Kufa Journal for Veterinary Medical Sciences Vol.(6). No.(2) 2015

Kufa Journal for Veterinary Medical Sciences
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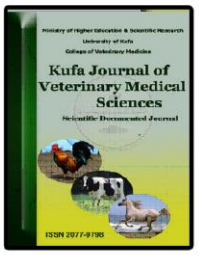

Effect of different doses of ethanolic extract of date palm pollen grains on serum gonadotropin and total Glutathione in mature female rats

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Summary

This study was focused on the effect of different doses of DPP on antioxidant status and gonadotropin hormones of adult female Wister rats, sixty male rats were randomized into 6 groups (A-F). Group A received distilled water (control group). while groups B-F were administration 50, 100 , 150 , 200 and 250 mg/kg of DPP extract respectively for twenty eight (28) days. Blood samples were drawn prior to administration (day zero) and every fourteen days for the estimation of serum FSH,LH and Glutathione concentration. 28-day daily oral administration of ethanol crude extraction of date palm pollen to female rats is associated with high circulating GSH concentration. And an elevation in serum FSH and LH concentrations in groups received 100mg/kg of DPP extract .

Conclusions: Data from this study identified that in a dose more than 100mg/kg there were no dose related effects.

Key words: Glutathione , Gonadotropin,Date Palm Pollen.

تأثير الجرع المختلفة من المستخلص الكحولي لحبوب طلع النخيل في تركيز الهرمونات المغذية للقند والكلوتاثايون في مصل الدم في اناث الجرذان البالغة

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الخلاصة:

وضعت هذه الدراسة لتعيين الجرعة المؤثرة للمستخلص الكحولي لحبوب طلع النخيل والتي ادت الى ارتفاع تركيز الهرمونات المحرضة للقند والكلوتاثايون الكلي في مصل الدم في اناث الجرذان. استخدمت لهذا الغرض ستون حيوانا قسما الى ستة مجاميع متساوية اعطيت الاولى ماء مقطر واعتبرت مجموعة سيطرة اما المجاميع الخمسة الاخرى فقد اعطيت 50, 100 ,150, 200 و 250 ملغم /كغم من وزن الجسم من المستخلص ولمدة 28 يوم . سحبت عينات دم من القلب مباشرة بعد تخدير الحيوان قبل البدء بالتجربة وفي اليوم الرابع عشر وفي اليوم الثامن والعشرون وبعد كل سحبة يفصل مصل الدم وتجرى عليه الاختبارات. اظهرت النتائج ارتفاع ارتفاع

تركيز كل من الهرمونات المحرضة للقند وكذلك الكلوتاثايون في المجاميع باستثناء تلك التي اعطيت مستخلص بجرعة 50 ملغم /كغم ولكن لا توجد فروقات معنوية بين المجاميع الاربعة الاخيرة لذا اعتبرت ال100ملغم هي الجرعة الاكثر تأثيرا.
نستنتج من هذه الدراسة ان الجرعة الؤثرة للمستخلص الكحولي لحبوب طلع النخيل هي 100 ملغم /كغم وان الزيادة في جرعة المستخلص اكثر من 100 ملغم ليست ذي جدوى .
الكلمات الافتتاحية : الكلوتاثايون , الهرمونات المغذية للقند , حبوب طلع النخيل

Introduction

Date palm pollen (DPP) application in the rites, and its uses in traditional and herbal medicine, have been recorded throughout history. They contain concentration of photochemical and nutrients and are rich in carotenoids flavonoids and phytosterols alkaloids, phenol, steroids, ,saponins, flavonoids, tannins and glycosides (Abedi *et al*., 2013; Abbas& Ateya,2011; Alrikabi 2011 & Broadhurt,1999)..Moreover, they are good source of protein ,amino acid ,vitamins ,dietary fiber, fatty acid, enzymes, hormones and minerals,(Alferz and Campos,2000). Many researchers have documented the antioxidant property of DPP (Al-arrak, 2010), The antioxidant activity by the DPPH method indicated that the DPP extract showed a strong antioxidant against DPPH radicals (Abbas and Ateya, 2011). also it used to enhancement of reproductive function and fertility in male of laboratory animals(Alrikabi 2011), and female rats (Hammed *et al*., 2012) .

