

## Effect of aqueous extract of Date Palm Pollen (DPP) on the sperm characteristic and Serum Testosterone, FSH and LH Values in albino male rats treated with sodium floride

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### Summary

The present study was undertaken to evaluate the effect of aqueous extract of Date Palm Pollen DPP on the testicular function and serum testosterone, FSH and LH hormones value. Thirty five male rats were divided randomly into five equal groups. Group 1: received 0.5 ml of distilled water (control group), group 2: was treated orally 0.250 p.p.m of sodium florid (NaF) (with volume of 0.5 ml / rat), Group 3: was treated with 0.250 p.p.m of NaF and 50 mg/kg. B.W. of DPP extract (0.5ml D.W \rat), Group 4: was treated with 0.250 p.p.m of NaF and 100 mg/kg. B.W. of DPP extract and Group 5: was treated with 0.250 p.p.m of NaF and 150 mg/kg. B.W. of DPP extract. The results showed significant ( $P < 0.05$ ) decrease in sperm concentration, motility and significant ( $P < 0.05$ ) increases in dead and abnormal sperm in the group 2 in comparison to control, while all groups of DPP extract showed significant ( $P < 0.05$ ) increase in sperm concentration, motility and decrease in dead and abnormal sperm. Maximum effect was observed in animals treated with a dose of 150 mg/kg of DPP extract, also the results revealed significant ( $P < 0.05$ ) increase in testosterone, FSH and LH hormones in groups treated with DDP in comparison to G1 and G2. Male rats received DPP for 50 days showed significant ( $P < 0.05$ ) increases in body and testes weight as compared to G1 and G2. In conclusion the results revealed that the aqueous extract of DPP pollen can be used as a sex enhancer and seems to cure male infertility.

**Keywords:** Date Palm Pollen, Testosteron, Rats, Sperm characteristic.

### Introduction

Medicinal plants continue to provide valuable therapeutic agents, both in modern and in traditional medicine (1). Traditional medicines are gaining importance and nowadays are being studied to find the scientific basis of their therapeutic actions (2). Plant-derived chemicals are used to relieve sexual dysfunction and they have sex enhancing potentials. These phytochemicals increase libido, sexual potency and sexual pleasure; and seeds are used to restore sexual potency. Pollen grains of date Palm were also used to promote fertility in fertile women (3). The use of herbal medicine has become popular worldwide especially in the Asian and African countries (4). The various parts of *Phoenix dactylifera* are used widely in traditional medicine for the treatment of various disorders which include memory disturbances, fever, inflammation, paralysis, loss of consciousness and nervous disorders (5).

Suspension of *Phoenix dactylifera* date palm pollen (DPP) is herbal mixture that is widely used as a folk remedy for curing male infertility in traditional medicine. Date palm fruit suspensions improve the sperm count, motility, morphology, and DNA quality with a concomitant increase in the weights of testis and epididymis (4). The aphrodisiac activity of aqueous extract of *Phoenix dactylifera* pollen grain on the sexual behavior in male rats in recent years has been suggested that oestrogen may be involved in the regulating renewal spermatogonial stem cells (6) and male reproductive tissue with oestrogen receptor (7).

Investigations have revealed that date Palm pollen extract contain oestrogenic materials as gonad stimulating compound that improve male infertility (8). It has been suggested that estrogen might be involved in regulating the renewal of spermatogonial stem cells and male reproductive tissues with estrogen receptors (7)

Investigations have revealed that palm kernels and date pollen grains extracts contain estrogenic materials as gonad stimulating compounds that improve male infertility. Reports have also pointed that isolation of micro elements from DPP has estrogen, sterols, and other agents that may influence male fertility (9). Experimentally, date extracts have been shown to increase sperm count in guinea pigs and to enhance spermatogenesis and increase the concentration of testosterone, follicle stimulating hormone, and luteinizing hormone in rats. Date palm pollen (DPP) cure male infertility by improving the quality of sperm parameters (4).

