



## Effects of Date Palm Fruit (*Phoenix Dactylifera L.*) as a Dietary Additive on Some Physiological Parameters and Radiographic Bone Density in Heat Stressed Male Rats

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### ABSTRACT

Heat stress is a life-threatening condition with a detrimental impact on the physiological functions of both humans and animals. The present study aimed to investigate the effect of date palm fruit on physiological functions and bone density and to document the protective effect of date palm in mitigating the negative impact of heat stress in rats. Thirty-two mature male Sprague Dawley rats weighing 170–200 g were randomly divided into four groups (8 rats/group): group 1 (control) was provided with standard diet pellets; group 2 (heat stress) rats received a standard diet and were exposed to artificial heat stress (43°C for 60 min/day); group 3 (date palm fruit) rats were given date palm fruit at a dose of 1 g/kg body weight; and group 4 (date palm fruit + heat stress) rats received date palm fruit with the same dose and were exposed to the same protocol of heat stress. Diet protocols started from the beginning of the experiment and continued till the end of the study at 2 months. Heat stress was induced (groups 2 and 4) daily during the second month of the experiment. Hematobiochemical and oxidative stress parameters, histopathological examination of the liver, kidney, and adrenal gland, and quantitative evaluation of radiographic bone density were evaluated at the end of the study. Results demonstrated heat stress resulted in significantly increased leucocyte count, decreased RBCs, platelet count, and Hb concentration ( $P < 0.01$ ). Significant increases in ALT, AST, ALP, urea and creatinine levels ( $P < 0.01$ ) with concurrent histopathological changes in the liver and kidney were also recorded in heat-stressed rats. Oxidative stress biomarkers, glucocorticoids, were increased with heat stress ( $P < 0.01$ ). Serum calcium level and radiographic bone density were significantly decreased in the heat stress group. Rats supplemented with date palm and subjected to heat stress exhibited an insignificant change in physiologic parameters ( $P > 0.05$ ) compared to control rats. In conclusion, date palm fruit demonstrated a protective effect against the deleterious changes of heat stress in rats by maintaining physiological parameters and improving bone turnover. Dietary supplementation with date palm prior to exposure to heat stress is safe and effective in protecting against the life-threatening adverse effects of heat stress.

**Keywords:** Bone, Date fruit, Heat-stressed, kidney, Liver, Oxidative stress.

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### INTRODUCTION

Global warming and exposure to high environmental temperatures may be life-threatening, with a detrimental impact on the physiological functions of both humans and animals (Garnett *et al.*, 2009). When a homoeothermic organism is unable to release excessive heat from its body, it experiences a

condition called "heat stress," which leads to an increase in body temperature (Renaudeau *et al.*, 2012). Animals' nutritional intake, energy, body temperature, water intake, hormonal status, antioxidant concentration, and mineral balance are all impacted by stress due to high environmental temperatures (Zheng *et al.*, 2020a).

In the Middle East and North Africa, date palm fruit (*Phoenix dactylifera L.*) is an important part of the diet. Dates are a high-nutrient, low-cost source of macro- and micronutrients (Al-Farsi and Lee, 2008a and 2008b). Date fruits are rich in carbohydrates, including soluble sugars and dietary fibre, as well as low in lipids and proteins (Ghnimi *et al.*, 2017). Additionally, dates contain significant nutrients that have a variety of beneficial effects such as anti-mutagenic, anti-microbial, anti-inflammatory, hepatoprotective, gastroprotective, anti-cancer, and immune-stimulatory action (Khalid *et al.*, 2017). Also, dates are rich in flavonoids, procyanidins, p-coumaric, ferulic and sinapic acids, all of which have strong antioxidant effects (Yun *et al.*, 2006). The added nutritional value of dates is also based on the presence of vitamin C (Allaith, 2008). Date fruit provides essential minerals such as calcium, iron, magnesium, phosphorus, potassium, zinc, selenium, and manganese (Hussain *et al.*, 2014). In fact, due to their potential positive effects on health, there is an interest in scientific research for the use of natural antioxidants to replace synthetic antioxidants, especially those of plant origin (Najafian and Babji, 2012).

Animals need multiple disciplines and strategies that prioritise housing, nutrition, and health management to reduce heat stress (Zheng *et al.*, 2020b). By comparison, dietary manipulation is the simplest and most affordable method (Aderao *et al.*, 2020) to minimize the detrimental consequences of heat stress (Tian *et al.*, 2022).

The aim of the present study was to investigate the effect of date palm fruit on haematological parameters, liver and kidney functions, as well as bone density. Additionally, the role of date palm fruit as a natural additive in strengthening male rats' ability to cope with heat stress was tested.

## MATERIALS AND METHODS

### Ethical approval

The present study was carried out at the Department of Physiology, Faculty of Veterinary Medicine, Cairo University. All study procedures were approved by the Institutional Animal Care and Use Committee of Faculty of Veterinary Medicine, Cairo University (VET.CU.IACUC, approval number Vet CU 20092022495). Experimental procedures were carried out in accordance with the National Institutes of Health (NIH) guidelines for the Care and Use of Laboratory Animals in Scientific Investigations.