Materials And Methods

Preparation of plant material

Pollen grains of date palm (*Phoenix dactylifera* L.) was collected from Alzubaidyia district 90 Km east of Baghdad in Wasit covernorent ,Iraq country.in period through last March and along April and left to air dried under dark condition then stored in frozen until use.

Preparation of ethanol extract of DPP grains (*Phoenix Dactylifera*)

The air dried powdered were extracted in soxhlet extractor successively with 70% ethanol for 16 hrs. using double-thickness cellulose extraction thimbles. The successive extracts were evaporated by a rotary evaporator at temperature below 45c̊ the crude extract was kept at -20c̊ till use.(Jiheel,2010).

Animal Grouping and Extract Administration

Sixty female rats weighing 150-200g, were randomly grouped into 6 (A-F) consisting of 10 animals each, and drenching treatments orally by gavage needle daily for 28 days as follow: Animals in group A, which served as the control received 0.5 ml of distilled water,. Animals in groups B, C, D, E and F were treated with doses of 50, 100, 150, 200 and 250 mg/kg of the extract, respectively. The experimental rats allowed free access to rat pellets and water.

Blood sample collection

Blood sampling from each group was collected at zero time and after 14& 28 day to determination of serum FSH, LH and GSH concentrations.

Determination of FSH by ImmunoRadiometric Assay(IRMA) KIT by Beckman Coulter

Determination of LH by ImmunoRadiometric Assay(IRMA) KIT by Beckman Coulter

Determination of Serum Reduced glutathione concentration (GSH).

Reduced glutathione (GSH) was determined by the method of Ellman (Burtis and Ashood, 1999). To 100 µl of serum 800 µl DW plus 100 µl of **50 %** TCA was added in a test tube. Tubes were mixed on vortex

intermittently for 10-15 minutes and centrifuged contents for 15 minutes at 3000 rpm, then pipetted into test tubes as follows:

To 400µl Supernatant 800µl Tris-EDTA buffer plus 20µl DTNB reagent was added Tubes contents were mixed in vortex. The spectrophotometer was adjusted with a blank reagent to read zero absorbance (A) at 412nm, and the absorbance of standards and samples were read within 5 minutes of the addition of DTNB.

against a reagent blank. Absorbance values were compared with a standard curve generated from known GSH.

Results

The effect of five successive increasing doses of ethanol crude extract of DPP grains on serum FSH

crude extract of DPP grains on mean values of serum FSH concentration (IU) of female rats is shown in table (**1**). The results showed that after two weeks of treatment a significant(P>0.05) differences in serum FSH concentration were recorded between the treated groups GII, serum FSH concentration (7.97±0.8), GIII (7.61±0.47), that received 100 and 150 mg/kg crude ethanolic extract of DPP respectively, comparing with the control (6.37±0.74), and other groups,GI (6.65±0.8), GIV(6.65±0.8) and GV(6.92±0.35).After four weeks the result showed a continues elevation in the same groups(GII, GIII). comparing with the result of other groups in the same time . Moreover, the results showed a significant rises in serum FSH within group GII and GIII which received 100mg/kg DPP extraction after two and four weeks compering with zero time . While serum FSH concentration of control and other treated groups showed a non-significant (*P*<0.05) differences within each group compared with zero time .

Table (1) effects of different doses of crude ethanolic extract of DPP on serum FSH concentration IU/L of female mature rats, each dose mention with number of group.

