a concentration of 130 mg/kg and 160 mg/kg for 4 weeks. At the end of the experiment, the rats were anesthetized, the epididymis and testis were extracted. The positive control group significantly decreased body weight, sex hormones, and sperm count. However, a significant increase in fasting glucose and the number of abnormal sperm. The histological results confirmed tubular degeneration, Leydig cell atrophy, and tubular dilation. Decrease the basement membrane, the semenforuos epithelium, and tubular diameter, while the diameter of the lumen increased. Treatment with DPP suspension showed a significant decrease in the level of fasting glucose and the number of abnormal sperm. While the number of sperm increased significantly. The results of the histological study confirmed that the Seminiferous Tubules treated with DPP were intact, the germinal epithelium dense, seminiferous tubules did not expand. We concluded that the DPP has an influence on protecting testis from diabetes complication.

الإمكانية العلاجية لطلع نخيل في علاج تلف الخصية الناجم عن مرض السكري في ذكور الجرذان المصابة بالسكري

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الكلمات المفتاحية:
طلع النخيل
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تكوين الحيوانات المنوية
معايير الحيوانات المنوية
الدراسة النسيجية

الملخص

داء السكري هو احد الاسباب الرئيسة للوفيات على نطاق واسع. هذه المتلازمة لها تأثير سلبي على جميع الأجهزة الفسيولوجية، بما في ذلك الجهاز التناسلي الذكري. ويؤثر على عدد كبير من الرجال في سن الإنجاب ويسبب اضطرابات تناسلية خطيرة. هدفت هذه الدراسة إلى دراسة التأثير الوقائي لطلع النخيل على تلف الخصية الناجم عن مرض السكري في ذكور الجرذان. تم تقسيم الفئران إلى 4 مجموعات. تمثل المجموعة الأولى المجموعة الضابطة السالبة والمجموعة الثانية هي الضابطة الموجبة، في حين تمثل المجموعتان التاليتان المجموعات المعالجة بمعلق بتركيز 130 ملغم / كغم و160 ملغم / كغم لمدة 4 أسابيع. في نهاية التجربة تم تخدير الفئران واستخراج البربخ والخصية. في المجموعة الضابطة الموجبة انخفض بشكل ملحوظ كل من وزن الجسم، الهرمونات الجنسية، عدد الحيوانات المنوية. كما عانت هذه المجموعة زيادة كبيرة في معدل السكر الصائم وعدد الحيوانات المنوية غير الطبيعية. اظهرت النتائج النسيجية للمجموعة الضابطة الموجبة تفسخ في الانيبيبات، ضمور خلايا ليدج، توسع الانيبيبات. نقص سمك الغشاء القاعدي والظهارة المنوية وقطر الانيبيبات المنوية بينما زاد قطر تجويف الانيبيب. أظهر العلاج بمعلق طلع النخيل انخفاضًا ملحوظًا في مستوى السكر الصائم

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وعدد الحيوانات المنوية غير الطبيعية. بينما زاد عدد الحيوانات المنوية بشكل ملحوظ. في حين اظهرت نتائج الفحص النسيجي للخصية الانيبيبات المنوية سليمة، الظهارة الجرثومية كثيفة و لا يوجد توسع في الانيبيبات المنوية، استنتجنا من هذه الدراسة ان للطلع فدرة على حماية الخصية من مضاعفات السكري.

1-Introduction

Recent attention has been focused on reproductive dysfunction associated with diabetes, as more people are diagnosed and affected by diabetes at reproductive age [1]. Increasing evidence suggests a higher incidence of infertility and spontaneous abortion rates in the diabetic population due to dysfunctional spermatogenesis and about 50% of men with diabetes exhibit relative subfertility. The increased evidence suggests that the incidence of infertility and spontaneous abortion in diabetes patients is higher due to spermatogenesis dysfunction and that about 50% of diabetes patients have relative subfertility [2].

