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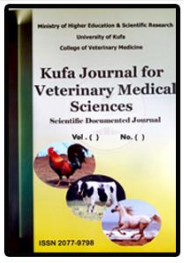

Ameliorating effects of DPP (*Phoenix dactylifera* L.) extract on serum gonadotropins and Prolactin hormones in mature female rats exposed to sodium nitrite

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Abstract

This study was accomplished to explore the role of crude ethanol extract of date palm pollen grains (DPP) in ameliorating the induction of oxidative stress by sodium nitrite in female rats.

To detection the effect of sodium nitrite and crude ethanolic extract of DPP in the concentration of serum FSH, LH and Prolactin, forty mature female rats divided into five equal groups, first group served as control where the second received 100mg/kg DPP extract daily, third received 100mg/kg DPP extract plus 100mg/kg sodium nitrite, fourth received100mg/kg sodium nitrite, for 42 days, while the fifth group received 100mg/kg sodium nitrite for 28 days then shift to 100 mg/kg DPP extract for another 14 days .the result showed an elevation of serum FSH and LH of the second group, and reduction in fourth and fifth groups . while the elevation of serum prolactin were clarified in fourth and fifth groups.

In conclusions, treatment by extracts of DPP grains leads to improvement fertility in female rats. Exposure to sodium nitrite at a dose of 100mg/kg leads to an elevation in serum prolactin and reduction in gonadotropin hormones in female rats.

Key wards: FSH, LH , Prolactin, DPP and NO_2 .

التأثيرات الحسنة لمستخلص طلع النخيل على الهرمونات المغذية للاقناد وهرمون البرولاكتين في مصل الفئران الناضجة المعرضة لنتريت الصوديوم .

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الخلاصة:

أجريت هذه الدراسة لمعرفة الدور الوقائي والعلاجي للمستخلص الكحولي لحبوب طلع النخيل في اناث الجرذان البالغة المعرضة للإجهاد التأكسدي بواسطة التجريع اليومي بنتريت الصوديوم. وتأثيرهما على الهرمونات المحرضة للقند والهرمون المدر للحليب في مصل الدم ولهذا الغرض أخذت اربعون من اناث الجرذان الناضجة وقسمت الى خمسة مجاميع متساوية , واعتبرت مجموعة الأولى سيطرة جرعت ماء مقطر فقط ، والثانية جرعت يوميا 100ملغم / كغم مستخلص والثالثة جرعت يوميا 100ملغم / كغم نتريت الصوديوم بالإضافة إلى التجريع اليومي بالمستخلص الكحولي بنفس الجرعة السابقة ، حيوانات المجموعة الرابعة جرعت يوميا 100ملغم / كغم نتريت الصوديوم ولمدة 42 يوم اما المجموعة الخامسة فقد جرعت يوميا 100ملغم / كغم

نتريت الصوديوم ولمدة 28 يوم ثم تحولت المعالجة الى التجريع اليومي بالمستخلص الكحولي بجرعة 100ملغم / كغم لمدة 14 يوما اضافية . أظهرت الدراسة ارتفاعا معنويا في تركيز الهرمونات المحرضة للقند في مصل دم حيوانات مجموعة المعالجة الثانية والثالثة وانخفاض في المجموعتين الرابعة والخامسة , اما تركيز الهرمون المدر للحليب فقد ارتفع بشكل ملحوظ في مصل دم حيوانات المجموعتين الرابعة والخامسة .
نستنتج من الدراسة الحالية ان للمستخلص الكحولي لحبوب طلع النخيل دورا في تحسين الخصوبة لدى الفئران التي تعرضت للإجهاد التأكسدي بواسطة نتريت الصوديوم ,كما بينت الدراسة ان هناك ارتفاعا ملحوظا في تركيز الهرمون المدر للحليب في مجاميع الحيوانات التي جرعت بنتريت الصوديوم .

Introduction

Nitrate (NO_3^-) and Nitrite (NO_2^-) are referred to transcendently as undesired buildups in the natural way of life with possibly cancer-causing effects[1], or as idle oxidative final results of nitric oxide digestion system. Regardless, from exploration performed over the earlier decade, it is as of now evident that nitrite and nitrate are physiologically reused in blood and tissues. This jumps out at frame NO and other bioactive nitrogen oxides [2]. In this manner, they should be seen as capacity pools for NO like bioactivity, from now on supplementing the NO synthase (NOS)- subordinate pathway.

