

Effect of Chronic Administration of Date Palm Seeds Extract on Some Biochemical Parameters, Oxidative Status and Caspase-3 Expression in Female Albino Rats

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Date palm seeds have been debated for their beneficial effects on body health. Present study discussed the influences of chronic exposure to palm date seeds extract on hepato-renal integrity and feed efficiency. Twenty one Albino female rats were randomly splitted into 3 groups. Group I, is the control and were given oral propylene glycol 10%. Group II, were given palm date seeds extract (200 mg/kg body weight) dissolved in 10% propylene glycol. Group III, were given (400 mg/kg body weight) palm date seeds extract dissolved in 10% propylene glycol. Treatments were given by intra gastric route daily for 60 days. Body weight, weight gain, feed efficiency ratio (FER) were determined. Serum liver and kidney functions tests were detected. Serum super oxide dismutase (SOD) and glutathione peroxidase (GPx) activities were estimated. Caspase-3 protein expressions in kidney and liver were detected by immunohistochemistry (IHC). Results revealed improved FER in group II and III than control. Reduction in serum liver and kidney enzymes were evident in group II and III with improvement in albumin and total protein levels. Caspase-3 IHC in liver and kidney exhibited significant ($P < 0.05$) reduction in palm date seeds extract treated groups especially in group III. Palm date seeds extract, seemed to be safe after chronic administration and has beneficial influences on liver and kidney. The beneficial effects of palm date seeds extract seemed due to its antioxidant effect that reduced hepato-renal caspase-3 protein expression.

Keywords: Caspase-3; Chronic Exposure; Kidney; Liver; Rat.

Phoenix dactylifera (date palm) is widely grown in both semi-arid and arid regions all over the world. They found in North Africa, the Near East, and the American continent, where they are grown in large quantities there (Al-Farsi, 2011). The fruits of date palm were consumed by several

countries all over the world. About 60- 150 g of seeds were obtained from one kilogram of ripen dates (Habib and Ibrahim, 2009). A huge amount of date seeds were produced yearly that were estimated to be approximately about 800000 tons (FAO, 2004). These huge amounts were used for

animal feed (cattle, camel, sheep and poultry), processing caffeine-free beverages that have flavor like coffee beside a considerable amount was wasted (Rahman *et al.*, 2007). Therefore, exploring new applications for date seeds can benefit the date producing countries (Habib and Ibrahim, 2011).

Several studies had demonstrated the nutritional value of palm date seeds especially antioxidant contents. They were found to contain moisture about 3.1-10.3 g/100 g, protein about 2.3-6.4 g/100 g, fiber about 73.1 g/100 g, fat nearly about 5.1-13.2 g/100 g, ash 0.9-1.8 g/100 g and phenolic compounds approximately 3942 mg/100 g (Baliga *et al.*, 2011; Al Farsi and Lee, 2008; Al-Farsi and Lee, 2011). They were estimated to have relatively great quantities of fat and protein, of high biological value, compared to date flesh. In addition to the beneficial effects of palm date seeds which attributed to their dietary fiber contents and phenolic compounds thus making them a good constituent for functional foods (Al-Farsi *et al.*, 2007). Although the date palm fruits had been used by millions of people as a cheap food source for centuries ago, researches on their benefits and nutritional advantages are insufficient (Vayalil, 2012; Takaeidi *et al.*, 2014).

Favorable effects of date palm seeds were previously shown by some researchers as they were proven to improve feed efficiency and reproductive hormones when they were incorporated in animal and poultry feed (Ali *et al.*, 1999; Elgasim *et al.*, 1995; Sawaya *et al.*, 1984). There are no available studies demonstrating the adverse effect of palm date seeds on human or animal health. Therefore, the present study aimed to investigate the effect of chronic exposure of female Albino rats to palm date seeds extract on feed efficiency, body weight, serum parameters for renal and hepatic function, serum antioxidant enzymes and histopathology of liver and kidney. Also, caspase-3 immunohistochemical staining in both liver and kidney was assessed.