Three major pathways are involved in the pathogenic effects of hyperglycemia on damage caused by various organs: the circulation of the polycarbon pathway, the formation of advanced glycation end products (AGE), and the activation of protein kinase C (PKC) by the synthesis of diacylglycerol (DAG). All of these reflect the excessive production of superoxide by the electron transport chain of the mitochondria, which in turn inhibits glyceraldehyde phosphate dehydrogenase (GAPDH) and ultimately causes organ damage [3]

The damage caused by high levels of glucose in the testis includes sperm DNA damage, testicular oxidative stress, mitochondrial impairment, hypothalamic pituitary gonadal axis dysfunction, and factors such as increased advanced glucose end products [4]. However, it is not yet clear whether these findings can be replicated in human clinical samples. Therefore, there are no targeted drugs that directly restore sperm development in diabetic male patients [2].

Date palm pollen (Phoenix dactylifera) is a natural product from palm trees grown in different Arab regions and collected at the end of spring. [5]. It was used as a regeneration factor and a dietary supplement around the world [6]. DPP consists of 31.11% crude protein, 20.74% crude fat, 1.37% crude fiber, 13.41% carbohydrate, 28.80% moisture, and 4.57% ash, as well as 57.9 mg essential oil/g total phenolic content [5]

It has been utilized by the initial Chinese and primeval Egyptians to treat infertility in men [6]. Experimental studies have revealed that DPP increases both sperm count and quality. Phytochemical studies showed that the presence of flavonoids, sterol derivatives, and amino acids[7], estradiol, and several types of vitamins, such as vitamin A, vitamin E, and minerals such as manganese, zinc, and selenium may be responsible for the pharmacological effect of DPP. Indeed, these ingredients can increase the levels of LH, and testosterone consequently leads to improve sexual function [8].

2- Materials and methods

2.1- Plant Material

DPP powder was collected, identified, and authenticated at the Department of Botany, Sebha University Herbarium. The collected powder was kept in the dark bottle under 4°C until used.

2.2- Preparation of DPP suspension

The DPP suspension was freshly prepared daily in two different concentrations (130 mg/kg and 160 mg/kg) by mixing DPP and distilled water by using a magnetic stirrer for 10 minutes to get a homogenous mixture[9] and [10].

2.3- Animal and experiment design

Fourth, adult Western albino rats Weight 200g were used in this study. All rats were obtained from the animal house in the faculty of the Pharmacy, University of Misurata, under constant environmental conditions. With an alternating 12 h light/dark cycle. The animals were supplied with fresh water and fed with standard pellets.

2.3.1- Induction of diabetes experimentally

Streptozotocin (STZ) (Sigma-Aldrich Egypt) was used for induction of diabetes mellitus in rats (25-0.45 mg/kg) by intraperitoneal injection in fasted rats (6-8 hours). The animals were considered diabetic if the blood glucose rate exceeded 200 mg [11].

2.3.2- Experimental design

Fourth, adult male rats were divided into four groups (n = 10) as follows:

Group I: Healthy rats received the equivalent volume of vehicle (distilled water) daily by oral gavage for four weeks (negative control).

Group II: Diabetic rats received the equivalent volume of vehicle (distilled water) daily by oral gavage for four weeks (positive control).

Group III: Diabetic rats were treated with DPP suspension (130 mg/kg) by oral gavage for four weeks.

Group IV: Diabetic rats were treated with DPP suspension (160 mg/kg) by oral gavage for four weeks.

2.3.3- Measuring body weight and fasting blood glucose level

The body weight was taken weekly at the end of each week for four weeks. The fasting blood glucose of rats was measured by an On-Call Plus® blood glucose meter. All rats fasted overnight after taking a drop from the end of the tail and placing it in the designated place in the device. This process was repeated weekly for a period of 4 weeks [12].

2.3.4- Sperm Sample Collection and preparation

After four weeks, rats were anesthetized with Hallotan, sperm samples were obtained from the cauda epididymis by cutting the epididymis with anatomical scissors in 5ml of pre-warmed (37^0C) physiological saline and incubated for 15 min. to allow the sperms to become motile and swim out from the right epididymis [13] and [14]. Sperm count was examined by taking from the sperm sample suspension and then inserted into the space between the cover glass and haemocytometer and the number of sperms was counted with a light microscope at the magnification of 400 × [15] and [16]. Sperm abnormal morphology was evaluated using eosin-nigrosin by mixing 20 µl sample of the sperm suspension with 7 µl eosin on a glass slide. A total of 200 sperm cells were counted on each slide under a light microscope at 400 × magnification. Sperms with abnormal head and/or tail were considered abnormal [13],[17] and [18].