The affirmation of this mammalian nitrogen cycle has driven the scientists to take a gander at the piece of nitrite and nitrate in physiological procedures that are known not controlled by NO. The bio activation of nitrate from dietary or endogenous sources obliges its beginning lessening to nitrite. This in light of the fact that warm blooded animals need specific and convincing nitrate reductase chemicals, this change is basically done by commensal microbes in the digestive tract and body surfaces [3].

On the off chance that nitrite is framed, there are various pathways in the body for its further decrease to NO, including hemoglobin [4]. Xanthine xidoreductase [5]. Ascorbate [6]. Polyphenols [7] and protons [8].

The era of NO by these pathways is by and large overhauled in the midst of hypoxia and acidosis, thusly ensuring NO creation in circumstances for which the oxygen-subordinate NOS compound exercises are bargained [9]. Nitrite lessening to NO and NO altered proteins amid physiological and neurotic hypoxia seem to add to hypoxic physiological flagging, vasodilation, balance of cell breath and the cell reaction to ischemic anxiety [10].

Materials and methods

Animal Grouping and Extract Administration

In this experiment 40 albino mature female rats were used. Each animal of groups treated orally by gavage needle daily for 42 days as follow:

GI: control group received DW, **GII:** received 100mg/kg of crude ethanolic extract of DPP, **GIII:** received 100mg/kg of a crude ethanolic extract of DPP plus 100mg/kg of sodium nitrite,**GIV:** received 100mg/kg of sodium nitrite, **GV:** received 100mg/kg of sodium nitrite for 28 days then shift to100mg/kg of a crude ethanolic extract of DPP for 14 days.

Blood sample collection

Whole blood samples were obtained in non-heparinized tubes to detect serum FSH,LH, and Prolactin, from each group blood was collected at zero time and after 14, 28 and 42 days of treatment to determination of serum FSH, LH and Prolactin concentrations.

Determination of each hormone was done by ImmunoRadiometric Assay(IRMA) KIT by Beckman Coulter.

Statistical analysis.

Values are listed as mean ± standard error. Statistical analysis was done by Kruskal Wallis test (ANOVA). The comparison of means between control and each experimental group (one way ANOVA). $P < 0.05$ was regarded as significant. GenStat software and Excel 2010 were used for analyzing.

Results

Effect of ethanolic extract of DPP on serum FSH concentration

While there were no noteworthy differences in serum FSH mean value in all groups at zero time and control group as well at all times of the experiment, but the view for table (1) appears an elevation in mean value of serum FSH of GII animals after four(7.37±0.46) and six (7.65±0.41) weeks that treated with100mg/kg DPP extract alone as compared with the control group (6.39±0.66), (6.42±0.32) at the same time , but there was a significant reduction in serum FSH mean values of GIII, GIV and GV animals after 2 weeks (5.35±0.67), (3.22±0.0.82), (3.22±0.0.82), after4weeks (5.26±0.67), (3.21±0.95) ,(3.21± 0.95),and after 6weeks (5.45±0.46),(3.75±0.24), (4.75±2.4) respectively as compared with the control group (6.64±0.98) after 2 weeks, (6.39±0.66) after 4weeks and (6.42±0.32) after 6 weeks of experimental periods.

Moreover, there was a significant($P<0.05$) reduction in GIV(3.21±0.95) and GV (3.21±0.95) at four weeks of treatment as compared with the mean value of G III (5.26±0.67). Serum FSH mean value of GV animals returned to elevation after six weeks of treatment in a noteworthy degree ($P<0.05$) as compared with mean value of same group at the second week(3.22±0.82) but less than mean value of control group (6.42±0.32) at the same time.

Table (1) Effects of crude ethanolic extract of DPP on serum FSH concentration IU/L of female mature rats exposed to sodium nitrite

G / T	GI D.W	GII 100mg DPP	G III 100mgDPP + 100mg $NaNO_2$	G IV 100mg $NaNO_2$	GV100mg $NaNO_2$ after 4w 100mgDPP
Zero	**6.76±0.74 A a**	**6.85±0.34 A b**	**6.28±0.41 A a**	**6.28±0.56 A a**	**6.28±1.11 A a**
14 days	**6.64±0.98 A a**	**6.75±0.68 A b**	**6.35±0.67 A b**	**3.22±0.0.82 B b**	**3.22±0.82 B c**
28 days	**6.39±0.66 B a**	**7.37±0.46 A a**	**5.26±0.67 C b**	**3.21±0.95 D b**	**3.21±0.95 D c**
42 days	**6.42±0.32 B a**	**7.65±0.41 A a**	**5.45±0.46 C b**	**3.75±0.24 E b**	**4.75±2.4 D b**