### Animals

The study included thirty-two male Sprague Dawley rats weighing 170–200 g that were obtained from a parasite-free animal centre (Egyptian Holding Company for Biological Products and Vaccines—VACSERA, Giza, Egypt). Rats were fed a standard rat diet with calculated nutritional parameters (casein 200 g/kg, L-cystine 3 g/kg, corn starch 150 g/kg, sucrose 500 mg/kg, cellulose 50 g/kg, corn oil 50 g/kg, mineral mix S10001 35 g/kg, vitamin mix V10001 10 g/kg, choline bitartrate g/kg).

Rats were kept in a separate room with natural ventilation, a 12-hour light/dark cycle, a room temperature of 22–25 °C, and a humidity of 60–65%. They were grouped randomly and housed in plastic cages (30.80 × 59.37 × 22.86 cm) with galvanized iron filter tops. Rats were given two weeks to adjust to lab conditions before the experiment began.

### Experimental design

Rats were randomly allocated into 4 groups (n = 8). Group 1 (control group) was provided with standard diet pellets and drinking water *ad libitum*. Group 2 (heat stress group) was provided with standard diet pellets and drinking water *ad libitum* and exposed to heat stress during the second month of the experiment (43°C) for one hour daily. Group 3 (date palm fruit group) received date palm fruit after its preparation with a concentration of 1 g/kg body weight, and drinking water was provided *ad libitum*. Group 4 (date palm fruit + heat stress) received date palm fruit with the dose and was exposed to the same protocol of heat stress, and drinking water was provided *ad libitum*.

For all groups, diet protocols started from the beginning of the experiment and continued till the end of the study at 2 months. Heat stress was induced (groups 2 and 4) daily during the second month of the experiment.

### Preparation of date palm fruit

The plant material was rendered free from soil, the date palm fruits were manually separated from the pits, and the flesh of the fruits was cut into small pieces and coarsely ground using a grinding machine (Moulinex®, SEB Group, France). The grinded date palm extract was thoroughly mixed with the rat's ration and was given daily at a concentration of 1 g/kg body weight according to Fathy *et al.*, (2018) for each rat separately in groups 3 and 4.

### Heat stress protocol

During the 2<sup>nd</sup> month of the experiment, heat stress was induced in groups 2 and 4 using an artificial heating chamber without anaesthesia, according to **Halder et al., (2020)** with some modification. Heat stress was performed at 43°C for one hour daily using a heater that was fitted at the top-lid of the chamber and temperature was continuously monitored by a thermometer that was fixed at the corner of the chamber.

### Sampling

#### Blood samples and measured parameters

Fasting blood samples were collected from each rat by puncturing the retrograde orbital venous plexus using capillary tubes in the morning at the end of the experiment. Blood samples were collected (for all haematological and biochemical analyses) just prior to euthanasia. The 1<sup>st</sup> blood sample was taken on anticoagulant (0.1 mg EDTA/5 mL blood) and used for haematological examination. The 2<sup>nd</sup> blood sample was taken without an anticoagulant and left to clot. The sera were divided into 2 parts; 1<sup>st</sup> one was centrifuged at 4000 rpm for 15 min and stored at -20°C until the biochemical examinations were performed. The 2<sup>nd</sup> part was stored at -80°C until antioxidant parameters (MDA and TAC) were determined.

#### Determination of a complete blood count

Red blood cells (RBCs), white blood cells (WBCs), and platelet counts as well as haemoglobin (Hb) concentration were measured using a Coulter counter (automated haematology analyzer) according to **Longanbach et al., (2007)**.

#### Determination of the biochemical parameters

All commercial kits used in this part were purchased from Spectrum Company-Egypt. Biochemical parameters in the serum were measured using a UV spectrophotometer (Jasco, v-730, Japan). The measurement of AST was determined according to the method of **Saris (1987)**, ALT according to the method of **Bergmeyer and Horden (1980)** and alkaline phosphatase (ALP) according to the method of **Tietz (1986)**. Total protein (TP) was measured according to **Weichselbaum (1946)** and albumin was estimated according to **Doumas et al., (1971)**. Urea nitrogen concentration and creatinine level were determined according to the method of **Spencer and Price (1980)**. Serum glucose was determined according to the method of **Trinder (1969)**.

Serum calcium concentration was measured spectrophotometrically at 565nm using QCA calcium kits. At alkaline pH, calcium forms a coloured complex with O-cresolphthalein. 8-

hydroxyquinolein is added to the reagent as a chelating agent for magnesium ions, which can interfere with the reaction. Serum inorganic phosphate was measured spectrophotometrically at 625nm, according to the method described by **Risteli et al., (2015)**.

#### Determination of osteocalcin and corticosterone

Rat-specific (osteocalcin) Sandwich-ELISA Kit (Catalogue No. E-EL-R0243, Elabscience Company) was used in the determination of osteocalcin level in serum (**Aydin, 2015**). Rat-specific CORT (corticosterone) Sandwich-ELISA Kit (Catalog No: E-EL-0160, Elabscience company) was used in determination of corticosterone level in serum (**Kinn Rød et al., 2017**). All commercial kits used in this part were purchased from Sigma Chemical Co., Cairo, Egypt.

#### Determination of oxidative stress parameters

Determination of malondialdehyde concentration in serum according to the method of **Ohkawa et al., (1979)**. Total antioxidant capacity in serum was estimated according to the method of **Koracevic et al., (2001)** using commercial kits purchased from Bio-diagnostic Company, Cairo, Egypt and CAT NO (MD25 29 and TA 25 13, respectively).