2.3.5- Testis harvest and tissue processing for light microscopy

The testes were removed and cleaned of accessory tissue, taking care to handle the specimens to minimize trauma to the delicate seminiferous tubules. The testis was fixed with modified Davidson's fluid (MDF) for 48 hours. Prior to placement of the testis into the fixative, the tunica albuginea was shallowly pierced at each pole 5 times with a 21-gauge needle to aid in the penetration of the fixative, then briefly washed in tap water before being transferred to 10% neutral buffered formalin for storage prior to trimming and processing [19], [20] and [21].

The testis tissues were cut into pathological sections and stained using a routine hematoxylin–eosin staining method. Stained sections were studied by Carl Zeiss light microscopy (Axioskop Plus, magnification ×10, × 40, and × 100) to assess spermatogenesis and histopathological studies. Johnsen's score was used to categorize the spermatogenesis[22] [23], morphometric analysis of seminiferous tubular [24] [25], Recommended terminology for histopathological findings [26] [27] and Assessment severity grading of spermatogenic changes [28].

2.4- Statistical analysis

Data obtained from laboratory tests were entered, edited, and analyzed using the statistical software SPSS® version 20. Results represent the mean ± standard deviation. One-way analysis of variance (ANOVA) with the least significant difference (LSD) test was conducted to determine the significant differences between groups. A value of $P<0.05$ was considered statistically significant.

3- Results

3.1- Effect of DPP on progressive Body weight

The results showed that the treatment with DPP significantly increased body weight compared with the positive control group over a period of 4 weeks (Table 1).

3.2- Effect of DPP on Blood Glucose Level

The glucose level in the diabetic groups treated with DPP decreased significantly compared with the positive control group (Table 2).

(Table 1) effect of DPP on progressive body weight.

GROUPS	1ST WEEK	2ND WEEK	3RD WEEK	4TH WEEK
PC	242±46.15	228±51.40	198.7±40.9	161.7±8.4
NC	199±25.07	213±15.45	225.7±23.4	244±14.6
DP 130	279.2±101.05	216±44.69	258.4±26.14	272±35.5
DP 160	286.7±112.37	305.7±74	307.7±58.36	313.5±48.5
P-VALUE	0.112	0.007	0.109	0.031

PC: Positive control ،**NC**: Negative control, **DPP 130**: date palm pollen 130 kg/mg, **DPP 160**: date palm pollen 160 kg/mg.

3.3- Effect of DPP on sperm count and sperm morphology

The results show that the treatment with DPP increases sperm count significantly ($P < 0.05$) compared with the control group. Sperm abnormal morphology (Fig. 1) was significantly lower in the treatment group compared with the positive control group, which had higher sperm abnormal morphology. The results of sperm examinations are summarized in Table 3.

(Table 2) effect of DPP on blood glucose level.

GROUPS	1ST WEEK	2ND WEEK	3RD WEEK	4TH WEEK
PC	535.7±47.2	451.7±25,06	412.50±34.9	424.50±29.7
NC	62±4.91	70±4.06	65.25±5.87	68.75±2.86
DP 130	357.6±195.1	232.6±104.6	100.6±33.9	83.8±65.5
DP 160	365±169.1	164.7±31.2	122.5±39.8	69.7±12.01
P-VALUE	0.010	0.004	0.009	0.000

PC: Positive control ،**NC**: Negative control, **DPP 130**: date palm pollen 130 kg/mg, **DPP 160**: date palm pollen 160 kg/mg.

(Table 3) effect of DPP on sperm count and sperm morphology.

GROUPS	SPERM COUNT	ABNORMAL MORPHOLOGY
PC	1.60E+06±2.53E+06	179.75±5.37
NC	1.78E+07±7.03E+06	45±3.34
DPP 130	2.12E+07±9.89E+06	40±6.16
DPP 160	7.65E+06±5.17E+06	42.75±6.01
P-VALUE	0.014	0.00

PC: Positive control ،**NC:** Negative control, **DPP 130:** date palm pollen 130 kg/mg, **DPP 160 :** date palm pollen 160 kg/mg, **1.60E+06±2.53E+06:** 1600000±2533416, **1.78E+07±7.03E+06 :** 17800000±7025667, **2.12E+07±9.89E+06:** 9891814 ± 21240000**, 7.65E+06±5.17E+06:** 5170106 ± 765000.

