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Effect Of Date Palm (Phoenix Dactylifera) Seeds Extracts On Hematological, Biochemical Parameters And Some Fertility Indices In Male Rats

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Abstract

The date seeds used in some traditional medicines and has been investigated for potential health benefits. The present work was carried out to evaluate the impact of date palm pit on hepatic, renal, hematological parameters hormone testosterone and antioxidant status in testis in male rats. Twenty male rats were involved in this study and were divided randomly into two groups (10 rats in each group) and treated as following Group 1 served as control and received basal diet without any date seeds. Group 2 received date seed extract in a dose of 2 ml / kg orally for 60 days. The results showed that the daily oral administration of pits of date palm caused a significant increase in hemoglobin concentration, MCH and MCHC while caused a significant decrease in total protein, ALT and creatinine. The daily oral administration of seeds extract decreased malondaldehyde level in testicular tissue of male albino rat. On conclusion the date seeds has the potential to improve serum biochemical values, testosterone level and antioxidant status in testis.

Keywords: Antioxidant; Date seed; hematological parameters; liver; kidney; testosterone

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1. Introduction

Egypt is considered to be one of the date-producing countries. The fruit of the date palm is composed of a fleshy pericarp and seed [1].The date seeds represent about 15% of the total weight of the date fruits [2]. The seeds of many fruits are used in complementary and alternative medicine (CAM) to prevent or reduce stress and side effects diseases [3]. The date seed contain different chemical compounds such as saturated fatty acids as stearic and palmitic acid, unsaturated fatty acids such as linoleic and oleic acids which could inhibit the 5-α reducates enzyme, Zinc (Zn), Cadmium (Cd), Calcium (Ca) and potassium (K) [4]. Date seed contains 3.1–7.1% moisture, 2.3–6.4% protein, 5.0–13.2 fat, 0.9–1.8% ash and 22.5–80.2% dietary fiber. Also, seeds contain high levels of phenolics (3102– 4430 mg gallic acid equivalents/ 100 g), antioxidants and dietary fiber (78–80 g/100 g) [5].

The seed powder is also used in some traditional medicines and has been investigated for human potential health benefits [6], also included in animal feed to enhance growth and plasma level of testosterone [7]. 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 [8]. The pollen grains of date palm have been used by Egyptians to improve fertility in women [9]. In addition, date seed extract shows an ability to restore the normal functional status of the poisoned liver, and also to protect against subsequent carbon tetrachloride hepatotoxicity on the liver in rats [10]. Dietary antioxidants are important in controlling and ameliorating the harmful effects of oxidative stress, high intake of fruits and vegetables with high antioxidant content contributes to reduced risk of oxidative stress-mediated diseases such as cardiovascular disease and cancer [11, 12]. Date seeds have been shown to contain significant amounts of antioxidants [5, 13].

In vivo studies on the effects of date seeds on blood picture, kidney function test and antioxidant potential are lacking. Therefore the present study was undertaken to determine the effect of date seeds on complete blood count, oxidative damage and antioxidant status in testicular tissue, serum biochemical parameters and hormone testosterone in male albino rats.

2. Material and methods:

2.1. Preparation and Extraction of date palm (Phoenix Dactylifera L.) seeds:

Date fruits were obtained from the Al-tahhan Dates Factory in El wady government; the pits were collected, rinsed well then left to dry and roasted. The dried pits were ground into a fine powder which added to distilled water to make a mixture of 50 gm / L, the mixture was boiled until it becomes brownish in color then filtration [14].

2.2. Animal:

Experiment was performed using 20 male rats; weighting 120 gm. Rats were randomly selected and transferred to an animal house having standard conditions. Animals were quarantined and allowed to acclimate for a week

prior to experiment. The animals were handled under standard laboratory conditions of a 12-h light/dark cycle in a temperature and humidity-controlled room. Water and feed were supplied ad libitum.

2.3. Experimental conditions:

Rats were randomly divided into two groups of ten animals each (n=10). Group 1 served as control and received basal diet. Group 2 received date seed extract in a dose of 2 ml / kg orally [14] for 60 days.

2.4. Blood sampling:

At the end of the test period, blood sample collected from retro-orbital puncture after diethyl ether anesthesia**,** blood samples were drawn into dry tubes (for obtaining serum) and heparinized tubes (for obtaining whole blood). Serum were separated after centrifuging the blood sample and stored at -20°C for subsequent analysis.