The date Palm pollen mainly contain cholesterol, rutin carotenoid as estrone which is known to exhibit gonadotrophic effect in rats. The date palm (*Phoenixd actylifera* L.) is considered the most important source of food for both humans and animals (10). Dates contain a high percentage of sugars reaching 88% in some varieties (11). Dates are also rich in mineral salts and vitamins for the date pit, the percentage of non-reducing sugars is 3.82% and in glucose and fructose is 1.68 and 1.53, respectively. In local medicinal practices, dates are considered a tonic; some consider it to be an aphrodisiac, and the flower of the plant is used as a purgative (12). Date pits have been included in animal feed to enhance growth, an action that has been ascribed to an increase in the plasma level of testosterone. Studies indicated that the aqueous extracts of dates have potent antioxidant activity (13). The antioxidant activity is attributed to the wide range of phenolic compounds in dates including p- coumaric, ferulic and sinapic acids, flavonoids and procyanidins (14). Therefore this experiment was designed to evaluate the role of DPP ameliorating the effect of NaF in adult male rats.

### Materials and methods

Date palm pollen grain is a small and oval shape gametocyte with a fine bark. After removing the bark, pollen grain washed with distilled water and then dried. Dried pollen grain was pulverized with a small electric blender. A hundred (100) gram of powder was extracted in

0.5 liter of warm distilled water (30° C) with constant shaking (magnetic shaker model). The solution was passed through a filter paper. After vaporizing of water, the resultant yield was reconstituted in normal saline to give the required doses of 50, 100, and 150 mg/kg body weight. Solutions were stored in refrigerator and protected from light at 4°C until use (15).

Thirty five male rats, aged 4-5 months, weighing 230±5g were obtained from the animal house of Biotechnology Research Center Al-Nahrain University. Animals were housed in a well-ventilated, temperature-controlled room at 22 ± 3°C with a 12 h light-dark cycle. They were provided with standard rat chow pellets ad libitum. Male rats were randomly divided into five groups (n =7). Group 1: served as control group and received 0.5 ml of distilled water, group 2: was administered orally 0.250 p.p.m of NaF (with volume of 0.5 ml / rat), Group 3: was administered orally with 0.250 p.p.m of NaF with volume of 0.5 ml / rat) and 50 mg/kg b.wt. of DPP extract (1ml/rat), Group 4: was administered orally with 0.250 p.p.m of NaF (with volume of 0.5 ml / rat and 100 mg/kg b.wt of DPP extract Group 5: was administered orally with 0.250 p.p.m of NaF (with volume of 0.5 ml / rat) and 150 mg/kg b.wt of DPP extract (15), for 50 consecutive days for each group, at the end of the of experimental sperm were collected by epididymis flushing (2ml of Roswell Park Memorial Institute-1640) to determine total count motility, viability and morphologically abnormalities of sperm. Viability and morphologically abnormalities were detected by smear stained with (1% eosin, 5% negrosin) on 37 °C slide. Body weight was determined at the end of the experiment.

Sperm motility and count: To determine sperm motility and sperm counts, caudal epididymis was minced in 2 ml of RPMI-1640 media. One drop of an evenly mixed sample was applied to a Neubauer's counting chamber under a cover slip. Quantitative motility expressed as an index was determined by counting both motile and immotile sperm per unit area. Epididymal counts was made by routine procedure and expressed as million/ml

of suspension. To determine Sperm viability and abnormal sperm: 50  $\mu$ l of freshly obtained epididymal sperm solution was thoroughly mixed with 10  $\mu$ l of eosin-nigrosin (Merck, Germany), viable and abnormal sperm was recorded according (16 and 17). Three milliliters of blood was drawn in 5 ml disposable syringe by cardiac puncture. It was allowed to coagulate at room temperature for one hour before centrifuging at a speed of 3000r/pm for 10 minutes. The clear, non-haemolysed supernatant serum was quickly removed divided into three portions for each individual, and stored at -20°C for the measurement of testosterone, FSH and LH using immunoassay technique.

**Hormones concentrations assay:** The concentration of FSH hormone was measured by Enzyme Linked Immuno Assay (ELISA) technique according to instructions of Bio Check, Inc (Foster city USA). The LH hormone was measured by ELISA technique according to instructions of Bio Check, Inc (Foster city USA). Serum testosterone was assayed using Coat-a-Count Radioimmunoassay kits (Active Testosterone RIA DSL-4000, Diagnostic System Laboratories Inc., Texas, USA). The amount of testosterone was expressed as ng/ml. Statistical analysis was performed to compare different groups using (ANOVA) –one way analysis of variance, Statical significance was differenced at  $p < 0.05$  (18).