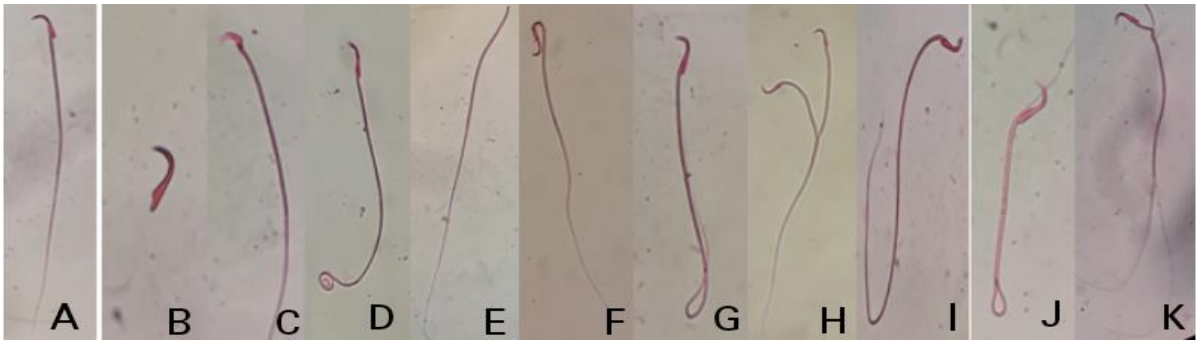

Fig. 1. shows sperm morphology. **A**: Normal, **B** :Tailless, **C:** Amorohous Head, **D** :Banana Head, **E**: Headless, **F** : Ring Shaped Head, **G** : Coiled Tail, **H** :Two Heads, **I** : Bent Tail, **J** : Hairpin Loop, **k** : Two Tails.

3.4- Spermatogenesis evaluation

Spermatogenesis was assessed using the Johnsen's score, as well as morphometric analysis of the seminiferous tubules.

The results of the Johnsen's score showed a significant difference (P-value 0.001) between the positive control and treated animals with DPP (Table 4), scores of 10 and 9 were found more frequently in the treatment group. No cross-sections showed a Johnsen's score of less than 6.

The basement membrane, the semenforuos epithelium, tubular diameter, and diameter of the lumen were measured. The results of these morphometric evaluations of seminiferous tubules showed a significant difference between the positive control and treated animals with DPP (Table 5).

(Table 4) the results of the Johnsen's score.

GROUPS	JOHNSEN'S SCORE
PC	3.16±0.84
NC	9.49±0.14
DPP 130	9.38±0.12
DPP 160	9.06±0.30
P-VALUE	0.001

PC: Positive control, **NC**: Negative control, **DPP 130**: date palm pollen 130 kg/mg, **DPP 160**: date palm pollen 160 kg/mg.

(Table 5) morphometric evaluations of seminiferous tubules.

Groups	Basement Membrane	Lumen Diameter	Semenforuos Epithelium	Tubular Diameter
PC	12.91±5.7	626±269.45	165.31±28.40	567.05±163.94
NC	47.84±1.36	240.20±40.82	659.44±32.25	1489±52.66
DPP 130	43.13±1.70	335.98±45.63	557.89±13.52	1388.52±32.61
DPP 160	44.75±3.93	356.05±63.04	635.77±25.59	1480.04±123.46
P-value	0.001	0.001	0.001	0.001

PC: Positive control, **NC**: Negative control, **DPP 130**: date palm pollen 130 kg/mg, **DPP 160**: date palm pollen 160 kg/mg

3.5- Recommended terminology for histopathological findings.

The histopathological results of the negative control group and the treated group with DPP suspension showed that the seminiferous tubules were intact, the germinal epithelium was dense and contained layers of germ cells as well as spermatocytes attached to the basement membrane, and the sertoli cells were visible, and attached to the sperm cells. Interstitial tissues and Leydig cells were normal and showed no decrease or increase in size. The seminiferous tubules did not suffer from expansion, and the testicular network appears normal. (Fig. 2).

