

## Effect of Date Palm Pollen Suspension on Ovarian Function and Fertility in Adult Female Rats Exposed to Lead Acetate

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### Abstract

This study present the effects of Date Palm Pollen grains (DPP) (*Phoenix dactylifera*) on ovary function and fertility in adult female rats exposed to lead acetate. Forty adult female albino rats were divided randomly into four equal groups. The first control group was given orally (1 ml) distal water,(T1) group given orally 150 mg / kg .B.W. DPP ( 0.5ml),(T2) group given orally (10) mg / kg. B.W. lead acetate (1 ml) ,(T3) group given daily by oral administration of both DPP150 mg/kg B.W. and 10 mg / kg. B.W. lead acetate .all animals treated via gavages needle for 6 weeks.At the end of experiment blood were collected to determine serum LH & FSH level .

Treated( T1) group showed the increase levels of LH were on significant importance at a level of  $< 0.05$  in comparison with zero time of the same group and in comparison with control group . To contrast to treated(T1) group, the level on LH showed a significantly decrease levels in animals on treated (T2) group exposure lead acetate at a dose rate of 10 mg / kg. B.W daily . The significant decrease in LH were showed in comparison the value with that at zero day of the same group and at a time on experiment 14, 28, 42 day post exposure in comparison with control group and with (T1) group exposed to DPP. Animal on treated (T3) group exposed of both DPP at 150 mg /kg. B. W. and lead acetate 10 mg / kg. B.W. for 6 weeks didn't show any significant changes during the experiment.

Animals on( T1) group that exposed to DPP at a dose rate on 150 mg / k. B.W. for 6 weeks showed a significant increase FSH level in serum of exposed animal in comparison with zero time of the same group and in comparison with contrl group . While those on

(T2) group which exposed to lead acetate at a dose rate of 10 mg / kg. B.W. for 6 weeks showed a significant decrease in FSH level in serum on day 14 and 42 following the exposure the decrease in FSH level in serum may significant in comparison with zero day of same group and with level on T1 group and control group. Animal on group T3 didn't show any significant changes during the experiment.

This study conclude that DPP has obvious improvement effect on fertility hormones of Adult female rats exposed to lead acetate.

**Key word :** Date Palm Pollen Grains ( *Phoenix Ductylifera*), Ovarian Function, Fertility in Rats .

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## الخلاصة

تناولت الدراسة تأثير المحلول المائي للقاح طلع النخيل على وظيفة المبايض و الخصوبة في إناث الجرذان البالغة المعاملة بخلات الرصاص . (٤٠) أنثى الجرذ البالغة قسمت عشوائيا إلى أربعة مجاميع متساوية، المجموعة الأولى السيطرة أعطيت (١ مل) فمويا من الماء المقطر، المجموعة المعالجة الأولى أعطيت فمويا (٥٠ مل) من ١٥٠ ملغم/كغم من وزن الجسم من المحلول المائي للقاح طلع النخيل، المجموعة المعالجة الثانية أعطيت فمويا (١ مل) من ١٠ ملغم/كغم من وزن الجسم من خلات الرصاص ، المجموعة المعالجة الثالثة أعطيت يوميا بالتجريب الفموي كلا المحلول المائي لطلع النخيل و خلات الرصاص ،كل الحيوانات عولجت بالتجريب الفموي لمدة ٦ أسابيع . في نهاية التجربة تم جمع نماذج الدم من القلب مباشرة لغرض قياس مستوى الهرمون اللوتيني والهورمون المحفز للجريبات المجموعة المعالجة الأولى لوحظ زيادة معنوية في مستوى الهرمون اللوتيني والهورمون المحفز للجريبات إذا ما قورنت بوقت الصفر في نفس المجموعة ومجموعة السيطرة ما بين المجاميع ، المجموعة المعالجة الثانية لوحظ نقصان معنوي في مستوى الهرمون اللوتيني والهورمون المحفز للجريبات إذا ما قورنت بوقت الصفر في نفس المجموعة و مجموعة السيطرة والمجموعة المعالجة الأولى ما بين المجاميع،إما المجموعة المعالجة الثالثة لم تبدي إي تغير معنوي خلال فترة المعالجة لكلا الهرمونين .