2.5. Hematological and Biochemical Investigations

Hematological parameters were determined by standard methods. Hemoglobin concentration was determined by the Cyanomethemoglobin Method [15]. Packed cell volume (PCV) was determined by microhematocrit method as described by Feldman et al [16] using microhematocrit centrifuge. The red cells (RBC) were counted under the high power of microscope by using double improved Neubauer counting chamber [16] and white blood cells (WBC) were counted under the high power of microscope by using double improved Neubauer counting chamber [17].

Mean corpuscular hemoglobin (MCH), mean corpuscular volume (MCV) and mean corpuscular hemoglobin concentrations (MCHC) were calculated according to the following equations *1, 2, 3* [16]:

$$MCH = \frac{Hb \times 10}{RBCs} \qquad (1)$$

$$MCV = \frac{PCV \times 10}{RBCs} \qquad (2)$$

$$MCHC\ \% = \frac{Hb \times 100}{PCV} \qquad (3)$$

The activities of serum AST, ALT were estimated by kinetic kits by the method of Young [18], Total protein was estimated by the method of Lowry et al. [19]. Urea was estimated by the method of Patton and Croush [20] and creatinine was estimated by the method of Young [18].

The serum testosterone level was assayed using immunoassay technique. The amount of testosterone was expressed as ng/ml.

2.6 Preparation of testicular tissue samples

The testis was removed and quickly excised, minced with ice cold saline, blotted on filter paper and homogenized in phosphate buffer (pH7.4). The supernatant were frozen at -20 C for further determination of antioxidant enzymes activities and MDA level. Tissue homogenate was prepared according to Combs et al. [21].

2.7 Lipid Peroxidation and Antioxidant Enzyme

Measurement of testis Malondialdehyde (MDA Concentration):

Testis lipid peroxidation product such as malondialdehyde (MDA) was determined by the method of Yashkochi and Masters [22]. MDA reacts with thiobarbituric acid (TBA) in an acid medium giving a colored TBA-complex measured colorimetrically at 520-535 nm against blank and MDA values were expressed as n moles MDA/gm tissue protein

Measurement of testis Superoxide Dismutase Activity:

Superoxide dismutase (SOD) activity was estimated according to Giannopolitis and Ries [23]. The optical absorbance was measured at wave length 560 nm against blank reagent. SOD= Reading (absorbance) of (SOD)/ mg protein.

Protein Determination: The total protein concentration of supernatant was determined by the method of [19].

Statistics: The values were expressed as means ± standard error (SE). T test was used to compare between the values of control group (G1) with that of the group 2 (G2) All statistical analyses were performed using SPSS (Statistical package for Social Sciences 10.0 for windows) [24].

3. Results

Date seed causes significant increase (P<0.05) of hemoglobin concentration while no significant difference in PCV%, WBCS and RBCs was found between date seed group and control groups (table 1).

The rats were administrated date seed exhibited a significant increase (P<0.05) of MCH and MCHC as compared to the normal rats (table 2). Date seed result in a significant decrease (P<0.05) in total protein and ALT while no significant difference in albumin and AST was found between date seed group and control groups (table 3).

Date seed causes significant decrease (P<0.05) in creatinine while no significant difference in urea was found between date seed group and control groups (table 4).

Date seed causes significant increase in testosterone level in serum while caused a significant decrease (P<0.05) in MDA level and there was no significant difference in SOD activity in testis was found between date seed group and control groups (table 5).

Table 1. Comparison of mean ± SD of hematological parameters between rats received date seed and controls.

Experimental group	Hemoglobin g/dl	PCV%	WBCs 1×10^3 /ml	RBCs 1×10^6
Control	15.64±0.76[b]	40.40±1.69	5.725±0.98	7.15±0.64
Date seed	18.99±0.41[a]	44.00±0.32	7.500±0.81	7.08±0.45

- g/dl = gram per deciliter, ml = milliliter

-Different letters in the same column show significant difference at the level of <0.05.

Table 2. Comparison of mean ± SD of hematological indices between rats received date seed and controls.