The seminiferous tubules of the positive control group were severely damaged (Fig. 3) as a result of diabetes, which made it impossible to determine the stages of spermatogenesis, only spermatocytes appear in these tubules, some of these terms depend on the type of stage and the spermatogenesis process was not used.

The results of the histological examination in the positive control group showed the presence of tubular degeneration/atrophy, where apoptosis was observed in germ cells and multinucleated giant cells (Fig. 4), exfoliation or descent of germ cells into the tubule lumen (Fig. -5), germinal macro-vacuolation and Sertoli cell Micro-vacuolation vacuoles (Fig. -6), decrease in the diameter of the seminiferous tubule (Fig. -3), dilatation of the blood vessel (Fig. -6) Most of the tubules contain only Sertoli cells (Fig. 4).

The results showed that interstitial tissue in the positive control group severity damaged. The Leydig cell cytoplasm appeared fairly and damaged (Leydig cell atrophy) (Figures 4 and 6). While the samples of the treatment group were healthy Leydig cells and the interstitial tissue, in general, was normal and did not have any abnormalities (Fig. 2).

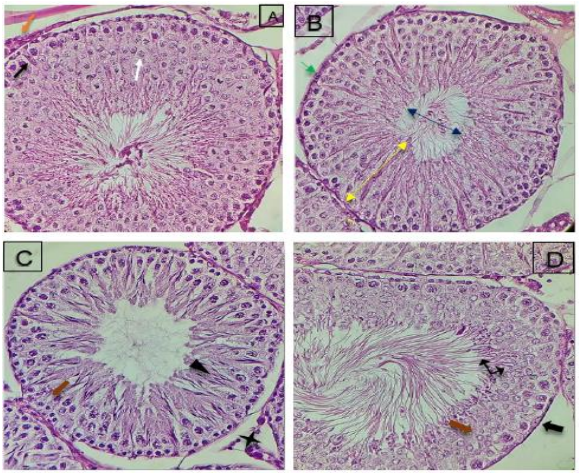

Fig. 2. shows the composition of the seminiferous tubules **A**: negative control groupa , **B** and **C**: DPP group, 160g/mg, **D**: DPP group, 130 kg/mg. Sertoli cells (black arrow), Round Spermatids (white arrow), interstitium (orange arrow), tubular lumen (double blue arrow), seminiferous epithelium (double yellow arrow), basement membrane (green arrow), Spermatocytes (double black arrow), Elongated Spermatids (arrowhead),leydig cell (star), 500x.

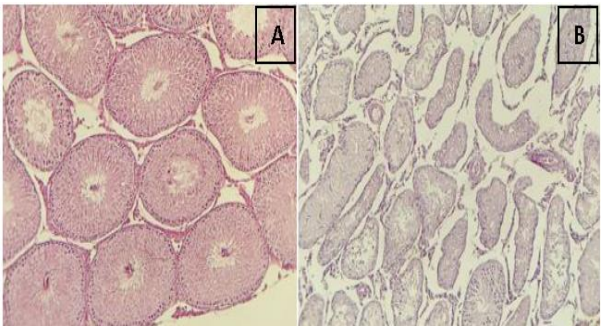

Fig. 3. shows a cross-section of testis **A**: negative control, group, **B**: DPP group (200X).

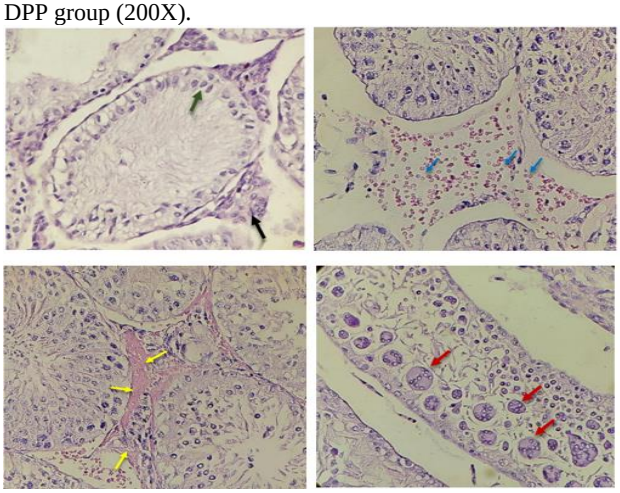

Fig. 4. shows seminiferous tubules in the positive control group. Seminiferous tubules containing only Sertoli cells (green arrow), Leydig cells with fair cytoplasm (black arrow), Multinucleated giant cells (red arrow) and Inflammation (yellow arrow), hemorrhage in the testis containing echinocytes (blue arrow). 500X.