MATERIAL AND METHODS

Extraction of palm date seeds

Date palm (*Phoenix dactylifera*) fruits and seeds were obtained from Saudi Arabia (identified and authenticated by Botany & Microbiology Department, Faculty of Science, Suez Canal University, Egypt). The fruits were thoroughly

washed with running water, dried in dark place and then the seeds were manually removed. Seeds were weighted, efficiently dried and re-weighted. The seeds were crushed into fine particles to be extracted as follow 500 g of crushed seeds were packed in separating funnel and completely submerged with 99.9% ethanol (about 750 mL). Funnels were kept in dark cold place for 24 h then the ethanol was collected and another amount was placed in funnels. This step was repeated 3-4 times till the seeds and the fruits were extensively extracted. The collected ethanolic extracts were evaporated for desiccation under reduced pressure at 25°C using rotator evaporator (rotavapor RE111, BÜCHI). The harvested extract crusts (17.62 g) were reconstituted in 10% propylene glycol (10 volume propylene glycol: 90 volume distilled water) to be suitable for oral administration in rats, this suspension was prepared fresh daily.

Animals and experiment design

Twenty one female Albino rats weighing (120-130 g) obtained from the Animal House of Theodor Bilharz Research Institute, Cairo, Egypt, were used for the present study. All animals were housed in standard plastic cages at the Laboratory Animal House, Faculty of Veterinary Medicine, Suez Canal University, Ismailia, Egypt. All experimental protocols were executed according to the "Guide for the Care and Use of Laboratory Animals". After accommodation for 14 days, rats were randomly divided into three equal groups, each containing 7 rats and treated as follows:

Group I, served as control and given oral propylene glycol 10% daily for 60 days.

Group II, given palm date seeds extract (200 mg/kg body weight) dissolved in 10% propylene glycol via intra gastric route daily for 60 days.

Group III, given (400 mg/kg body weight) palm date seeds extract dissolved in 10% propylene glycol via intra gastric route daily for 60 days.

Body weight and feed efficiency ratio (FER)

Experimental rats were weighed weekly during the whole experimental period. The weight gain was got by subtraction of final body weight from the initial one. Food intake was recorded all over the sixty days of the experimental time. Feed efficiency ratio (FER) was obtained according to the formula of Helmy *et al.* (2018) as follow:

FER = body weight gain (g) after 60 days/

food intake (g) for 60 days. The liver and kidney weights were recorded then relative hepatic and kidney weights in relation to body weight were calculated.

Sampling

At the end of the study, serum samples and tissue specimens were collected from diestrus females. Animals were exposed to mild tetrahydrofurane inhalation anesthesia. Blood samples were collected from retro-orbital venous plexus in plain centrifuge tubes by a clean sterile capillary tube that was inserted at the inner canthus of the eye. The blood was left for 20 minutes to coagulate then centrifuged at 3000 rpm for 15 minutes to separate the serum; then, serum was stored at -20°C to be used for further biochemical analysis. After blood collection, rats were dissected and the postmortem findings were recorded; then samples from the internal organs (liver and kidney) were taken. Liver and kidney sections were fixed in 10% neutral buffered formalin for the histopathological and immunohistochemical examination.

Liver and kidney function tests

Alanine aminotransferase (ALT), gamma glutamyl transferase (GGT), aspartate amino transferase (AST), and alkaline phosphatase (ALP) were calorimetrically measured using commercial kits DIACHEM Ltd. Co., Hungary. Both serum total proteins (TP) and albumin levels were measured according to the methodology of Gornall *et al.* (1949) and Westgard and Poquette (1972), respectively using Biodiagnostic Co., (Egypt) kits. Furthermore, serum level of urea and creatinine were determined by Diamond diagnostic kits, Egypt.

Antioxidant enzymes

Serum super oxide dismutase (SOD) and glutathione peroxidase (GPx) activities were

determined according to Nishikimi *et al.* (1972) and Paglia and Valentine (1967). Previous kits were got from Oxis Research Co., USA.

