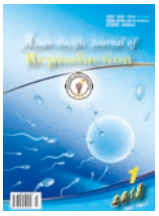

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Effect of water extract of dates palm (*Phoenix dactylifera*) on semen characteristics and oxidative status in serum of male New Zealand rabbits under heat stress

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ABSTRACT

Objective: To estimate the effects of the water extract of dates palm (*Phoenix dactylifera*) (DWE) on sperm quality parameters, testosterone level and serum antioxidants activities of New Zealand rabbits under heat stress. **Methods:** A total of 30 bucks of New Zealand White rabbits were randomly divided into three equal groups as follows: Group 1 was treated as control group and fed on balanced commercial ration. Groups 2 and 3 were treated with 10 and 20 mL of dates extract substituting water in the early morning before watering and fed on balanced commercial ration. This schedule was performed daily for 5 days/week, for an experimental period of 5 weeks. Fertility parameters such as reaction time, potential of hydrogen ion (pH), mass motility, individual progressive motility %, percentage of live sperm and abnormal sperm (%) were measured. Blood serum testosterone level, serum glutathione reduced, nitric oxide, ascorbic acid and malondialdehyde were also determined. **Results:** The daily oral administration of 10 mL DWE significantly increased the pH, the mass motility and individual progressive motility % compared to the control group. Although, the consumption of 20 mL DWE significantly (P<0.000 1) increased the live sperm% and decreased the abnormal sperm % compared to the other two treatments. The administration of date extracts (10 and 20 mL) had significantly (P<0.000 1) decreased nitric oxide and glutathione reduced levels compared to the control. On the other hand, it increased significantly the lipid peroxidation, ascorbic acid and testosterone level compared to the control. **Conclusions:** The aqueous extract of date palm (10-20 mL) could enhance the rabbit bucks fertility and its health performance.

1. Introduction

High temperature stimulates physiological stress in rabbits and decreases its production[1]. Rabbits are homoeothermic animals and very sensitive to high temperatures. They have few functional sweat glands that limit their ability to eject excessive body heat resulting from higher environmental temperature stress. Heat stress may lead to increased production of transition metal ions, which can make

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electron donations to oxygen forming superoxide or H_2O_2 which is further reduced to an extremely reactive OH^- radical causing an oxidative stress[2]. The former can affect male reproductive function through activating an oxidative stress cascade that assist in an arrest of sperm function.

The date palm has played an important role in the lives of many people due to their numerous health benefits, as it is rich in mineral salts and vitamins[3] such as vitamins C, B_1 (thiamine), B_2 (riboflavin), nicotinic acid and some phenolic compounds[4]. Studies have indicated that phenolic compounds are a main source of natural antioxidants in foods of plant[5] and display a wide range of biochemical activities such as antimicrobial, antimutagenic, and anticarcinogenic. So, incorporating antioxidants in the rabbit diets can improve male reproductive performance by declining the oxidative damage[6]. Consuming more antioxidants can improve the semen quality which includes motility and high number of sperms[7]. Thus, the aim of this study is to verify the ability of dietary date palm extract and its antioxidant capability in ameliorating sperm characteristics and total antioxidant blood capacity in the rabbits.

2. Materials and methods

2.1. Experimental animals

The present study was conducted between June and September 2016 at National Research Centre experimental animal house, Dokki, Giza, Egypt. Thirty apparently healthy, sexually mature and fertile New Zealand White male rabbits were obtained from a known archived herd in a popular farm. Rabbits aged 20 weeks and weighed 2.0-2.5 kg served as initial weight. Bucks were individually housed in metal wire meshed cages provided with separate facilities for feeding and water supply. Average ambient temperature and relative humidity were (34.00±0.27) ℃ and (70.24±0.88)%, respectively. All rabbits were offered with a commercial ratio (berseem hay, yellow corn, wheat bran, soybean meal, molasses, minerals and vitamins) in the form of small dry pellets with a diameter of 2 mm. This formulation had afforded 18% crude protein, ~2 600 kcal/kg with a C/P ratio of 144. Chemical analyses of diets were determined according to AOAC[8]. It provides normal growth and maintains adult body weight. Fresh tap water was supplied ad libitum. All managements care was carefully served.

2.2. Date palm water extract (DWE)

The 200 g of soft dates were emptied from their seeds, chipped in small parts and merged in 400 distilled water, then incubated at 5 ℃ for 48 h and shacked every 4 h. After incubation, the whole substances were filtered in a piece of gauze (20 cm × 20 cm) to remove the bulky fibers then filtered in Whatman paper No. 5 to remove minute debris. The filtrated dates extract was segregated into aliquots of 10 and 20 mL capped tubes and kept in refrigerator to be used daily for 5 days/week, for an experimental period of 5 weeks.

2.3. Experimental design

The 30 bucks were allocated in three equal groups (n=10) according to the randomized complete block design. Group 1 was treated as control group and fed on balanced commercial ration. Groups 2 and 3 were treated with 10 and 20 mL of dates extract, respectively, substituting water in the early morning before watering. Body weight of bucks was measured weekly during the experiment to maintain healthy management control. Bucks were inspected for any disease observations to be cured if necessary.