#### Tissue samples

After the collection of blood samples, rats were euthanized for tissue sampling by cervical dislocation that was humanely performed under the effect of isoflurane anaesthesia for only a few seconds. For histopathological studies, the liver, kidney, and adrenal gland were fixed in neutral buffered formalin for 48 hours. They were subsequently washed in distilled water and processed through a graded series of alcohol, cleared in xylene, and embedded in paraffin wax. Sections of 5µ thickness were cut and stained with hematoxylin and eosin. Stained sections were examined with a light microscope for histopathological changes (**Dapson and Horobin, 2009**).

#### Radiographic bone density measurement

Digital radiographs were taken for the left femur of all rats using the Poskom machine (PCMAX-60H (LED), Poskom Co., Gyeonggi-do, Korea) using the same automated setting and read by the digital radiography system. Standardization of radiographic density was made through an aluminium step wedge that was positioned next to bone samples during the same radiographic exposure. The obtained radiographic images were

analysed using Carestream MI application software (version 5.0.2.30, Carestream Health Inc.). To create a calibration curve, the radiographic density was measured in a circular region of interest on each step of the wedge. The bone density of all left femurs was then measured in triplicate from lateral radiographs using a circular region of interest selected at the proximal third of the femur. After calibrating the images to optical density, the automatic selection tool was employed to delineate the bone area. The mean grey intensity of each pixel within the outlined region was then recorded (**Kinds et al., 2011; Castro et al., 2020**).

**Statistical analysis**

Data were tabulated and presented as mean ± standard error. Normality of the data was tested using Kolmogorov-Smirnov test. A one-way analysis of variance (ANOVA) was used to determine statistically significant differences between groups. When statistically significant differences were detected, LSD test was used for pairwise comparison. Data were considered statistically significant when P < 0.05. Data were analyzed using the Statistical Package for Social Sciences (SPSS Windows Version 28, SPSS Inc., Chicago, IL, USA).

**RESULTS**

**Effect of date palm fruit alone and after exposure to heat stress on some haematological parameters in male rats**

The heat-stressed rats (group 2) showed a significant decrease (P<0.01) in RBC count, Hb concentration, and platelet number. Also, group 2 showed a significant increase (P<0.01) in WBC number compared to group 1 (control). The date palm fruit group (group 3) showed a significant increase (P<0.01) in RBCs count and haemoglobin concentration when compared to group 1 (control group). Group 4 (date fruit + heat stress) revealed a significant increase (P<0.01) in RBCs number, Hb concentration and platelet number and a significant decrease (P<0.01) in WBCs, when compared to heat-stressed rats (group 2), as shown in Table 1.

Table 1: Effect of date palm fruit alone and after exposure to heat stress on some serum hematological parameters of male rats.

Groups Parameters	Group 1 (Control)	Group 2 (Heat stress)	Group 3 (Date palm)	Group 4 (Date palm + Heat stress)	P-Value
RBCs (10 <sup>6</sup> /mm <sup>3</sup> )	7.25±0.11 <sup>b</sup>	6.36±0.14 <sup>c</sup>	9.06±0.20 <sup>a</sup>	9.06±0.20 <sup>a</sup>	P<0.01
Hb (g/dl)	12.61±0.27 <sup>b</sup>	12.12±0.15 <sup>b</sup>	16.13±0.17 <sup>a</sup>	16.13±0.17 <sup>a</sup>	P<0.01
Platelet (10 <sup>3</sup> /mm <sup>3</sup> )	630±29.17 <sup>b</sup>	782±27.82 <sup>a</sup>	543±11.65 <sup>c</sup>	543±11.65 <sup>c</sup>	P<0.01
WBCs (10 <sup>3</sup> /mm <sup>3</sup> )	9.02±0.22 <sup>c</sup>	11.41±0.51 <sup>a</sup>	9.82±0.17 <sup>b,c</sup>	10.20±0.14 <sup>b</sup>	P<0.01

Each value is expressed as mean ± standard error.

Different superscripts in the same row show significant difference at P<0.05.

**Effect of date palm fruit alone and after exposure to heat stress on some serum biochemical parameters in male rats**

The data are presented in Table 2. The data revealed that there were significant increases (P<0.01) in ALT, AST, and ALP in heat-stressed rats and significant decreases in serum TP (P<0.01) and albumin (P = 0.04) levels when compared with the corresponding values in the control group. There was no significant difference (P > 0.05) recorded between date palm fruit and the control group in the following: ALT, AST, ALP activity, total protein and albumin levels. Group 4 (Date palm fruit + heat stress) showed significant decreases (P<0.01) in the serum ALT, AST, and ALP levels and significant increases in the serum TP (P<0.01) and albumin (P = 0.04) compared with Group 2 (heat stress group), as shown in Table 2.

There was a significant increase ( $P<0.01$ ) in creatinine and urea levels in heat-stressed rats when compared with the corresponding values in the control and date palm fruit groups. There was no significant difference between group 3 (date palm fruit) and group 1 (control group) in the creatinine and urea levels. While group 4 (Date palm fruit + heat stress) showed a significant decrease ( $P<0.01$ ) in the serum creatinine and urea levels compared with group 2 (heat stress group), as shown in Table (2).