L.S.D = 0.85 Values are expressed as mean ±SE, n=8 each group. Capital letters denote significant differences between group(P<0.05), small letters within groups

Effects of crude ethanolic extract of DPP of serum LH concentration

The results of Table (2)showed a significant(P<0.05) elevation of serum LH concentrations after 2 weeks (0.28±0.07) from treatment in GII group (which received DPP extract only) compared with control(0.15±0.07), and other groups GIII(0.14±0.15) ,GIV (0.11±0.05) and GV(0.11±0.01), the elevation of serum LH mean value of GII group were continued after 4 weeks(0.29±0.07) , while LH mean values of group GIII (0.15±0.16)remained without any change but other groups showed non-significant reduction like GIV (0.10±0.05) and GV (0.10±0.01). After six weeks the mean value of LH concentration of GII(0.27±0.03) showed a non-significant reduction. While serum LH concentration in the other groups revealed no significant differences between groups (except GII) and within groups in different times.

The result showed a non-significant reduction in serum LH mean value of GIV and GV groups after the fourth and sixth weeks of the experimental period.

After four weeks of experiment period treatment of GV was shifted from sodium nitrite (100mg/kg) to DPP extract at a dose of 100mg/kg for another two weeks then serum LH mean value of GV (0.12±0.03) after 6 weeks clarified non-significant elevation as compared with the same group mean value after 2 weeks (0.11±0.01) and after 4 weeks(0.1±0.01) mean value.

Table (2) Effects of crude ethanolic extract of DPP on serum LH concentration IU/L of female mature rats exposed to sodium nitrite

G / T	GI D.W	G II 100mg DPP	G III 100mgDPP+ 100mg $NaNO_2$	G IV 100mg $NaNO_2$	GV 100mg $NaNO_2$ after 4w100mg DPP
Zero	**0.15±0.05 A a**	**0.15±0.07 A b**	**0.15±0.04 A a**	**0.15±0.07 A a**	**0.15±0.06 A a**
14 days	**0.15±0.07 B a**	**0.28±0.07 A a**	**0.14±0.15 B a**	**0.11±0.05 B a**	**0.11±0.01 B a**
28 days	**0.15±0.06 B a**	**0.29±0.07 A a**	**0.15±0.16 B a**	**0.10±0.05 B a**	**0.1±0.01 B a**
42 days	**0.15±0.07 B a**	**0.27±0.03 A a**	**0.14±0.05 B a**	**0.10±0.05 B a**	**0.12±0.03 B a**

L.S.D = 0.08 Values are expressed as mean ±SE, n=8 each group. Capital letters denote significant differences between group(P<0.05) small letters within groups

Effects of crude ethanolic extract of DPP in serum Prolactin concentration of female mature rats exposed to sodium nitrite

There are no adverse effects on serum prolactin concentrations of animals exposed to sodium nitrite at significant levels (P<0.05), the results clarified table (3) non-significant reduction in serum prolactin mean values of GII that received DPP extract only and GIII which received DPP extract plus nitrite along experimental period while the elevation of serum prolactin mean values (0.71±0.11) of GIV animals and GV(0.70±0.12) appeared after two weeks less than significant levels as compared with control group, but considered significant if compared with serum prolactin mean values of GII(0.56±0.08) and GIII (0.47±0.07) mean values at the same time, the result also showed a significant elevation in serum prolactin mean value of GIV animals(0.75±0.09) and GV(0.74±0.12) after 28 days of sodium nitrite drenching as compared with GII(0.55±0.05) and GIII(0.52±0.07) mean values at the same time so as with control(0.58±0.08) mean value.

After 42 days of experimental period, serum prolactin mean values were (0.61±0.08) for the control group ,(0.61±0.08) GII, (0.51±0.1) GIII, (0.74±0.09) GIV and (0.57±0.12)GV, statistical analysis appeared (except GIV)no significant differences between group mean values, but showed significant elevation in serum prolactin mean value of GIV that received only sodium nitrite on one side as compared with the control, GII, GIII, and GV mean values that received extract alone or extract with sodium nitrite in another side. Although serum Prolactin mean value of GV that received nitrite showed significant (P<0.05) differences between mean values of GII and GIII at 2 and 4 weeks of experimental period, but after 6 weeks of experimental period when treatment of GV shift from nitrite to DPP extract the result showed a significant decline in serum prolactin means value as compared with the previous period of the same group results, the same was with GIV mean value at the same time

Table (3) Effects of crude ethanolic extract of DPP on serum Prolactin concentration IU/L of female mature rats exposed to sodium nitrite