Histopathology and immunohistochemistry (IHC)

Formalin fixed kidney and liver tissues were paraffinized in blocks then sliced into 5- μ m-thickness sections to prepare histological slides. These slides were subjected to hematoxylin and eosin (H & E) staining procedures according to Drury and Wallington (1980) and examined carefully under microscope.

Immunohistochemical staining for kidney and liver slides were performed according to the methodology of Abdel-Rahman *et al.* (2018) using 1 : 1000 caspase-3 (Thermo Fisher Scientific Co., USA) primary antibody and secondary anti-rabbit antibody (Dako, USA), Immunoreactive staining area percentage (ISA%) was measured using ImageJ software according to Elgawish *et al.* (2015).

RESULTS

Body weight and feed efficiency ratio (FER)

The initial and final body weights of experimental rats were not differed at the start or at the end of experiment. Feed intake was significantly ($P < 0.05$) lower in low and high doses of palm seeds extract than control. Weight gain was significantly ($P < 0.05$) increased in high dose palm seeds extract than control, however low dose palm seeds extract exhibited non-significant alteration than control and high dose palm seeds extract. The FER exhibited significant ($P < 0.05$) increase in rats administered low and high doses palm seeds extract (Table 1).

Table 1. Effect of date palm seeds extract on body weight, feed intake, weight gain and feed efficiency ratio (FER) in female Albino rats

| Groups/ Parameters | Control | Low dose (200 mg/kg) | High dose (400 mg/kg) |
|-------------------------|----------------------------------|----------------------------------|----------------------------------|
| Initial body weight (g) | 124.7 \pm 1.52 ^a | 124.7 \pm 1.51 ^a | 124.7 \pm 1.2 31 ^a |
| Final Body weight (g) | 181.9 \pm 5.1 ^a | 190.3 \pm 4.2 ^a | 196.32 \pm 2.53 ^a |
| Feed intake (g) | 2514.3 \pm 85.7 ^a | 2221.4 \pm 57.6 ^b | 2112.91 \pm 34.62 ^b |
| Weight gain (g) | 57.1 \pm 4.2 ^b | 65.6 \pm 3.0 ^{ab} | 71.91 \pm 3.21 ^a |
| FER | 0.0229 \pm 0.0019 ^a | 0.0300 \pm 0.0016 ^b | 0.0341 \pm 0.0018 ^b |

Different superscripts in the same row are considered significant at $P < 0.05$

Table 2. Effect of date palm seeds extract on antioxidant enzymes, serum liver and kidney functions biomarkers in female Albino rats

| Groups/ Parameters | Control | low dose (200 mg/kg) | High dose (400 mg/kg) |
|----------------------|----------------------------|----------------------------|----------------------------|
| Urea (mg/dL) | 28.67 ± 1.94 ^a | 19.77 ± 1.03 ^b | 20.20 ± 0.87 ^b |
| Creatinine (mg/dL) | 0.865 ± 0.030 ^a | 0.795 ± 0.034 ^a | 0.838 ± 0.033 ^a |
| GGT (U/L) | 32.50 ± 0.65 ^a | 29.75 ± 0.48 ^a | 24.00 ± 1.58 ^b |
| ALT (U/L) | 32.00 ± 1.08 ^a | 26.50 ± 0.65 ^b | 18.75 ± 0.85 ^c |
| AST (U/L) | 37.50 ± 0.65 ^a | 31.75 ± 0.85 ^b | 22.75 ± 0.85 ^c |
| ALP (U/L) | 149.3 ± 3.6 ^a | 154.8 ± 15.2 ^a | 104.0 ± 3.0 ^b |
| Total protein (g/dL) | 6.43 ± 0.17 ^b | 7.26 ± 0.03 ^a | 7.34 ± 0.19 ^a |
| Albumin (g/dL) | 3.69 ± 0.17 ^b | 4.44 ± 0.15 ^a | 4.45 ± 0.16 ^a |
| SOD (U/mL) | 5.83 ± 0.20 ^b | 6.66 ± 0.14 ^a | 7.18 ± 0.13 ^a |
| GPx (nmol/mL) | 72.47 ± 2.21 ^c | 84.21 ± 2.40 ^b | 105.15 ± 2.86 ^a |