Regarding serum calcium level, heat-stressed rats in group 2 showed a significant decrease ( $P<0.01$ ) in calcium level compared to group 1 (control group). Group 3 (date palm fruit group) showed a significant increase ( $P<0.01$ ) in serum calcium level compared to group 1 (control group). While calcium levels returned to normal levels in Group 4 (date fruit + heat stress group), as shown in Table 2, the heat stress group revealed a significant decrease ( $P<0.01$ ) in serum phosphorous level compared to other experimental groups (Table 2). Concerning serum glucose level, the heat-stressed rats showed a significant increase ( $P<0.01$ ) in serum glucose level compared to the control and date palm fruit groups, while group 4 (date palm fruit + heat stress) restored the serum glucose to the normal level as shown in Table 2.

Table 2: Effect of date palm fruit alone and after exposure to heat stress on some serum biochemical parameters of male rats.

Groups Parameters	Group 1 (Control)	Group 2 (Heat stress)	Group 3 (Date palm)	Group 4 (Date palm+Heat stress)	P-Value
ALT (U/L)	51.06±1.01 <sup>b</sup>	62.63±0.62 <sup>a</sup>	52.32±0.56 <sup>b</sup>	53.95±1.02 <sup>b</sup>	P<0.01
AST (U/L)	90.10±1.82 <sup>b</sup>	146.25±6.40 <sup>a</sup>	86.01±1.33 <sup>b</sup>	100.87±1.73 <sup>b,c</sup>	P<0.01
ALP (U/L)	182±26.71 <sup>b</sup>	363±16.38 <sup>a</sup>	206±19.08 <sup>b</sup>	260±27.64 <sup>b</sup>	P<0.01
Total proteins (g/dl)	8.17±0.22 <sup>a</sup>	4.88±0.51 <sup>b</sup>	8.03±0.17 <sup>a</sup>	8.34±0.14 <sup>a</sup>	P<0.01
Albumin (g/dl)	5.58±0.22 <sup>a</sup>	4.87±0.51 <sup>b</sup>	5.62±0.17 <sup>a</sup>	5.61±0.14 <sup>a</sup>	P=0.04
Urea (mg/dl)	44.56±1.05 <sup>b</sup>	59.37±1.49 <sup>a</sup>	45.58±0.96 <sup>b</sup>	47.96±0.35 <sup>b</sup>	P<0.01
Creatinine (mg/dl)	0.72±0.01 <sup>b</sup>	0.87±0.01 <sup>a</sup>	0.73±0.01 <sup>b</sup>	0.75±0.01 <sup>b</sup>	P<0.01
Calcium (mg/dl)	8.86± 0.09 <sup>b</sup>	7.40± 0.20 <sup>c</sup>	10.88± 0.22 <sup>a</sup>	9.30± 0.09 <sup>b</sup>	P<0.01
Phosphorous (mg/dl)	4.50± 0.22 <sup>a</sup>	3.53± 0.51 <sup>b</sup>	4.45± 0.17 <sup>a</sup>	4.59± 0.14 <sup>a</sup>	P<0.01
Glucose (mg/dl)	107.50 ± 2.12 <sup>c</sup>	147.37±1.91 <sup>a</sup>	117.75±0.79 <sup>b</sup>	118.0±2.20 <sup>b</sup>	P<0.01

Each value is expressed as mean ± standard error.

Different superscripts in the same row show significant difference at  $P<0.05$ .

**Effect of date palm fruit alone and after exposure to heat stress on serum oxidative stress parameters in male rats**

The serum MDA and TAC levels were significantly higher ( $P = 0.00$ ) in group 2 (heat-stressed rats) than in the control and date palm fruit groups. Moreover, group 4 (date fruit + heat stress) revealed a significant decrease ( $P = 0.00$ ) in serum MDA and TAC levels compared to group 2 (heat stress group), as shown in Table 3.

Table 3: Effect of date palm fruit alone and after exposure to heat stress on some serum oxidative stress parameters of male rats.

Groups Parameters	Group 1 (Control)	Group 2 (Heat stress)	Group 3 (Date palm)	Group 4 (Date palm + Heat stress)	P-value
MDA (mM/L)	5.64± 0.12 <sup>c</sup>	12.67±0.22 <sup>a</sup>	5.77±0.07 <sup>c</sup>	7.84±0.19 <sup>b</sup>	P=0.00
TAC (mM/L)	0.77±0.01 <sup>a</sup>	0.44±0.01 <sup>c</sup>	0.76±0.01 <sup>a</sup>	0.65±0.01 <sup>a</sup>	P=0.00

Each value is expressed as mean ± standard error.

Different superscripts in the same row show significant difference at  $P<0.05$ .

**Effect of date palm fruit alone and after exposure to heat stress on osteocalcin and corticosterone levels in male rats**

Serum osteocalcin level in group 2 (heat stress group) showed a significant decrease in serum osteocalcin level compared to the control and date palm fruit groups ( $P = 0.00$ ). While the serum osteocalcin level was significantly higher ( $P = 0.00$ ) in the date palm fruit group than the control group, However, group 4 (date fruit + heat stress) could restore serum osteocalcin to its normal level.