## Introduction

*Phoenix dactylifera* L (Date Palm) belong to family Arecaceae, called Nakhla and the Tree of Life by the Arabs. It is member of the monocotyledon family Arecaceae (Phillips on, 2001). The Palm family is a symbol of prosperity and love to Muslims (Al-Qarawiet *et al.*, 2004) ..In fact, Muslims believes that he who eats seven Dates every morning will not be affected by poison or magic on the day he eats them (cited by Miller *et al.* 2003). The early Egyptians and ancient Chinese used DPP as a rejuvenating medicinal agent .It has been called a fountain of youth. Pollen preparations are distributed worldwide for dietary purposes and as diet supplement by increasing the total dietary intake (Kroyer and Hegedus, 2001). DPP application in the rites, and its uses in traditional and herbal medicine, have been recorded throughout history. Varsity of pollen containing food products, such as candy and

chocolate bars, are commercially available in health food stores in the Western world (Stanley and Linkens,1974).They contain concentration of photochemical and nutrients and are rich in carotenoids flavonoids and phytosterols (Broadhurts,1999)..Moreover, they are good source of protein ,amino acid ,vitamins ,dietary fiber, fatty acid , enzymes ,hormones and minerals (Alferez and Campos,2000).. Many researchers have also documented the antioxidant property of *Phoenix dactylifera* (Vayalil, 2002); Mohamed and Alokbi, 2004; Allaith and Abdul, 2005).Many researchers have documented the antioxidant property of DPP (Asii, 2009; Al-arrak, 2010).also it used to enhancement of reproductive function and fertility in male of laboratory animals. **Lead** is considered as one of the most hazard and cumulative environmental pollutants that affect all biological system through exposure from air, water and food source (Patra and

rup,2000) and it is one of the oldest known and most important environmental pollutions which are toxic and may affect body organ for several years even in the absence of continued exposure (Todd,1994; Tuormaa, 1995; Han *tal.*,1997;Vig and Hu, 2000; Gidlow, 2004).

**Lead** is a heavy metal naturally found in Earth crust and also providing from anthropogenic source in environmental, feeds and foods of vegetal and animal origin and has toxic effects on many system of the body, particularly on the developing nervous system, the hematological and cardiovascular system, and the kidney (Atsder, 2005).

Data are about the negative impact on integrity and performance of male reproductive system are more than those of female reproductive system (Pinon *et al* .,1995.Taupeau *et al* .,2001;Dearth *et al* .,2002;Pillai and Gupta,2005).Many studies have shown that reproductive toxicity is an important feature of lead toxicity ( Boscolo *et al* .,1988.Adhikari *et al* ., 2001;Batra *et al* .,2001).

### **Aims of study**

The aims of this study are:-

- i. Study the effect of DPP on reproductive function and fertility of adult female rats.
- ii. Study the toxicity effect of lead acetate on reproductive function and fertility of adult female rats.
- iii. Study the protective role of DPP to prevention or decrease the toxicity effects of lead acetate on reproductive function and fertility of adult female rats.

### **Materials and Methods**

#### **Preparation of Date Palm Pollen Suspension:**

Date palm pollen were purchased from the local market in Baghdad, Iraq and certified at the National Herbarium, in Abu Grab . The water suspension was prepared as 1ml of suspension contains (75 mg) of Date Palm

Pollen. Each animal of treated group received DPP 75mg/500 gm Body Weight once daily.

#### **Preparation of Lead Acetate Solution:**

Lead acetate purchased from Gonne office for medical devices-Iraq, BDH Co. (England). The water solution was prepared as 1ml of solution contains 1mg of lead acetate (3g/litter) and each animal of treated groups (T2 and T2) given1ml/100 gm from weight of animal.

#### **Experimental Design:**

##### **Animals:**

Fourty Albino female rats aged 12-14 weeks; weighed 250-300 gms was used. Rats obtained from the National Center Of Drug Observation Search and housed in animal house, College of Veterinary Medicine, Diyala University/Department of Physiology and pharmacology. Rats housed in plastic cages 70×50×15 cm in a room for two weeks for adaptation of 21 - 25 °c, air of the room was changed continuously by using ventilation vacuum and with light/dark cycle of 12:12 hrs per day. The litter of the cages was changed every week. Rats were fed on pellet of freshly prepared ration and animals were adlibitum to water along the experimental period.

##### **Study protocol:**

Fourty female rats were divided randomly into (4 groups), of 10 animals each:

##### **Control group:**

Ten adult female rats treated once daily by oral administration of (1 ml) distal water given via gavages needle for 6 weeks.