2.4. Sampling

At the end of the experimental period (after 35 days), semen samples were collected on an artificial vagina (40-42 ℃) provided with a clean graduated collecting glass tube. On the other hand, blood samples were collected from the middle ear vein in plain clean tubes, and spinned at 3 500 rpm for 15 min. The serum were collected in 2 mL eppendorf vials and stored at -80 ℃ until assayed.

2.5. Semen evaluation

Males were trained for semen collection on an artificial vagina (adjusted inner temperature, 42-45 ℃)[9] for 2 weeks. Two ejaculates per male and per week were collected with an interval of 3 days between them. The reaction time (in seconds) was the interval calculated from the introduction of the "teaser" doe into the male's cage till the first mounting with vigorous thrust and was considered as an indicator for libido. Ejaculates gel plugs were removed. All ejaculates were stored in a water bath at 37 ℃ until evaluation for 15 min maximum after collection. The volume of each ejaculate was recorded, after removal of the gel mass, in the graduated collecting tube. Potential of hydrogen ion (pH) was determined immediately after collection using pH paper (Universalindikator pH 0–14 Merck, Merck KgaA, 64271 Darmstadt, Germany).

Mass motility was assessed according to a subjective scale ranging from 0-9, and individual progressive motility % of the ejaculate was measured in aliquots under a microscope with a phase-contrast optic (Nikon) at × 400. Aliquots (10 μL) of raw semen were mixed with equal volume of vital nigrosin–eosin staining[10] and spread a thin film to allow the measurements of sperm quality traits (percentage of viable spermatozoa, percentage of total sperm abnormalities) by

examining 200 spermatozoa under a light microscope at × 1 000 (oil immersion).

2.6. Blood collection and biochemical analyses

Blood samples were collected into clean sterilized plain tubes from the ear vein of each buck on days 35 from the start of the experiment. Serum was separated from blood by centrifugation at 700 × *g* for 20 min and stored at -80 ℃.

Blood serum testosterone level was determined by using solid-phase enzyme immunoassay (ELISA) total testosterone commercial kit (Biosource, Testo ELISA, Belgium). The sensitivity of the assay was 0.05 μg/L and intra-assay coefficients of variation were 6.3 and 8.3, respectively.

Serum reduced glutathione (GSH), nitric oxide (NO), lipid peroxideindicator (Malondialdehyde, MDA) and ascorbic acid were measured using commercial kits (Bio Diagnostic, Egypt).

2.7. Statistical analysis

Replicates for all parameters, in the three groups, were statistically analyzed using the SAS[11] computerized program version 9.2. ANOVA test was performed for their means with a comparison between means using the Duncan Multiple Range test for a significant difference at $P<0.05$.

3. Results

Data in Table 1 showed that the 10 mL DWE was significantly ($P<0.036\ 7$) different from drinking of 20 mL DWE, while both were not significantly different from the control group. Non-significantly difference was shown for the ejaculate volume between the three groups. The pH of the 2nd and 3rd DWE treated groups (8.60 and 8.54, respectively) were significantly ($P<0.000\ 1$) increased compared to the control group. The drinking of 10 mL DWE had increased significantly ($P<0.000\ 1$) the mass motility score and individual motility% of the second group (8.36 score and 85.00%, respectively) compared to the other two groups. Although, the drinking of 20 mL DWE for the third group significantly ($P<0.000\ 1$) increased the live sperm% (94.17%) when compared to the two other groups. The significant ($P<0.000\ 1$) lowest abnormal sperm % was obtained on using 20 mL DWE (5.16%) compared to the other two treatment groups.

Data in Table 2 showed that the NO was significantly ($P<0.000\ 1$) decreased, in case of using date extracts (10 and 20 mL), compared to the control. On the other hand, the ascorbic acid showed a significant ($P<0.000\ 1$) increase, in case of using 20 mL date extracts, compared to the control. GSH was significantly ($P<0.000\ 1$) decreased compared to the control. MDA and testosterone, in case of using 20 mL extract, were increased significantly ($P<0.000\ 1$ and $P<0.008\ 0$, respectively) compared to the control and the 10 mL extract groups.

4. Discussion

As a scope of view, antioxidants are the compounds and reactions that include scavenging, disposing, and suppressing the formation of reactive oxygen species, or combating their actions. They can shatter the oxidative chain reaction, thus reducing the oxidative

Table 1

Effect of DWE on semen characteristics of male New Zealand rabbit (Mean ± SE, n=9).