G / T	C D.W	G I 50mg/kg DPP	G II 100mg/k DPP	G III 150mg/kg DPP	G IV 200mg/kg DPP	G V 250mg/kg DPP
Zero	**6.54 ±0.68 A a**	**6.65±0.49 A a**	**6.69±0.59 A b**	**6.64±0.49 A b**	**6.71±0.54 A a**	**6.34±0.59 A a**
14 days	**6.37±0.74 B a**	**6.65±0.8 B a**	**7.97±0.8 A a**	**7.61±0.47 A a**	**6.65±0.8 B a**	**6.92±0.35 B a**
28 days	**6.03±0.54 B a**	**6.79±0.73 B a**	**8.24±0.94 A a**	**7.73±0.5 A a**	**6.45±0.23 B a**	**6.61±0.42 B a**

L.S.D = 0.65 Values are expressed as mean ±SE, n=8 each group. Capital letters denote significant differences between group(P<0.05) , small letters within groups

Effect of selected doses of ethanol extract of DPP on serum LH

The effect of five successive increasing doses of ethanol crude extract of DPP grains on mean values of serum LH concentration (IU) of female rats is shown in table (**2**). The results revealed that a significant

(P<0.05) elevation in serum LH mean values in GII , GIII,GIV and GV(0.26 ± 0.05), (0.24 ± 0.05),(0.24 ± 0.02), (0.26 ± 0.07) respectively after two weeks of treatment compared with control (0.17 ± 0.09) at same time and with zero time of each group GII (0.17 ± 0.05),GIII(0.17 ± 0.06), GIV(0.17 ± 0.07) and GV(0.17 ± 0.05).The elevation of serum LH mean value were continued in GII and GIII after four weeks of treatment but at non-significant differences with that results of last two weeks. Serum LH mean value of GI animals group showed no significant differences at all periods.The results showed non-significant (P>0.05) differences in LH mean values between groups of GII, GIII, GIV and GV after two and four weeks of treatment.

The effects of crude ethanolic extract in serum reduced glutathione level an antioxidants status was shown in table (1) The mean values of serum reduced Glutathione concentration in the control and treated groups along the experimental period are depicted in table (4-4). The statistical analysis indicated that the mean values of GSH in treated groups significantly (P<0.05) increased after two weeks of treatment and the recorded mean values were (40 ±1), (311 ± 9) , (307 ± 13),(311±4) and (308±6) for GI, GII , GIII,GIV and GV respectively comparing with the control (33 ± 2).

Table (2) effects of different doses of crude ethanolic extract of DPP on serum **LH** concentration IU/L of female mature rats each dose mention with number of group.

G / T	C D.W	G I 50mg/kg	G II 100mg/kg	G III 150mg/kg	G IV 200mg/kg	G V 250mg/kg
Zero	**0.17 ± 0.06** **A a**	**0.17 ± 0.07** **A a**	**0.17 ± 0.05** **A b**	**0.17 ± 0.06** **A b**	**0.17 ± 0.07** **A b**	**0.17 ± 0.05** **A b**
14 days	**0.17 ± 0.09** **B a**	**0.16 ± 0.05** **B a**	**0.26 ± 0.05** **A a**	**0.24 ± 0.05** **A a**	**0.24 ± 0.02** **A a**	**0.26 ± 0.07** **A a**
28 days	**0.17 ± 0.09** **B a**	**0.18 ± 0.06** **B a**	**0.27 ± 0.06** **A a**	**0.27 ± 0.07** **A a**	**0.24 ± 0.03** **A a**	**0.25 ± 0.08** **A a**

L.S.D =0.07 Values are expressed as mean ±SE, n=8 each group. Capital letters denote significant differences between group(P<0.05) , small letters within groups

The result of serum GSH concentration in table (**3**) was showed a significantly elevations after four weeks of treatment in all treated groups nearly the same value of result after two weeks of treatment periods. on the other hand there was a significant increase (P<0.05) of GSH concentration within group at 14 and 28 days compared with zero time. There were no significant differences in serum GSH concentrations in animal groups that received 100mg/kg of DPP extract and more after two as well as after four weeks of treatment.

Table (3)effects of different doses of crude ethanolic extract of DPP on serum GSH concentration (µmol/l)of female mature rats each dose mention with symbol of group.