Concerning serum corticosterone concentration, the heat stress group (group 2) exhibited a significant increase ( $P = 0.00$ ) in serum corticosterone concentration compared to other experimental groups. Moreover, group 4 (date fruit + heat stress) reduced serum corticosterone concentration ( $P = 0.00$ ), as shown in Table 4.

Table 4: Effect of date palm fruit alone and after exposure to heat stress on serum Osteocalcin and Corticosterone levels of male rats.

Groups \ Parameters	Group 1 (Control)	Group 2 (Heat stress)	Group 3 (Date palm)	Group 4 (Date palm + Heat stress)	P-value
Osteocalcin (ng/ml)	4.99± 0.31 <sup>c</sup>	2.95±0.28 <sup>d</sup>	10.84±0.21 <sup>a</sup>	8.69±0.41 <sup>b</sup>	P=0.00
Corticosterone(ng/ml)	17.37±0.18 <sup>c</sup>	21.96±0.17 <sup>a</sup>	17.58±0.09 <sup>c</sup>	18.70±0.18 <sup>b</sup>	P=0.00

**Effect of Date palm fruit alone and after exposure to heat stress on radiographic bone density in male rats**

Radiographic bone density of the femur differed significantly among different groups ( $P = 0.010$ ), as shown in Figs. 1 and 2. Rats subjected to heat stress demonstrated decreased bone density compared to control rats. However, this decrease was not statistically significant ( $P = 0.582$ ). A statistically significant increase in bone density was recorded in rats treated with date palm either with or without heat stress compared to that of control and heat-stressed rats ( $P = 0.020$  and  $0.005$ , respectively). No statistically significant difference was recorded in the bone density of rats treated with date palm alone or treated with date palm and subjected to heat stress ( $P = 0.966$ ).

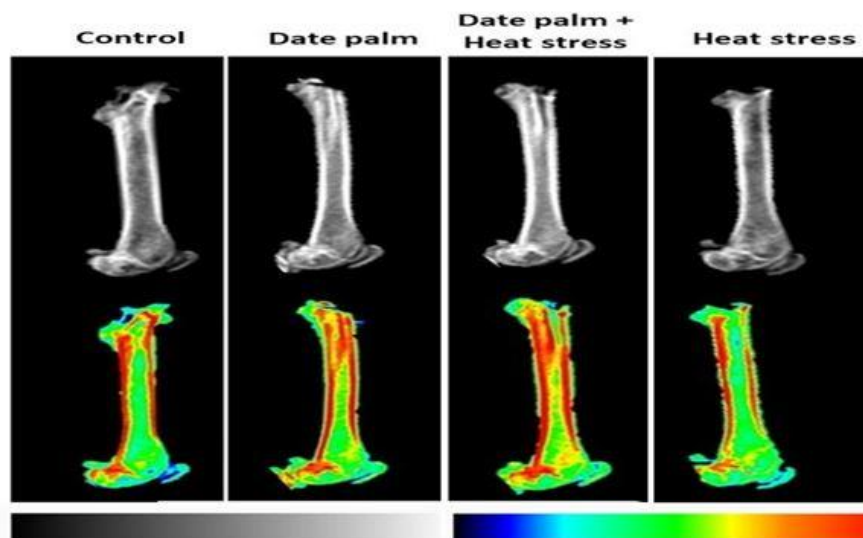


Fig.1: Representative digital radiographs of the left femur of rats in different groups. Radiographic bone density is represented in colour scale correlated to bone radiodensity and radiolucency.

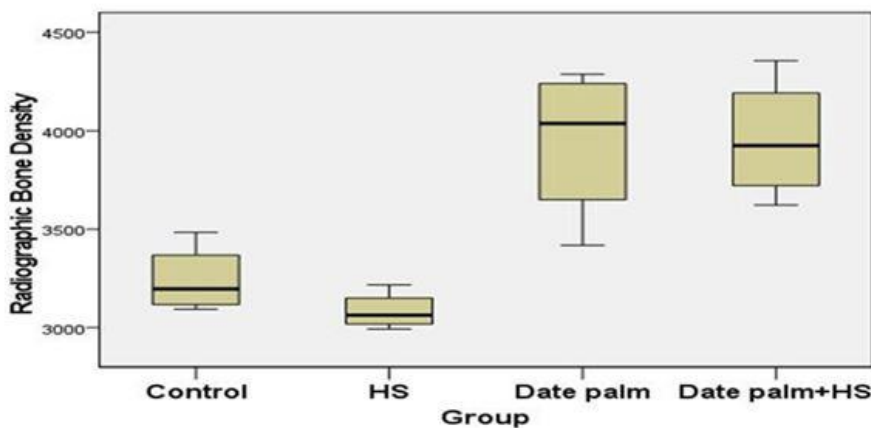


Fig.2: Boxplot visualization demonstrating radiographic bone density of date palm fruit group alone and after exposure to heat stress.