##### **Treated group 1(T1):**

Ten adult female rats treated once daily by oral administration of(0.5 ml )150 mg / kg .B.W. DPP suspension given via gavages needle for 6 weeks.

##### **Treated group 2 (T 2):**

Ten adult female rats treated once daily by oral administration of(1ml ) 10 mg / kg.

B.W. lead acetate given via gavages needle for 6 weeks.

### **Treated group 3 (T 3):**

Ten adult female rats treated once daily by oral administration of both DPP150 mg/kg B.W. and 10 mg / kg. B.W. lead acetate given via gavages needle for 6 weeks.

### **Blood collection:**

At zero time, 14, 28, 42 days post exposure to agents, animals were anesthetized by intramuscular injection of (ketamine 90mg/Kg B.W & Xylazine 40mg/Kg B.W) (Ogata *et al*, 1985) Three milliliter blood samples were collected via cardiac puncture technique from each (rpm) for 15 minute, and the obscured serum samples were stored in a freezer at (-18 C) till used for.

### **Hormones concentrations:**

#### **(A)-Serum FSH concentration:**

The concentration of FSH hormone was measured by Enzyme Linked Immuno Assay (ELISA) technique according to instructions of Bio Check, Inc(Foster city USA).

#### **(B)-Serum LH concentration:**

The LH hormone was measured by ELISA technique according to instructions of Bio Check, Inc(Foster city USA).

### **Results**

Treated group T1 showed a significant increase level of LH on day 14 post exposure to date palm pollen at a dose rate on 150mg / kg. B.W. for 6 weeks, which continued till the end on experiment (42day) post exposure. As it increase from ( $4.54 \pm 0.33$  ng / ml ) at zero day to ( $6.52 \pm 0.2$  ng / ml ) at 14 day post exposure , Attended maximum level of ( $7.77 \pm 0.6$  ng/ml ) on day 28 post exposure then decrease but still higher time a level on

zero day (  $7.10 \pm 0.6$  ng /ml). The increase levels were on significant importance at a level of  $< 0.05$  in compassion with zero time of the same group and in compassion with control group at the same time of compassion (14, 28) and 42 day post exposure. To contrast to treated group 1, the level on LH showed a significantly decrease levels in animals on group 2 exposure lead acetate at a dose rate On 10 mg / kg. B.W. The significant decrease in LH were showed in compassion the value with that at zero day of the same group and at a time on experiment 14, 28, 42 day post exposure in compassion with control group and with group T1 treat exposed to DPP. Animal on group T3 treat exposed of both DPP at 150 mg /kg. B. W. and lead acetate 10 mg / kg. B.W. for 6 weeks didn't show any significant changes during the experiment as shown in **table (1).**

Animals on group T1 that exposed to DPP at a dose rate on 150 mg / k. B.W. for 6 weeks showed a significant increase FSH level in serum of exposed animal from day 14 to day 42 post exposure attended time maximum level ( $17.54 \pm 0.6$  ng / ml) on day 28 post exposure. While those on group T2 which exposed to lead acetate at a dose rate of 10 mg / kg. B.W. for 6 weeks showed a significant decrease in FSH Level in serum on day 14 and 42 following the exposure the decrease in FSH level in serum may significant in compassion with zero day of same group and with level on group 1 and control group. Animal on group T3 didn't show any significant changes during the experiment as shown in **table (2).**

**Table (1):** showed serum LH level (ng/ml) in adult female rats exposed to date palm pollen (group T1), Lead acetate (groupT2) and both (groupT3).

Groups Time	Control Group	(T1) group	(T2) group	(T3) group
Zero time	4.80 ± 0.3 B	4.54 ± 0.33 B	4.78 ± 0.22 A	4.80 ± 0.44 a
14 day	5 ± 0.36 B	6.52 ± 0.2 Ab	3.20 ± 0.23 b C	4.60 ± 0.3 b B
28 day	5.06 ± 0.4 B	7.77 ± 0.6 Aa	2.10 ± 0.3 c C	4.40 ± 0.3 b B
42 day	5.08 ± 0.4 B	7.10 ± 0.6 Aa	3.64 ± 0.5 b C	4.86 ± 0.3 b B

**L.S.D. =1.00**

- Values are expressed as mean ± SE, n = 10 each group.
- T1: Animal given date palm pollen 150 mg. / kg. B.W once daily .
- T2: Animal given lead acetate 10 mg. /kg. B.W once daily .
- T3: Animal given Date Palm Pollen 150 mg. /kg. B.W. and Lead acetate 10 mg. /kg. B.W once daily .
- Capital litter denote difference between groups, p<0.05 vs. control.
- Small litter denote significant difference within group p<0.05.