Different superscripts in the same row are considered significant at $P < 0.05$

Table 3. Effect of date palm seeds extract on caspase-3 Immunoreactive staining area percentage (ISA%) in female Albino rats

| Groups/Parameters | Control | low dose (200 mg/kg) | High dose (400 mg/kg) |
|-------------------|-------------------------|-------------------------|-------------------------|
| Liver ISA% | 45.9 ± 2.4 ^a | 28.5 ± 1.3 ^b | 23.8 ± 1.1 ^b |
| Kidney ISA% | 32.0 ± 1.0 ^a | 22.5 ± 1.4 ^b | 19.2 ± 0.9 ^c |

Different superscripts in the same row are considered significant at $P < 0.05$

Liver and kidney function tests

Table (2) demonstrated significant ($P < 0.05$) reduction in serum urea levels in both low and high doses palm seeds extract than control level while creatinine values exhibited non-significant alteration among groups. Serum activities of ALT and AST exhibited significant ($P < 0.05$) reduction in both low and high doses palm seeds extract than control with a significant ($P < 0.05$) decrease at high dose palm seeds extract group than the low dose. Serum GGT and ALP activities demonstrated significant ($P < 0.05$) decrease in high dose palm seeds extract group than control and low dose palm seeds extract group. However, total protein and albumin levels in sera showed significant ($P < 0.05$) improvement in both low and high doses palm seeds extracts than control. The serum antioxidant enzymes activities of SOD and GPX were significantly ($P < 0.05$) improved in both low and high doses palm seeds extract than control with significant ($P < 0.05$) improvement of GPX activity in High dose than the low dose palm seeds extract.

Histopathology and IHC

Liver of control group showed normal histo-architecture; central vein, radiating hepatocytes cords and sinusoids. The administration of low (200 mg/kg) and high (400 mg/kg) doses of palm seeds extract had no adverse influence on hepatic architecture (Figure 1 a,b&c). Kidney structure of control group demonstrated normal histo-architecture glomeruli and renal tubules. The administration of low (200 mg/kg) and high (400 mg/kg) doses of palm seeds extract had no adverse influence on renal architecture was observed among groups with best one in high dose palm seeds extract (Figure 2 a,b&c).

Immunohistochemical sections of caspase-3 revealed intra-cytoplasmic signal of caspase-3 protein that was represented by brownish colourations in hepatocytes (Figure 1 d,e&f), renal tubules and glomeruli (Figure 2 d,e&f). The ISA% exhibited significant ($P < 0.05$) reduction in liver and kidney of low and high doses of palm seeds extract groups than control. The high dose palm