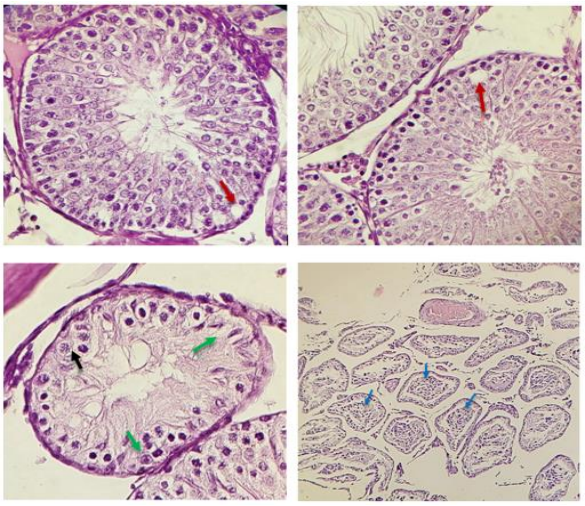

Fig. 5. shows macro-vacuolations (red arrow) and seminiferous tubules containing only Sertoli cells (green arrow) and spermatocytes (black arrow) and Exfoliation germ cells (blue arrow) 500X and 200X.

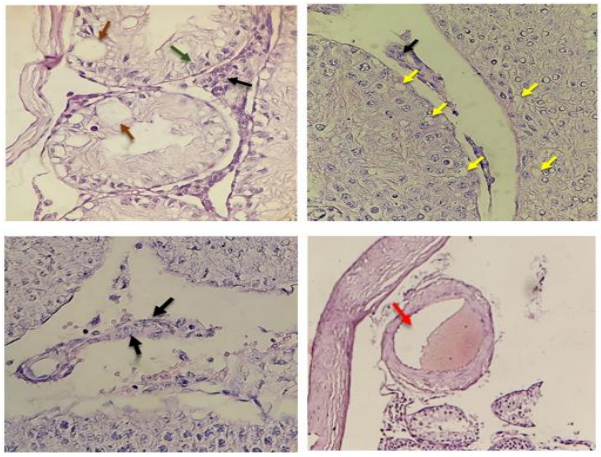

Fig.6. shows samples of the positive control group containing macro-vacuolation (brown arrow), Sertoli cell vacuoles or micro-vacuolation (yellow arrow), Leydig cells containing fairly cytoplasm (black arrow) and blood vessel dilated (red arrow) 500X.

3.6- Assessment severity grading

A general guide for severity grading of spermatogenic changes depends on the severity of changes in the spermatogenesis process based on the number of affected tubes. The seminiferous tubules in the samples of the treated groups were uninfected and appeared normally. The histological examination of the positive control group (Figs. 4 and 6) showed that most of the seminiferous tubules were damaged (80-100%) and were given a score of 5 (severe).

Identifying the histopathologic consequences of severe endocrine disruption. Sensitivity of the finding as an endpoint of endocrine disruption is provided to aid the investigator in deciding what is "within the normal range" and what the most sensitive indicators of endocrine disruption are.

Spermatid Retention: One of the earliest and most sensitive indicators of low intratesticular testosterone. In the samples of the positive control group, which suffered from severe damage, the presence of spermatid cells was not observed in the germinal epithelium.

Degeneration of Round spermatids and Pachytene spermatocytes: Despite the inability to determine the stage of spermatogenesis, these cells were observed in the positive control samples (Fig. 7), which indicates that they suffered from a severe decrease in testosterone.

Degeneration/depletion of elongated spermatids: The occurrence of this damage is an end-stage lesion of low intratesticular testosterone. In the positive control group samples, there was no presence of these cells in the seminiferous tubules, which only contained spermatocytes cells.