**Table (2):** serum FSH level (ng/ ml) in adult female rats exposed to date palm pollen (groupT1), Lead acetate (groupT2) and date palm pollen plus lead acetate (groupT3).

Groups Time	Control group	group T1)(	group T2)(	( T3) group
Zero time	13.74 ± 0.7 B	13.88 ± 0.26 B	13.76 ± 0.4 a	14.04 ± 0.4
14 day	14.40 ± 0.5	16.94 ± 0.6 A a	11.52 ± 0.6 b C	14.86 ± 0.8 B
28 day	15.04 ± 0.4	17.54 ± 0.6 A a	13.30 ± 0.6 A	14.96 ± 0.5 B
42 day	15.54 ± 0.5	17.16 ± 0.4 A a	10.76 ± 0.5 c C	14.90 ± 0.7 B

**L.S.D. =1.4 0**

- Values are expressed as mean ± SE, n = 10 each group.
- T1: Animal given date palm pollen 150 mg. / kg. B.W once daily .
- T2: Animal given lead acetate 10 mg. /kg. B.W once daily .
- T3: Animal given Date Palm Pollen 150 mg. /kg. B.W. and Lead acetate 10 mg. /kg. B.W once daily .
- Capital litter denote difference between groups, p<0.05 vs. control.
- Small litter denote significant difference within group p<0.05.



## Discussion

DPP mainly contains cholesterol, rutin, carotenoids, as well as estrone which is known to exhibit gonadotrophin activity in rats (Distal *et al.*, 1996), therefore, caused an increase of, FSH and LH, (Weinbauer *et al.*, 1991; O'shaughnessy and Sheffield 1999).

In the present study that a principal mechanism of action of lead toxicity at the level of the hypothalamic– pituitary axis or a combined defect involving the gonad and hypothalamic –pituitary sites (Thoreux – Manlay A, *et al.* 1995). Moreover, lead had significant reduced the binding of LH and FSH isolated granulosa but antioxidant supplementation had ameliorated the situation (Priya *et al.*.,2004).

## Conclusions

From the results obtained and discussed in this study, it could be concluded that :-

1-Oral administrated of lead acetate at a dose (10 mg/kg. B.W. ) lead to reduction in ovarian function and fertility of female rats manifested by decrement of reproductive hormones, increase of atretic follicles and decrease of development and maturation of follicles with a failed of fertility and pregnancy .

2-Oral administration of date palm pollen suspension with protective dose (150mg/kg. B.W.) lead to rebalance the harmful effects of lead acetate in female rats manifested by enhancement of the parameters above.

## Reference

- [1] Adhikari,N.;Sinha,N.;Narayan, R.and Saxena, D.K.(2001).lead induced cell death In testes of young rats.J.Applied Toxicol .,21:275-277.
- [2] Al-arrak,J.K.(2010). Effect of DPP on testis function and fertility in male rats. Iraqi Journal .vet. Medicine . vol 1 n 1.
- [3] Alferz, M.J.M. and Campos,M.S.(2000). Beneficial effect of pollen and or propels on the iron ,calcium, phosphorus and magnesium in rats with nutritional ferropernic anemia. J.Agric. Food. Chem., 48:5715-5722.
- [4] Alliath ,A. and Abdalla, A.A. (2005). In vitro evaluation of antioxidant activity of different extract of Phoenixdactylifera fruits as functional foods . Deutsche Lebensmittel Rundschau., 101:305-308.
- [5] Al-Qarawi,A.A.; Mousa, H.M.; Ali, B.H. ; Abdel-Rahman, H. and Elmougy,S.A. (2004). Protective effect of extracts from date (Phoenix dactylifera L.)on the carbon tetrachloride –induced hepato toxicity in rats . Intern.J.APPL.Res.Vet.Med., 2.(3) 176-180.
- [6] anti- inflammatory activity of different extract of dates fruits in adjuvant arthritis . Polish J. of Food and Nutr. Scie., 13:397-402.
- [7] Assi,M.A.(2009). Study the A thesis of master , college of veterinary medicine,University of Baghdad .
- [8] Atsdr,(Agency for Toxic Substance and Disease Registry) (2005). Toxicological profile for lead. (Draft for public comment).Agency for Health and Human Servises, Atlanta,GA.
- [9] Boscolo, P. ;Carmignani,M.; Sacchettoni-Logroscino, G.; Ranelletti, F.O.; Artese,L. and Preziosi, P. (1988 ) . Ultrastructure of the testis in rats with blood hypertension by long term lead exposure . Toxicol . Lett., 41:129-137.
- [10] Broadhurt, C.L.(1999). Bee products: medicine from the live. Nutr. Sci. News, 4:366-368.
- [11] Dearth,K.R.; Jill, K.; Hiney ; Srivastava Vinod; Sarah, B.; Burdick ; Bratton, R.G. and Dees Les, W.(2002). Effect of lead exposure during gestation and lactation on Female pubertal development in the rat . Reproductive Toxicology Vol.,16;Nr 4;P.343-352.
- [12] Han,S.; Qiao, X.; Kemp, F.W. and Bogden, J.D. (1997). Lead exposure at an early Lead and or cadmium on reproductive