G / T	A D.W	B 50mg/kg	C 100mg/kg	D 150mg/kg	E 200mg/kg	F 250mg/kg
Zero	33 ± 2 A a	32 ± 2 A b	33 ± 2 A c	32 ± 1 A b	34 ± 2 A b	34 ± 2 A b
14 days	33 ± 2 C a	40 ± 1 B a	311 ± 9 A b	307 ± 13 A a	311 ± 4 A a	308 ± 6 A a
28 days	32 ± 2 E a	39 ± 2 D a	315 ± 11 A a	302 ± 13 C a	309 ± 8 B a	309 ± 9 B a

L.S.D =7 Values are expressed as mean ±SE, n=8 each group Capital letters denote significant differences between group($P<0.05$) , small letters within groups

Discussion

Increase or decrease in endocrine function involve tissue response, alterations in secretory output from peripheral glands and alterations in the central mechanism controlling the temporal organization of hormonal release (Uboh *et al*., 2010).

Enhancement of serum FSH and LH hormones concentrations which clarified in animal groups that received crude ethanolic extract of Iraq DPP grains these results are consistent with the previous report: DPP has obvious improvement effect on fertility hormones(FSH and LH) of adult female rats (Hammed *et al*., 2012) and women (El-Neweshy, *et al*., 2013).

Improving effect of DPP is due to the presence of gonadotropins like substance or steroidal compound present in DPP (Hassan *et al*., 2012).

DPP has gonadotrphin effects (Adimoelja,2000). Steroidal saponins (one of DPP composition) increase LH and FSH levels, (Abedi, *et* al.,2013 & Samuel *et al*., 2013).

Hosseini *et al*., (2014) recorded a significant increase in the number of secondary follicles and the number of untral follicle, in infertility female mice treated with aqueous extract DPP due to increase of gonadotropins .

Many researchers showed improvement in serum FSH and LH of rats treated by herbal extraction that have same active ingredient of DPP such as *Tetracarpidium conophorum* that contain oils, carbohydrates, tannins, proteins, vitamins and minerals (Akpan and Anietie 2014).

Administration of crude extracts elevation serum LH,FSH and testosterone suggesting the stimulation of hypothalamic–pituitary–gonadal axis. The composition of the extract maintain the pulse episodes of GnRH, hence an increased level of FSH and

LH .The following two mechanisms are suggested for the elevation of serum LH and FSH were observed in this study . First, extract may have some constituents that act either as estrogen antagonist or as aromatase-inhibitor. The second possibility is that some components of the extract may act on kisspeptin neuron that controls the negative feedback mechanism(Chauhan *et al*., 2010).also some herbal constituents are instrumental for low production of inhibin lead to continuous inflow of FSH(Chauhan and Dixit 2009).

Follicle stimulating hormone level however decreased with increasing dose of the extract possibly due to negative feedback by the increase in estrogen,(AL-hassan 2012).that explain the dose related reduction.

The elevation in serum GSH in animal groups treated with crude ethanolic extract of date palm pollen grains may due to a high antioxidant effects were recorded by, Abbas and Ateya, 2011, The antioxidant activity by the DPPH method indicated that the DPP extract showed a strong antioxidant against DPPH radicals, other researchers record same results, DPP extracts have antioxidant and estrogen like activity (Ammar *et al*., 2009). the antioxidant effect of DPP through reduction of ROS level exerted by its content of flavonoids (Hassan *et al*., 2012), Flavonoids are excellent scavengers of free radicals and the number of hydroxyl group on the phenyl ring seems to enhance the antioxidant capacity of polyphenolic molecule (LeBlanc *et al*., 2009). The antioxidant effect of DPP is due to its high concentration of vitamin C, B (Thiamine), B (Riboflavin), nicotinic acid (Niacin) and vitamin A (Hassan, 2011).

Treatment with DPP counteracted the increases in antioxidant systems in rat testis as assessed by restoration of reduced glutathione (GSH), superoxide dismutase (SOD) and catalase (CAT), (Hassan *et al*., 2012).

Treatment of mice exposed to Incense smoke by DPP revealed a significant increase in GSH of mice splenocytes. (Soad Nady, *et al.*,2014), These results suggested that DPP act as a potent antioxidant .

Conclusions:

Data from this study identified that in a dose more than 50mg/kg there were no dose related effects in serum FSH, LH and GSH concentrations. ethanolic extract of *Phoenix dactylifera* pollen grain enhanced antioxidant status and gonadotropin hormones in female rats. The improved may be attributed, to the alkaloids, saponins, and or flavonoids .

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