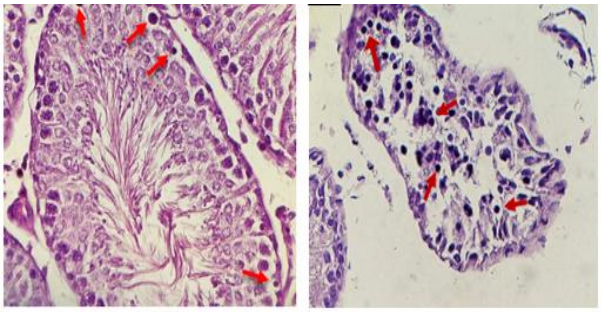

Fig.7. shows the pachytene spermatocytes in the positive control group (500X).

4- Discussion

Long-term diabetes causes male infertility through mechanisms that occur at three levels. The first mechanism occurs at the pre-testicular level, in which the patient initially develops hypogonadism. The second mechanism occurs at the testicular level; diabetes causes increased oxidative stress. The third mechanism occurs at the post-testicular level, where diabetes causes sperm damage and/or prevents semen release [29].

Medicinal plants and their active constituents can attack many different pathways of hyperglycemia, thus helping to regulate the level of glucose. In addition, they are inexpensive and safe, although the therapeutic use based on the evidence of many plants is undersized, and many plants have proven effective due to their antidiabetes effect that has been verified either experimental or clinical [30].

The weight of the body gradually decreases during the second week of STZ injection, as STZ accumulates selectively in the beta cells by a glucose transporter (GLUT 2) in these cells' membranes, after entering them, causing damage to DNA by the alkylation of the DNA by methyl nitrogen, which in turn leads to DNA fragmentation [31]. The recovery of body weight in the treatment group is believed to be achieved by the DPP content of proteins, carbohydrates, and fat [32]. DPP also contains amino acids [33] and high calcium concentrations required for the growth and development of the skeletal system [7].

As a result of insulin insufficiency or insulin resistance, diabetic patients suffer from intracellular hypoglycemia and extracellular hyperglycemia as a result of impaired glucose uptake by cells or due to elevated levels of hepatic Glucose-6-phosphatase (G6Pase), which catalyzes the glycogenolysis and gluconeogenesis [34] and [35]. The results showed a gradual decrease in the fasting glucose level, starting from the second week of the experiment, of the treated groups compared to the positive control group.

The ability of DPP suspension to adjust the fasting glucose levels may be due to the presence of many compounds that attack intestinal enzymes (α-glucose enzymes and α-amylase), such as Kefmerol, Quercetin, and Tannin [36]. Chlorogenic acid, which differentiates and proliferates beta cells [37] and inhibition of G6Pase in rats which reduces hepatic gluconeogenesis and glycogenolysis [38]. As well as quercetin and gallic acid, which protect against the harmful effects of ROS, in addition to many phenolic and flavonoid compounds that reduce oxidative stress, inflammation, and protecting beta cells from apoptosis, thus enhancing insulin secretion [36]. Both [39] ،[32] ،[7] and [40] studied the DPP content of these compounds, and the results of these studies showed that DPP contained high concentrations of them.

DM damages sperm through two main mechanisms: 1) an endocrine disorder that causes an imbalance in sex hormones as a result of steroidogenetic defects in Leydig cells. 2) Oxidative stress that damages DNA sperm, lipid peroxidation, and apoptosis [41]. Sperm cells contain a high amount of specific lipids (polyunsaturated fatty acids, plasmalogen, and sphingomyelin). This high amount of lipids without an antioxidant mechanism makes sperm more susceptible to oxidative damage, where DM is related to the overproduction of ROS that prompt testicular injury [42] and [43]. Moreover, sperm cytoplasm contained insufficient concentrations of scavenging enzymes [44].

The positive results obtained from treated by DPP suspension may be attributed to the fact that it contains phytoestrogens that can improve

reproductive function and increase gonadal activity in rat models [45]. Furthermore, DPP mainly contains cholesterol, rutin, carotenoids, and estrogen, which are known to exhibit gonadotropin activity in the rat [46].

DPP contains minerals, flavonoids, glycosides, alkaloids [39], vitamins A and C, proteins, and carbohydrates [47]. These substances may have different biological effects on spermatogenesis [48]. Flavonoids can act as agonists of the estrogen [49]. Whereas an alkaloid elevates testicular cholesterol in the testis [46]. DPP also contains a useful amount of zinc, making the DPP to be related to the stimulation of sperm motility and the progressive forward movement [7].