**Histopathological finding**

**Liver**

The sections of liver in groups 1 and 3 showed normal characteristics of hepatic architecture. Hepatocytes were arranged in cords radiating from the central veins and had rounded vesicular nuclei. Blood sinusoids were also observed (Fig. 3a and b). Histopathological examination of the liver sections of the heat-stressed group (group 2) showed moderate histopathological changes, congestion of the central vein, focal inflammatory cell haemorrhage of the blood sinusoids, and pyknotic nuclei (Fig. 3c). While group 4 showed a nearly normal structure associated with congestion in the central vein (Fig. 3d),

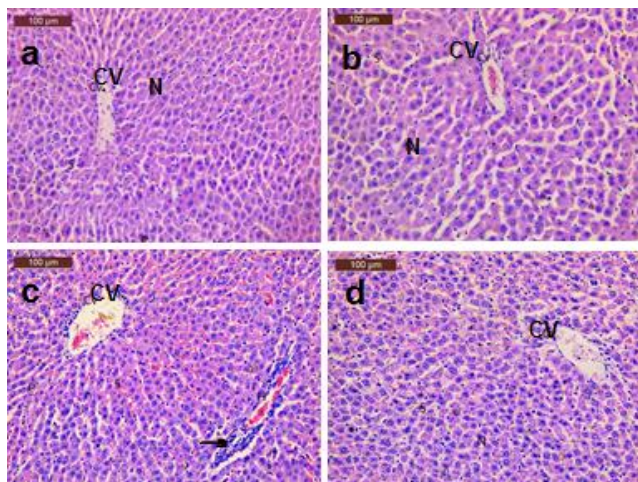


Fig.3: Photomicrograph of liver sections among different experimental groups. (a) Liver section of control group showing normal histological architecture, central vein (Cv) surround by hepatic cells and separated by blood sinusoids with prominent nuclei (N). (b) Liver section of date palm fruit group showing normal histological architecture of liver tissue, central vein surround by hepatic cells and separated by blood sinusoids with prominent nuclei. (c) Liver of heat stressed rat showing moderate histopathological changes, congestion of central vein, focal inflammatory cell, haemorrhage of blood sinusoids and pyknotic nuclei. (d) Liver section of pretreated rats with date palm fruit showing almost normal histological architecture of liver tissue, with congestion central vein.

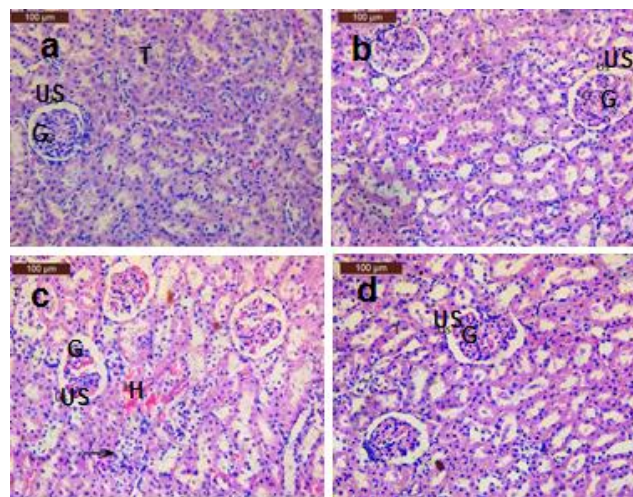


Fig.4: Photomicrograph of kidney sections among different experimental groups. (a) Kidney of control rat showing intact glomerulus basement membrane (G) normal urinary space (US) in between with normal tubular structures (T). (b) Kidney of rats in group 3 showing nearly normal of glomerulus, normal urinary space in between with normal tubular structures. (c) Kidney of heat stressed rats showing moderate histopathological changes with few shrunken glomeruli, mild dilated urinary Bowman's space and few foci of lymphocytic infiltration with interstitial haemorrhage (H). (d) Kidney of pre-treated rats with date palm fruit showing moderate ameliorative effect with few shrunken glomeruli, mild dilated urinary space and interstitial haemorrhage.

## Kidney

The sections of kidney of the control rats and rats treated with date palm fruit showed normal architecture of glomeruli, normal Bowman's space in between, and normal tubular structures (Fig. 4a and b). In addition, histopathological examination of the kidney sections of the heat-stressed group showed moderate histopathological changes with few shrunken glomeruli, mild dilated urinary space and few foci of lymphocytic infiltration with interstitial haemorrhage (Fig. 4c). While the pre-treated group with date palm fruit showed a moderate ameliorative effect with few shrunken glomeruli, mild dilated urinary space and interstitial haemorrhage (Fig. 4d),

## Adrenal gland

The sections of the adrenal gland in groups 1 and 3 showed a normal structure of the gland. The cortex layer consisted of three zones that had oval to rounded nuclei and acidophilic cytoplasm (Fig. 5a and b). Moreover, the heat stress-treated rats showed degeneration with inflammatory cell aggregation, vacuolated cells, haemorrhage, and pyknotic nuclei (Fig. 5c). While the pre-treated group with date palm fruit showed nearly normal gland structure with slight degeneration (Fig. 5d).