Semen characteristics	Control	DWE		*F*-cal	Sig. (*P*>*F*)
		10 mL/day	20 mL/day		
Reaction time (s)	$23.86^{ab} \pm 1.87$	$31.43^{a} \pm 5.08$	$18.57^{b} \pm 1.43$	3.99	0.036 7
Volume of ejaculate (mL)	$0.57^{a} \pm 0.04$	$0.54^{a} \pm 0.12$	$0.75^{a} \pm 0.09$	1.52	0.245 6
pH	$7.59^{b} \pm 0.15$	$8.60^{a} \pm 0.04$	$8.54^{a} \pm 0.04$	39.89	<0.000 1
Mass motility (score 0-9)	$6.29^{b} \pm 0.42$	$8.36^{a} \pm 0.18$	$6.50^{b} \pm 0.19$	15.90	<0.000 1
Individual motility (%)	$65.00^{c} \pm 1.54$	$85.00^{a} \pm 1.54$	$75.00^{b} \pm 1.89$	36.00	<0.000 1
Live sperm (%)	$89.61^{c} \pm 0.70$	$92.79^{b} \pm 0.46$	$94.17^{a} \pm 0.34$	20.31	<0.000 1
Abnormal sperm (%)	$8.23^{a} \pm 0.44$	$6.63^{b} \pm 0.40$	$5.16^{c} \pm 0.44$	11.53	<0.000 1

Different superscript letters (a, b…*etc*) within row are significantly different using Duncan multiple range test at $P<0.05$.

Table 2

DWE influence on serum antioxidants activities and testosterone of male New Zealand rabbit (Mean ± SE, n=9).

Group	Nitric oxide (nmol/ mL)	Ascorbic acid (mg/dL)	Glutathione reduced (mg/dL)	Malondialdehyde (nmol/ mL)	Testosterone (ng/mL)
Control	22.880 ± 0.920^{a}	3.760 ± 0.040^{b}	4.900 ± 0.820^{a}	8.400 ± 0.100^{a}	2.660 ± 0.280^{b}
DWE (10 mL)	14.260 ± 0.750^{c}	3.420 ± 0.070^{c}	1.620 ± 0.210^{b}	9.400 ± 0.100^{a}	4.180 ± 0.430^{a}
DWE (20 mL)	17.950 ± 0.710^{b}	4.520 ± 0.003^{a}	1.330 ± 0.020^{b}	5.100 ± 1.000^{b}	3.650 ± 0.190^{a}
F-cal	29.490	162.030	16.490	13.950	5.950
P	0.000 1	0.000 1	0.000 1	0.000 1	0.000 1

Different superscripts (a, b…*etc*) indicate significant difference between means using Duncan multiple range test at $P<0.05$.

stress[12,13]. Dates can be a source of antioxidants through several mechanisms, such as neutralizing and destroying free radicals (NO, OH, and H_2O_2) and its precursors, preventing lipid peroxidation and stimulating antioxidant enzymes activity[14]. This result agrees with Al-Farsi *et al*[15], who indicated that the aqueous extracts of dates had potent antioxidant activity and it can be considered an important dietary source of polyphenols particularly flavonoids[16]. The phenolic compounds have antioxidant activity which can play a great role in deactivating and captivating free radicals and decomposing peroxides[17].

Our study showed that date palm extract had an effect on sexual behavior and libido through decreasing the reaction time due to their androgenic effects (increase plasma level of testosterone). This result agrees with Bahmanpour *et al*[18] and Mills *et al*[19] who certified that erection and sexual behavior depend mainly on androgen which regulates the capability of penile erectile reaction, partly by adapting the venous outflow from cavernous tissue. So, aqueous date extract might ameliorate male libido through the increment of the serum testosterone concentration[18].

Sperm motility depends on the synchronized actions of proteins, sugars, ions and small organic molecules. It is one of the main factors that facilitate the journey of sperm toward the egg and the subsequent fertilization process[20]. The observed improvement in the present study concerning sperm characteristics, including motility and abnormality, may be due to the antioxidant properties of date extract which can prevent the superfluous generation of free radicals produced by sperm[21]. These findings supported the obtained results concerning improvement in sperm motility in association with marked reducing sperm abnormality without pronounced effect on sperm livability.

Testosterone level in blood increased by using date palm extract than control group. This consequence is similar with Kostyuk *et al*[22] who found an increase in plasma levels of testosterone on eating date palm. Also, Zargar *et al*[23] found that date extract increased the level of follicle stimulating hormone, luteinizing hormone and testosterone in rats.

MDA and GSH are indicators of lipid peroxidation and antioxidative stress, respectively. MDA could affect negatively some of the physiological mechanisms in the body as it may react with some molecules such as proteins and deoxyribonucleic acid. These molecules can be considered more than a lipid peroxidation product[24]. Decrease in MDA level in blood of date group compared to a control one may be attributed to the efficacy of the date on the testis function. This discovery was supported by Mansouri *et al*[24] who found that the aqueous extracts have a wide range of phenolic compounds including ferulic, sinapic acids, flavonoids, p-coumaric, and procyanidins that potentiate the antioxidant activity of the date extract as a dietary supplement[25].

Concerning the NO, it is found to play important roles in sperm physiology by its participation in the regulation of sperm motility and capacitation[26,27].

In conclusion,the low dose of date palm extract can im prove semen quality parameters and testosterone level which lead to increased male fertility. Accordingly, it is concluded that medicinal plants such as date palm pollen can be used as efficient protective agent to reduce the adverse effects of heat stress

Conflict of interest statement

The authors declare that they don't have any conflict of interest.

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