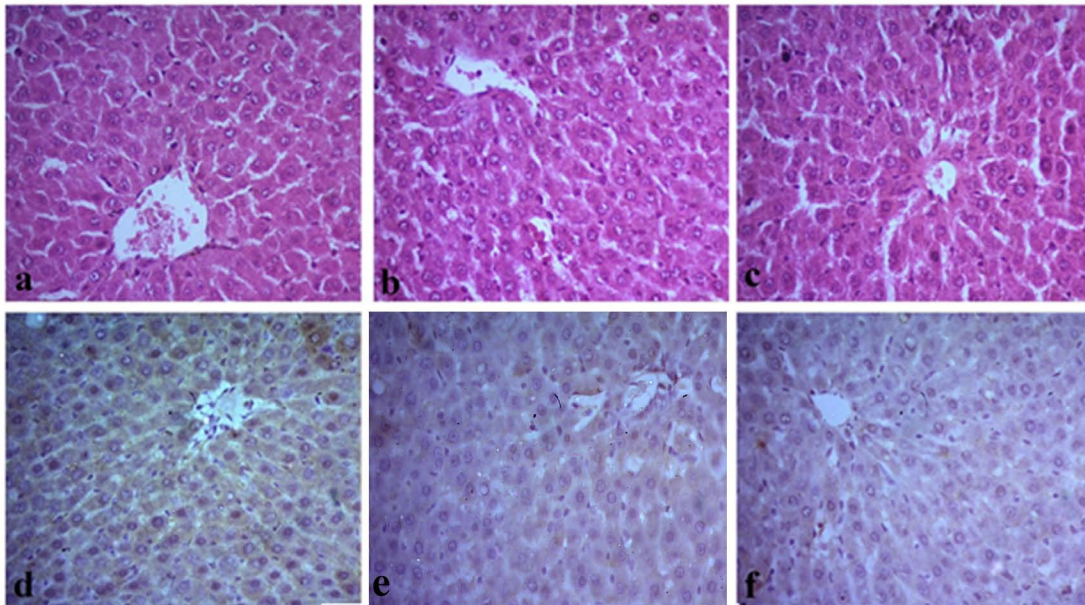


Fig. 1. Photomicrographs of liver histopathological sections of control (a), low dose palm seeds extract (b) and low and high doses palm seed extract (c). Normal histo-architecture (central vein, heopatoocytes and sinusoids) was observed among groups with best one in high dose palm seeds extract. Immunohistochemical sections for liver caspase-3 protein of control (d), low dose palm seeds extract (e) and low and high doses palm seed extract (f). The control group exhibited marked increase in caspase-3 protein staining than low and high doses palm seeds extract groups

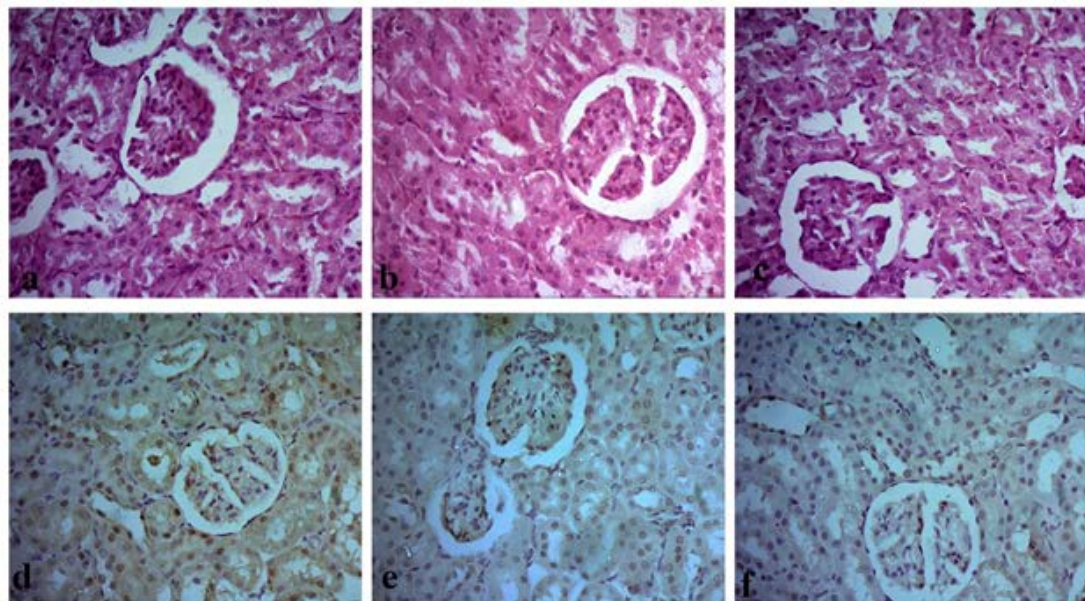


Fig. 2. Photomicrographs of kidney histopathological sections of control (a), low dose palm seeds extract (b) and low and high doses palm seed extract (c). Normal histo-architecture (glomeruli and renal tubules) was observed among groups with best one in high dose palm seeds extract. Immunohistochemical sections for kidney caspase-3 protein of control (d), low dose palm seeds extract (e) and low and high doses palm seed extract (f).the control group exhibited marked increase in caspase-3 protein staining than low and high doses palm seeds extract groups

seeds extract treated group exhibited significant ($P < 0.05$) reduction in caspase-3 ISA% than low dose palm seeds extract group (Table 3).