### Results and Discussion

The results showed significant ( $P < 0.05$ ) increase in total count of sperm ( $69.10 \pm 2.70$ ,  $73.00 \pm 1.75$ ) and motility ( $80.75 \pm 2.40$ ,  $85.42 \pm 2.50\%$ ) in treated groups compared with 100,150 mg/kg of DPP and NaF groups respectively, compared to group treated with only NaF (total count  $54.60 \pm 2.00$  million/ml and motility  $55.23 \pm 1.30\%$ ). And there was a significant ( $P < 0.05$ ) decrease in dead sperm in all groups treated with DPP and NaF ( $20.54 \pm 1.60$ ,  $19.70 \pm 1.30$ ,  $19.70 \pm 1.30$ ) % respectively, compared to group treated with NaF only ( $30.10 \pm 2.11$ ) % (Table, 1). Also the results showed a significant ( $P < 0.05$ ) decrease

in abnormal sperm in all groups treated with DPP and NaF ( $15.34 \pm 1.70$ ,  $15.21 \pm 1.58$ ,  $10.44 \pm 1.52$ ) % respectively, compared to group treated with only NaF ( $20.32 \pm 1.76$ ) % (Table, 1).

The results of the present study reveal significant ( $P < 0.05$ ) increase in testosterone, FSH and LH hormone serum levels in treated group with 150mg/kg of DPP and NaF ( $2.30 \pm 0.43$  ng/ml,  $2.10 \pm 0.41$  mIU/ml,  $1.97 \pm 0.22$  mIU/ml) respectively, compared to control group ( $1.30 \pm 0.09$  ng/ml,  $0.91 \pm 0.07$  mIU/ml,  $0.83 \pm 0.033$  mIU/ml) and the group treated with NaF only ( $0.80 \pm 0.05$  ng/ml,  $0.60 \pm 0.04$  mIU/ml,  $0.67 \pm 0.2$  mIU/ml) respectively (Table, 2). Body weight of all animals treated with DPP and NaF ( $260.81 \pm 2.07$ ,  $266.15 \pm 2.10$ ,  $268.15 \pm 3.03$ ) g show a significant ( $P < 0.05$ ) increase compared to control group ( $255.96 \pm 2.64$ )g and group treated with NaF only ( $248.87 \pm 2.54$ ) g (Table, 3). Also the results reveal a significant ( $P < 0.05$ ) increase in testes weight of animals treated with DPP and NaF ( $4.95 \pm 0.50$ ,  $4.80 \pm 0.62$ ,  $5.48 \pm 0.50$ ) g and the control group ( $5.89 \pm 0.20$ ) g compared to the group treated with NaF only ( $3.80 \pm 0.11$ ) g (Table, 3). The gonadotrophic effects of date palm pollen activate pituitary gland to secrete luteinizing hormone which affect Leydig cells to secrete androgen and may be helped by follicle stimulate hormone to stimulated sertoli cells to produce androgen binding protein increasing (19). Luteinizing hormone (LH) and FSH have important role for spermatogenesis and may lead to sperm concentration in mice treated with DPP and improving sperm motility as a result of increasing testosterone level in the blood. Sodium fluoride cause sperm DNA damage due to programmed death of germ cells (apoptosis) leading to decline in sperm number (20). Experimentally, date extracts have been shown to increase sperm count in guinea pigs and to enhance spermatogenesis and increase the concentration of testosterone, follicle stimulating hormone, and luteinizing hormone in rats. Date palm pollen (DPP) cure male infertility by improving the quality of sperm parameters. Gauthaman and Adaikan (21) found

that date extracts increased sperm count in guinea pigs and increased the concentration of testosterone, follicle stimulating hormone, and luteinizing hormone in rats. Date pits have been included in animal feed to enhance growth, an action that has been ascribed to an increase in the plasma level of testosterone (22).