Experimental group	MCH (pg)	MCV (fl)	MCHC %
Control	22.11±0.76[b]	57.96±4.01	38.51±1.45[b]
Date seed	27.32±2.14[a]	63.02±3.43	43.21±1.65[a]

- MCH = mean corpuscular hemoglobin, pg = Picogram, MCV = mean corpuscular volume, fl = femtoliter, MCHC= mean corpuscular hemoglobin concentration.

- Different letters in the same column show significant difference at the level of <0.05.

Table 3. Comparison of mean ± SD of Total protein, Albumin, AST (Aspartate transaminase) and ALT (Alanine transaminase) between rats received date seed and controls.

Experimental group	Total protein (g/dl)	Albumin (g/dl)	AST (IU/L)	ALT (IU/L)
Control	5.11±0.14[a]	2.52±0.05	127.3±3.53	53.09±3.61[a]
Date seed	4.71±0.07[b]	2.48±0.07	131.3±4.21	40.04±2.52[b]

- Different letters in the same column show significant difference at the level of <0.05.

Table 4. Comparison of mean ± SD of urea and creatinine between rats received date seed and controls.

Experimental group	Urea (mg/dL)	Creatinine (mg/dL)
Control	38.53±1.18	1.14±0.08 [a]
Date seed	37.05±0.43	0.82±0.07 [b]

- Different letters in the same column show significant difference at the level of <0.05.

Table 5. Comparison of mean ± SD of testesteron in serum, malondaldehyde and superoxide dismutase in testis tissue between rats received date seed and controls.

Experimental group	Testosterone ng/ml.	MDA nmol/g tissue protein	SOD IU/mg tissue protein
Control	2.30±0.09 [b]	3.28±0.71 [a]	0.039±0.004
Date seed	3.19±0.17 [a]	1.31±0.15 [b]	0.045±0.006

- Different letters in the same column show significant difference at the level of <0.05.

4. Discussion

From the obtained results, it was clear that date seed causes significant increase (P<0.05) in hemoglobin concentration, MCH and MCHC while no significant difference in PCV%, WBCS and RBCs.

Hemoglobin is the main component of red blood cells; a high hemoglobin concentration indicates an above-average concentration of the oxygen-carrying protein hemoglobin in blood. High hemoglobin concentration is somewhat different from a high red blood cell count, because each cell may not have the same amount of hemoglobin proteins. Therefore, you could have a high hemoglobin count even if your red blood cell count falls within the normal range.

AST and ALT are considered to be two of the most important tests to detect liver injury, although ALT is more specific to the liver than is AST.

Our results revealed that date seed causes a significant decrease (P<0.05) of total protein and ALT while no significant difference in albumin and AST was found between date seed group and control groups (table 3).

The decrease in serum total protein and ALT may have been due to decreased release of tissue specific enzymes and other intracellular proteins which secondary to oxidative stress during metabolism. The mechanism by which the date pits induces its hepatoprotective activity is not clear. However, it is possible that the recorded content of vitamin C in the date pits 0.137% may also play a role in hepatoprotection [25].

Creatinine is a substance that is produced during the body's natural activity (metabolism).

The present study results revealed that date seed causes a significant decrease ($P<0.05$) in creatinine in comparison to control groups (table 4). This may be attributed the ability of date seeds to promote the filtration process and increase the efficacy of the two kidneys.

Testosterone is the principal androgen in males [26]. The production of testosterone by the male testes is stimulated by luteinizing hormone (LH), which is produced by the pituitary. Testosterone levels change dramatically during the life cycle of males [27].

The present study results revealed that serum total testosterone level showed a significant increase in date seed administrated rats as compared with the control ones. This result agree with Kostyuk et al. [28] indicated that Date palm pollen suspension increases the plasma levels of testosterone and this hormone is found at high concentrations in rat testis and seminal fluids. Also, Zargar [29] found that date extracts increase sperm count in guinea pigs and increase the concentration of testosterone, follicle stimulating hormone, and lutanizing hormone in rats. Date pits have been included in animal feed to enhance growth and fertility by its stimulatory effect on plasma level of testosterone [30].

Decrease in MDA level in testicular tissue of date seed group in compare with control one may be attributed to the effectiveness of the date seed in normal functional status of the testis. This finding is supporting by Mansouri *et al.* [31] indicated that the aqueous extracts of dates have potent antioxidant activity. The antioxidant activity is attributed to the wide range of phenolic compounds in dates including p-coumaric, ferulic and sinapic acids, flavonoids and procyanidins [32, 33].

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