In this study, four histological criteria were used to evaluate the testis and link them with the obtained previous results and the reflection of those results on the testicular tissue. Johnson's scores were used to evaluate spermatogenesis. In the groups treated with DPP suspension, the results of Johnson's scores showed an improvement in the process of spermatogenesis (Table -4), while scores 10 and 9 were the most frequent (83.3%), which indicated that the seminiferous tubules are normal and exhibit spermatogenesis in different stages. There are all cell types including including Sertoli cells, spermatogonia, spermatocytes, round spermatids, and elongated spermatids. The results of the morphometric evaluations showed that the seminiferous tubules were normal and remained unaltered.

Terminology for histopathological findings which used to describe the histological sections were used with the samples of the positive control group, while the groups treated with PDD suspension did not need to be used because there was no evidence of any damage. It is believed that the tubular degeneration/atrophy in the positive control group occurred as a result of cytotoxicity (as a result of oxidative stress) and hormonal disruption [27]. Positive control groups suffered from apoptosis and androgen deficiency. Thus, we are believed that the degeneration of germ cells in this group is due to these reasons [50].

The histological finding of the positive control group showed a multinucleated giant cell. The appearance of these cells (Fig. 12) may be due to a deficiency in androgens and estrogens, which play an important role in the function of Sertoli cells [51] and [27]. Macro and micro vacuolation is generally an early morphological indicator of disturbance to the Sertoli cell. The vacuoles may be intracellular or intercellular and in both cases probably reflect a disturbance in the fluid balance of the Sertoli cell [27].

Leydig cell atrophy is detectable morphologically only when steroidogenesis has been severely decreased. The resulting androgen deficiency causes a decrease in the size and weight of the accessory sex glands and epididymides, which are androgen-dependent tissues. Leydig cell atrophy was accompanied by a decrease in spermatogenesis. (As illustrated by Johnson's score) and a decrease in weight of the sex organs [51].

DM induces iNOS production, which increases NO production by vascular endothelial smooth muscle cells, NO then interacts with other nitrogen and/or oxygen species, triggering oxidative stress. NF-κB, a transcription factor, serves as a critical link between oxidative stress, inflammation, and apoptosis. Upon activation by oxidative stress, NF-κB upregulates iNOS's level, leading to an increase in NO production. It is believed that the inflammation suffered by the positive control group is a result of oxidative stress and the occurrence of necrosis in the testis

Exfoliation of germ cells can be a specific response to certain Sertoli cell toxicants, which reflects a disturbance in the function of the Sertoli cell. Disturbance of Sertoli–germ cell intercellular junctions leading to loss of germ cell adherence to Sertoli cells. Positive control group suffered from a deficiency in androgens and estrogens, and thus a defect in the hypothalamic-pituitary-testicular axis may occur that maintains the formation of steroids, which in turn preserves the functions of Sertoli cells [52]

The mechanism for protection of testis by DPP suspension may involve phytoestrogens, which are essential materials for sex hormones, and thus play an important role in improving reproductive functions. DPP also contains flavonoids, Vitamin C, vitamins B1, B2, nicotinic acid, and vitamin A. Vitamin C is one of the antioxidants found in semen that scavenge free radicals [53]. Vitamin E, which is fat-soluble, inhibits the production of ROS in the sperm membrane [54]. In addition to the alkaloids, which may make the DPP play an essential role in the absorption and neutralization of free radicals and the quenching of mono-and triple oxygen or the decomposition of superoxide [53]. It also contains caffeic acid and catechins that provide protective benefits against lipid oxidation and may be helpful in preventing oxidative stress-related diseases such as diabetes [39].

5- Conclusion

The present results suggest that the DPP suspension administration improves sperm parameters and histology of testis in diabetic adult male rat, which lead to improvement spermatogenesis process. It also supports to the usage of DPP as a sexual function enhancing medicine. Thus, this study may prove to be an effective and safe alternative remedy in reproductive disorders. However, more studies are needed on the possible underlying mechanisms of action of DPP on spermatogenesis.

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