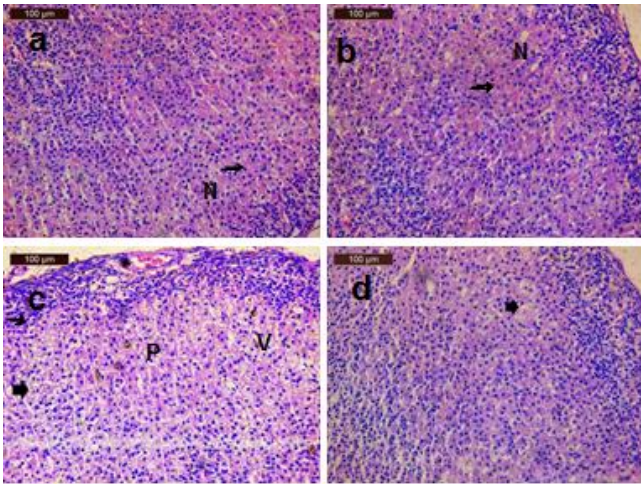


Fig.5: Photomicrograph of adrenal gland sections among different experimental groups: (a) Adrenal gland section of control rats showing normal structure. The cortex layer consists of three zones with oval to rounded nuclei (N) and acidophilic cytoplasm (arrow). (b) Adrenal gland section of date palm group showing nearly normal structure. The cortex layer consists of three zones with oval to rounded nuclei and acidophilic cytoplasm (arrow). (c) Adrenal gland section of heat stressed group showing degeneration (arrowhead), with inflammatory cells aggregation (arrow), vacuolated cells (V), haemorrhage (star), and pyknotic nuclei (P). (d) Adrenal gland section of pre-treated group with date palm fruit showing nearly normal structure with slight degeneration.

## DISCUSSION

Diet and lifestyle can affect the efficiency of different body functions and bone health. Persistent heat stress in the summer adversely affects domestic animals, leading to decreased productivity and metabolic activity by inhibiting certain physiological processes (Bhusari *et al.*, 2008). Recently, there has been an interest in supplementation with inexpensive and available alternatives that have a good impact on

health, bone metabolism, and overcoming the adverse effects of heat stress. Many studies have revealed that date palm fruit has a good impact on body health (Al-Mssallem, 2020; Fernández-López *et al.*, 2022). The aim of the present study was to investigate whether date palm fruit can ameliorate the adverse effects of heat stress on body functions and bone metabolism. The results of the present study revealed that date palm fruit administration significantly increased the RBCs count and Hb concentration compared to the control group. In this concern Zaakouk *et al.*, (2018) concluded that dates could boost RBCs count and Hb concentration and consequently, date palm fruit can restore anemia.

Another *in vivo* study by Paramitha and Machfudlo, (2020) indicated that date palm fruit can improve the RBCs number and Hb concentration due to its high iron content, so it can be used for the treatment of anaemia. Exposure of rats to heat stress caused a significant decrease in the count of RBCs, PLT and Hb concentrations compared to the control and date palm fruit groups. These results could be attributed to the ability of heat stress to cause osmotic swelling and erythrocyte hemolysis (Asmis *et al.*, 2005). Also, it might be due to increased destruction of mature cells, increased plasma volume, or decreased haemoglobin affinity for oxygen that induced hypoxia via diminished O<sub>2</sub> transport from the lungs to the blood and decreased O<sub>2</sub> release from oxyhaemoglobin to the tissues (Jagetia *et al.*, 2006). Exposure of rats to heat stress significantly increased total leukocytic counts (TLC). This result agrees with Haque *et al.*, (2013) who mentioned that exposure of animals to heat stress can activate the immune system and consequently increase TLC. Meanwhile, the pre-treated heat-stressed group with date palm fruit showed a return of the haematological values to normal values.

Concerning the effect of exposure to heat stress on liver functions, the obtained results indicated



that heat exposure significantly increased serum liver enzymes compared to the control and date palm fruit groups. This result is due to changes in membrane permeability and integrity of the cell membrane of hepatocytes that resulted in cellular leakage of these enzymes and increased their serum levels, which indicate liver necrosis as reported by **Malyar et al., (2021)**. Moreover, pre-treatment with date palm fruit showed improvements in liver function that prohibited prevented by heat stress. These results are consistent with **Ahmed et al., (2008)** and **Bashandy et al., (2018)** who recorded decreased serum liver enzyme levels in date palm fruit. This may be due to the phenolics and flavonoids in date palm fruit that help inhibit hepatic lipid peroxidation.

The current study showed that exposure to heat stress led to a significant decrease in serum TP and albumin due to hepatocellular damage, which caused an imbalance in the rate of protein manufacturing and breaking down in the liver (**Hashish and Elgaml, 2016**). While group 4 (date palm fruit + heat stress) showed a significant increase in the serum TP and albumin levels compared to group 2 (heat stress group), These results are in agreement with **Abdelaziz and Ali, (2014)** who found that aqueous date palm fruit treatment significantly boosted albumin and TP levels in rats following CCl<sub>4</sub>-induced hepatic damage because date palm fruit can preserve the liver's synthetic capacity and its flavonoids have membrane stabilizing activity.

Concerning the serum creatinine and urea levels, the result of the present study indicated that date palm fruit can maintain kidney function and ameliorate the damage occurring after heat stress exposure. This result is in accordance with **El Fouhil et al., (2013)** who reported that date palm fruit can reduce renal toxicity by restoring blood levels of urea nitrogen and creatinine to normal and minimizing proximal tubular deterioration.

The current study also showed that exposure to heat stress led to a decrease in serum calcium and phosphorous levels compared to control group which is consistent with the results obtained by **Desoky and Kamel, (2018)**. However, the pre-treated group with date palm fruit returned the serum calcium and phosphorous levels to their normal values.