From the present results, it is clear that chronic oral administration of pits of date palm caused recovery effect on testicular of male albino rats; these results may indicate that pits of date palm had beneficial effects on male reproductive activity and improve sperm quality, and therefore enhance fertility of the male adult rats. Bahmanpour (4) found that the comparative evaluation between control and experimental groups revealed that consumption of DPP suspensions improved the sperm count, motility, morphology, and DNA quality with a concomitant increase in the weights of testis and epididymis of the reproductive content (23). Studies indicated that the aqueous extracts of dates have potent antioxidant activity (13). The antioxidant activity is attributed to the wide range of phenolic compounds in dates including p- coumaric, ferulic and sinapic acids, flavonoids and procyanidins (14). In recent years, it has been suggested that estrogen may be involved in regulating the renewal of spermatogonial stem cells (7). The presence of anti-oxidants substances like vitamin A, E, C in DPP prevents oxidations of the germ cells in seminiferous tubules may explain the decline of dead sperm in treated mice with DPP. The Increase in testosterone and oestrogen level in

the serum indicated the improvement of semen parameter (quantity and quality) because the renewal action of oestrogen in epididymis by water absorbance and then increasing the sperm concentrations (23). It was suggested that DPP has some androgenic activity and thus has the ability to increase the lean body mass and weight, thus body weight of the experimental and control animals were monitored. Oral administration of DPP suspension resulted in increased body weight (Table, 3). This weight gain may partly be attributed to the androgenic effects of testosterone as its levels increased; this is in agreement with the study carried out by (23) who reported that androgens have a major role in the growth and differentiation of many tissues. Protein synthesis for maintaining muscle mass and bone formation requires testosterone addition to the organs of reproduction; testosterone is the main hormone having nitrogen-retaining (anabolic) properties which increases lean body mass and body weight.

In conclusion the administration of aqueous extract of DPP (100 and 150 mg/kg body weight) for 50 days caused a positive effect on some fertility parameters and pituitary-testicular hormone axis especially in higher dose. Dose 150 mg/kg had greater activity in improvement of total count and motility, and reduction of dead and abnormal sperm of male rats. DPP can enhance the testosterone, FSH and LH hormones values and increases the body and testes weight. Sodium florid (NaF) caused a decrease in weight and hormones levels as well as influence negatively on some criteria of sperm.

**Table, 1: Effect of administration of DPP (50,100,150) mg/kg b.wt. on sperm parameters in male rats after 50 days treated with NaF (0.250 PPM).**

Groups Test	Control (Distilled Water) Mean± SE	NaF 0.250 p.p.m for 50 days			
		NaF + (D.W) Mean± SE	50mg/kg DPP Mean± SE	100 mg/kg DPP Mean± SE	150mg/kg DPP Mean± SE
Motility %	90.60±2.25 a	55.23±1.30 b	77.43±2.30 c	80.75 ±2.40 c	85.42±2.50 d
Dead sperm %	10.10±1.50 a	30.10±2.11 b	20.54±1.60 c	19.70±1.30 c	19.70±1.30 d
Concentration ×106	79.70±2.75 a	54.60±2.00 b	55.40±1.70 b	69.10±2.70 c	73.00±1.75 d
Abnormal sperm %	5.32±0.56 a	20.32±1.76 b	15.34±1.70 c	15.21±1.58 c	10.44±1.52 d

Different letters refer to significant (p<0.05) differences compared between groups.

**Table 2: Effect of administration of DPP (50,100,150) mg/kg.b.wt on serum testosterone, FSH and LH blood levels in male rats after 50 days treated with NaF (0.250 PPM).**

Groups	Control (Distilled water)	NaF0.250 p.p.m for 50 days			
		NaF+(D. W) Mean± SE	50mg/kg DPP Mean± SE	100mg/kg DPP Mean± SE	150mg/kg DPP Mean± SE
Testosterone ng/ml	1.30±0.09 a	0.80±0.05 b	1.41±0.10 B	1.90±0.20 A	2.30±0.43 c
FSH mIU\ml	0.91±0.07 a	0.60±0.04 b	1.10±0.12 C	1.50±0.40 C	2.10±0.41 d
LH mIU\ml	0.83±0.03 a	0.67±0.2 a	0.90±0.2 A	1.47±0.2 B	1.97±0.22 b

Different letters refer to significant ( $p<0.05$ ) differences compared between groups.