- performance and hepatic oestradiol metabolizing enzyme, *Toxicol Lett.* 2005 Jan 15; 155(1):179-86.
- [13] Kroyer, G. and Hegedus, N. (2001). Evaluation of bioactive properties of pollen extracts as functional dietary foods supplements. *Innovative Food Sci. Emerging Technol.*, 2(3): 171-174.
- [14] McMurry, S.T., Lochmiller, R.L., Chandra, S.A. and Qualls, C.W. Jr Sensitivity of selected immunological, hematological, and reproductive parameters in the cotton rat (*Sigmodon hispidus*) to subchronic lead exposure. *J Wildl Dis*, Apr; 31(2): 193-204, (1995).
- [15] Miller, C.J.; Dunn, E.V. and Hashim, I.B. (2003). The glycogenic index of dates And dates / yoghurt mixed meals. *European Journal of Clinical Nutrition*. 57:427-430.
- [16] Mohamed, D.A. and Al-Okbi, S.Y. (2004). In vivo evaluation of antioxidant and
- [17] Patra, N.; Nehr, B. and Bansal, M.P. (2001). Influence of lead and zinc on rat male age substance increases lead retention in the rat. *Environ. Health. Prospect.* 105:412-417.
- [18] Phillipson, J.D. (2001). Phytochemistry and medicinal plants. *Phytochemistry* .56:237-243.
- [19] Pillai, A. and Gupta, S. (2005). Effect of gestational and lactational exposure to Reproduction at biochemical and histological levels. *J. Applied Toxicol.* 21:507-512.
- [20] Pinon – Lataillade, G.; Thoreux – Manlay, A.; Coffigny, H. and Soufir, J.C. (1995). Reproductive toxicity of chronic lead exposure in male and female mice; *Reproductive Toxicology*, vol; 14, 11, PP: 872-380.
- [21] Priya PN, Pillai A, Gupta S, Effect of simultance exposure to lead and cadmium on gonadotropine binding and steroidgenesis on granulose cells: an in vitro study. *Indian J Exp Biol*, 42(2): 143-8, 2004.
- [22] Stanley, R.G. and Linkens, H.G. (1974). *Pollen, Biology, Biochemistry Management.* Springer –Verleg Berlin Heidelberg, New-York, P.44
- [23] Taupeau Crystel ; Poupon, J.; Nome and Brigitte Lefebvre. (2002). Lead accumulation in the mouse ovary after treatment induced follicular atresia *Reproductive Toxicology*, vol.15, nr.4, PP: 385-39.
- [24] Todd, A.C. (1994). Lead poisoning .*West. J. Med.* 161:187-190.
- [25] Tuormaa, T.E. (1995). The adverse effects of lead. *J. Orthomol. Med* 10:149-164.
- Thoreux -Manlay A, Vélez de la Calle JF, Olivier MF, Soufir JC, Masse R, Pinon-Lataillade G. Impairment of testicular endocrine function after lead intoxication in the adult rat. *Toxicology* 1995; 26: 101-109.
- Vayalil, P.K. (2002). Antioxidant and antimutagenic properties of aqueous extract of date fruit (*Phoenix dactylifera* L. *Arecaceae* ). *J. Agric Food Chem.*, 50, 610-617.
- [26] Vig, E.K. and Hu, H. (2000). Lead toxicity in older adult. *Am. Geriatr. Soc.*, 48:1501-1506.