G / T	GI D.W	G II 100mg DPP	GIII 100mgDPP + 100mg $NaNO_2$	G IV 100mg $NaNO_2$	GV 100mg $NaNO_2$ after 4w100mg
Zero	0.60±0.29 A a	0.58±0.19 A a	0.60±0.08 A a	0.60±0.1 A b	0.60±0.08 A b
14 days	0.59±0.27 A a	0.56±0.08 A a	0.47±0.07 A a	0.71±0.11 A b	0.70±0.12 A b
28 days	0.58±0.08 B a	0.55±0.05 B a	0.52±0.07 B a	0.75±0.09 A a	0.74±0.12 A a
42 days	0.61±0.08 B a	0.61±0.08 B a	0.51±0.1 B a	0.74±0.09 A a	0.57±0.12 B b

L.S.D =0.14 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

The focal hormone of mammalian multiplication is follicle engaging hormone is essential for gonadal change and development at pubescence and gamete creation amid the fruitful period of life. It empowers the development and development of ovarian follicles by acting straightforwardly on the receptors situated on the granulosa cells. The diminishment in the levels of FSH by the nitrite may hamper folliculogenesis and delay advancement of the follicle in the pre-ovulatory phase[11].

The toxic impacts of nitrates and nitrites are very much archived in mammalians including weakness of conceptive capacity, and endocrine disturbance [12]. It is possible that, the concentrate may have applied its effect on the front pituitary or the hypothalamus since the emanation of FSH is controlled by the gonadotropic releasing hormone discharged by the hypothalamus [11].

Immobilization stress by nitrite caused a critical abatement in levels of luteinizing hormone (LH), [13]. Furthermore this study achieved a critical anxiety actuated diminishment in serum LH level. Previous studies showed that numerous stressors diminish LH by repressing LH-RH amalgamation and discharge from the hypothalamus [14]. Such extend impelled limitation of the hypothalamic-pituitary-gonad (HPG) pivot may be interceded by corticotropin-discharging variable (CRF) and endogenous opioids, fundamentally β-endorphins which are known not discharged from the hypothalamus in light of anxiety [15]. It has been exhibited that both CRF and β-endorphins can apply their impacts on the HPG pivot by restraining LH-RH release from the hypothalamus [16], repressing LH discharge from the pituitary [17].

The reduction in FSH and LH may be due to the elevation of prolactin,that revealed in groups which received sodium nitrite, these results agree with many searchers that record a relationship between serum prolactin elevation and the inhibition of ovarian function and ovulation [18].

In 2014, Arfat *et al* [19], revealed that DPP could enhance the levels of serum FSH, and LH in rats that may be due to the vicinity of gonadotropin-like substances in the DPP.

Prolactin(PRL) is a protein hormone made out of a polypeptide chain, secreted in various tissues. In the anterior pituitary acidophilic cells, known as mammotropes. PRL and PRL-like immunoreactivities were demonstrated over ten tissues other than the pituitary gland, The richest source of peripheral PRL is the placenta, the endometrium, myometrium and smooth muscle fibroid of uterus[19], immune system [20], Mammary gland, Adrenal gland, Corpus luteum[21], Prostate, Testes, Urethral gland, Lacrimal gland, Sweat gland, Pancreatic islets and brain [22].

The blend and arrival of prolactin by the anterior pituitary are controlled via autocrine and paracrine signals from the anterior pituitary itself and by gonadal steroids [23].

Dopamine holds a predominant role in the regulation of prolactin secretion, it inhibits the PRL high-secretory of the cell, by tying to D2 receptors communicated on the cell membrane of the lactotroph, enactment of which

results in a diminishment of PRL exocytosis and gene expression [24].

The consequences of the current study demonstrated a rise of serum prolactin in creatures got sodium nitrite just, this may be because of the lessening impacts of nitrite on dopamine, these results are consistent with a previous report which showed that nitrite, may contribute to the vulnerability of dopaminergic neurons to the oxidation of DA and change of protein[25]. Dopamine antagonists have been reported to increase prolactin concentration levels[26]. That sure the inhibitory effect of nitrite on dopamine activity. Thus, this result showed the elevation of PRL in the groups received sodium nitrite only. Veena *et al*. (2008)[27], demonstrated a positive correlation between serum prolactin and nitrite.

Oxidative stress caused Prolactin elevation that is a major stress-induced hormone, and it was secreted follows environmental, psychological, or physical stress[28].

High prolactin influenced an oxidative anxiety in the hypothalamus and reasons nitration of tyrosine hydroxylase, the chemical IS required for the combination of dopamine (DA) [29].

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