**Table 3: Effect of administration of DPP (50,100,150) mg/kg on Body and testes weight (g) in male rat after 50 days treated with NaF (0.250 PPM).**

Groups	Control (Distilled water)	NaF0.250 p.p.m for 50 days			
		NaF + (D. W) Mean± SE	50mg/kg DPP Mean± SE	100mg/kg DPP Mean± SE	150mg/kg DPP Mean± SE
Body weight(g)	255.96±2.64 a	248.87±2.54 b	260.81±2.07 C	266.15±2.10 d	268.15±3.03 d
testes Weight(g)	5.89±0.20 a	3.80±0.11 b	4.95±0.50 C	4.80±0.62 c	5.48±0.50 a

Different letters refer to significant ( $p<0.05$ ) differences compared between groups.

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## تأثير المستخلص المائي لحبوب طلع النخيل على صفات النطف ومستويات هرمونات التستوستيرون والمحفز للجريب واللوئيني في ذكور الجرذان المعاملة بفلوريد الصوديوم

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### الخلاصة

صممت الدراسة لتقييم تأثير المستخلص المائي لحبوب طلع النخيل على الوظائف الخصوية وهورمونات التستوستيرون والمحفز للجريب واللوئيني. قسمت الحيوانات وعددها خمسة وثلاثون جرذ عشوائيا الى خمسة مجاميع (سبعة لكل مجموعة). جرعت المجموعة الاولى بالماء المقطر (مجموعة سيطره) وجرعت المجموعة الثانية بفلوريد الصوديوم وبمقدار جزء من المليون بينما جرعت المجموعة الثالثة بفلوريد الصوديوم وبمقدار 0.250 جزء من المليون ومستخلص حبوب الطلع بتركيز 50 ملغم كغم من وزن الجسم (حجم المستخلص 0.5 مل) و جرعت المجموعة الرابعة بفلوريد الصوديوم وبمقدار 0.250 جزء من المليون ومستخلص حبوب الطلع بتركيز 100 ملغم كغم من وزن الجسم و جرعت المجموعة الخامسة بفلوريد الصوديوم وبمقدار 0.250 من المليون ومستخلص حبوب الطلع بتركيز 150 ملغم كغم من وزن الجسم. اظهرت النتائج وجود انخفاض معنوي ( $p < 0.05$ ) بالعدد الكلي وحركة النطف وزيادة اعداد النطف الميته وغير السويه في مجموعة الحيوانات التي جرعت بفلوريد الصوديوم فقط مقارنة بحيوانات السيطره في حين اظهرت النتائج وجو زياده ( $p < 0.05$ ) معنويه بالعدد الكلي وحركة النطف وانخفاض اعداد النطف الميته وغير السويه في مجاميع الحيوانات التي جرعت بالمستخلص المائي لحبوب طلع النخيل وكان اكثر التراكيز تأثيرا لمستخلص حبوب الطلع هو 150 ملغم كغم. كما اظهرت النتائج وجود زياده بتركيز هرمونات التستوستيرون، المحفز للجريب و اللوئيني في مجاميع الحيوانات التي جرعت بالمستخلص المائي لحبوب طلع النخيل مقارنة بمجموعة السيطره و مجموعة الحيوانات التي جرعت بفلوريد الصوديوم. كما اظهرت النتائج بعد التجريب لمدة خمسون يوما وجود زياده معنويه ( $p < 0.05$ ) في اوزان الجسم والخصى في مجاميع الحيوانات التي جرعت بالمستخلص المائي لحبوب طلع النخيل مقارنة بمجموعة السيطره ومجموعة الحيوانات التي جرعت بفلوريد الصوديوم. الدراسة تستنتج بان التجريع بالمستخلص المائي لحبوب طلع النخيل (100,150 ملغم كغم وزن الجسم) لمدة 50 يوم سببت تأثيرات ايجابية على بعض معايير الخصويه وعلى محور هرمونات النخامية-الخصويه وخصوصا الجرعه الاعلى 150 ملغم كغم وزن الجسم والتي لها فعاليه عاليه في تحسين العدد الكلي والحركه وخفض اعداد النطف الميته وغير السويه. اضافة الى ذلك فان مستخلص حبوب طلع النخيل حفز افراز هرمون التستوستيرون والهرمون المحفز للجريب والهرمون اللوئيني وايضا ادى الى زياده في وزن الجسم والخصى بينما ادت المعامله بفلوريد الصوديوم الى انخفاض الوزن ومستويات الهرمونات وكانت له تأثيرات سلبية على بعض معايير النطف.

الكلمات المفتاحية: حبوب طلع النخيل، التستوستيرون، الجرذان، صفات النطف.