The present study demonstrated that exposure to heat stress led to an increase in blood glucose levels compared to control group. This result is consistent with **Alhidary et al., (2012)**. This result may be due to greater blood insulin activity (**Baumgard et al., 2013**) or reduced feed intake. While the pre-treated group with date palm fruit before exposure to heat stress showed a return of the blood glucose level to

the normal range, The anti-diabetic activity of date palm fruit may be due to an increase in insulin output and a decrease in glucose absorption in the intestines (**Michael et al., 2013**). Date-derived diosmetin glycosides appear to increase insulin secretion and stimulate glycogen synthesis, which maintains blood-glucose homeostasis (**Singh et al., 2012**). According to that, date palm flesh may be used to replace the sugar in different food products to provide health benefits for diabetic patients (**Hussain et al., 2020**).

Regarding serum oxidative parameters, the heat-stressed rats showed a significant increase in serum MDA and TAC concentrations. **Kumbhar et al., (2018)** attributed this elevation to the increase of free radical production and the decrease of serum trace minerals associated with antioxidant defence in the body. On the contrary, date palm fruit restored MDA and TAC concentrations and returned their concentrations to their control values. **Benmeddour et al., (2013a)** reported that date palm fruit contains a lot of phenolic acids which are potent antioxidants that can capture free radicals and prevent different chronic diseases such as cancer and heart disease (**Kim et al., 2015**). Also, **Benmeddour et al., (2013b)** found that date palm fruits have a higher antioxidant capacity in comparison to other fruits when exposed to free oxygen radicals and so date palm is a powerful antioxidant.

Quantitative evaluation of radiographic bone density is widely utilized to evaluate bone density (**Huddleston, 2012**). Quantification of bone density relies on the principle of differential absorption of photons by tissues with varying radiodensities, including different regions within the same tissue. The amount of photon absorption is directly linked to the thickness and composition of the bone tissue. Consequently, as bone density decreases, there is a reduction in photon absorption, leading to the weakening of the radiographic signal (**De Klerk et al., 2009; Moorthi and Moe, 2013**). To the authors' knowledge, there has been no study till now aimed at using date palm fruit to improve bone health conditions and protect body function against heat stress. This study revealed an effective impact of the addition of date palm fruit to the diet on bone density, which reflects its benefit in bone turnover. In parallel to that, the current study proved the link between the corticosterone concentrations that increased in the heat stress group that reflected on the bone density induced by a decrease in osteocalcin level. The reason for those is that glucocorticoids have a direct effect on bone, causing inhibition of bone formation and enhancing bone resorption. Glucocorticoids decrease calcium absorption from the intestine and increase renal excretion (**Lukert and Raisz, 1990**). However,

pre-treatment rats with date palm fruit showed a protective effect, which reflected a decreasing corticosterone concentration that improved osteocalcin levels. That was ensured by the radiographic image, which showed the good impact of date palm fruit pre-treatment on bone density. Also, according to our results, we could conclude that date palm fruit has the protective property to face one of the most popular stresses, heat stress.

Concerning histopathological changes in the liver, the present study shows that exposure to heat stress induces congestion of the central vein, focal inflammatory cells, haemorrhage of the blood sinusoids, and pyknotic nuclei. **Malyar et al., (2021)** reported that heat stress induces nuclear damage and irregularly arranged liver cells. However, date palm fruit showed an improvement in histopathological changes and helped to get back to normal. In this regard, **Abdeen et al., (2021)** showed uniform polyhedral hepatocytes, regular sinusoids, and normal portal veins in the liver sections of date palm fruit-treated rats. **Abdeen et al., (2021)** also found that date palm fruit induces limited lymphocytic seepage and cytoplasmic vacuolations with un-noticeable sinusoidal changes and liver architecture relatively restores to a normal picture compared to gentamycin-treated rats.

Concerning the kidney, the result of the present study revealed that exposure to heat stress showed few shrunken glomeruli, dilated urinary space and foci of lymphocytic infiltration with interstitial haemorrhage. While date palm fruit showed an improvement in histopathological changes and returned to normal, **Abdeen et al., (2021)** found that date palm fruit induces mild missing of the brush border of the proximal tubules with slight inflammatory cell seepage and normal renal parenchyma compared to gentamycin-treated rats.

The present study showed degeneration with inflammatory cell aggregation, vacuolated cells, haemorrhage, and pyknotic nuclei in adrenal gland sections after exposure to heat stress, ensuring the protective role of pre-treatment with date palm fruit. Many studies have reported the protective effects of date palm fruit on the liver and kidney, but the present study is the first to record the protective effect of date palm fruit on the adrenal gland against the harmful effects of heat stress. Limitations of the present study may include the absence of the underlying molecular mechanisms of the protective role of date palm fruit in mitigating the negative impact of heat stress. Future studies will be directed towards understanding such molecular mechanisms and towards optimizing the

dose and duration of date palm supplementation to protect against heat stress.

## CONCLUSION

The present study is the first to document the beneficial role of date palm in mitigating the adverse effects of heat stress on liver and kidney functions, oxidative stress parameters, and radiographic bone density in a rat model.

## Conflict of interests

The authors declare no potential conflict of interest.

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