DISCUSSION

Palm date seeds have been recognized for their beneficial effects due to their, polyphenolics, anthocyanins sterols, flavonoids, procyanidins and carotenoids (Elgasim *et al.*, 1995). Adverse health consequences that may arise from chronic exposure palm date seeds extract were addressed in the present study through evaluation of hepatorenal functions as well as histopathology and IHC of caspase-3 in these organs.

Present study demonstrated that the body weights of experimental rats were not differing along the experimental period while feed intake was significantly lower in palm date seeds extract administered groups than control. This pointed to a high feed efficiency ratio as revealed in our result. Beside, weight gain was significantly promoted in high dose palm date seeds extract than control. The implications for this improvements may be due to protein content that mainly is lysine that has protein sparing effect to several cereals. Similar results were obtained by Ali *et al.* (1999) in male rats and Hussein *et al.* (1998) in broilers. Another attribution to the improved weight gain and FER is the anti-oxidant potential of palm date seeds extract that was demonstrated in the Herein study by promotion of serum activities of SOD and GPX in both low and high doses groups. The dietary antioxidants supplementation have been shown to reduce oxidative damage that impedes growth performance (Salami *et al.*, 2015).

Serum urea levels were significantly declined in both low and high doses of palm date seeds extract groups. The urea level is the last metabolic end result of the protein nitrogen balance process. Estimation of urea gives an idea about the efficiency of hepatic function through urea production cycle in metabolism of amino acids and proteins (González and González, 1994). The level of urea in blood gives an idea about its renal clearance efficiency; as it is primarily cleared from body via kidneys (Rivadeneira-Domínguez *et al.*, 2018). Creatinine is another kidney efficiency biomarker where it is produced from muscles and eliminated by kidney. Creatinine exhibited

non-significant alteration among groups. Both urea and creatinine results demonstrated non-toxic influence of palm date seeds extract chronic exposure on kidney function however, favorable influence may be confirmed. The expression of renal caspase-3 confirmed the later results where ISA% of caspase-3 exhibited a significant reduction in both palm date seeds extract treated groups. These results came in parallel with the increased antioxidant reserve of SOD and GPX. Caspase-3 is well recognized for its substantial role in apoptosis (Adedara *et al.*, 2017). Therefore present results augments the antioxidant and the anti-apoptotic influence of palm date seeds extract.

Serum activities of liver enzymes and biomarkers are good indicators for monitoring hepatic injury. Their proportions in blood were indicative for degenerative process in hepatic tissue (Hasan *et al.*, 2018). Also levels of ALP and GGT were indicative for infiltrative hepatic diseases or intrahepatic cholestasis (Posen and Doherty, 1981; Leonard *et al.*, 1984). All these enzymes were bound inside hepatocytes by their plasma membrane. They leaked to blood in case of hepatic degenerative conditions (Elgawish *et al.*, 2019). Present research revealed significant reduction in ALT, AST, GGT and ALP activities in palm seeds extract groups. These results lead us to the hepatoprotective effect of palm date seeds extract that could be mediated by its antioxidant potential. Antioxidant effect of palm date seeds extract could stabilize the hepatocytes cell membrane therefore prevent the later enzymes leakage to circulation (Eldeeb *et al.*, 2020). Parallel to these result, synthetic power of liver to the total protein and albumin (Oskoueian *et al.*, 2014) was significantly improved in both low and high doses palm date seeds extract than control. All previous results, sum up the hepatic improvement by palm date seeds extract via antioxidant potential which prevented apoptotic changes and reduced caspase-3 expression (Ahmed *et al.*, 2019) in hepatocytes treated with such extract.

CONCLUSION

Palm date seeds extract exerted hepatoprotection as well as reno-protection influences by its antioxidant potential. Chronic exposure to palm date seeds extract seemed to be safe for kidneys and

liver health. Palm date seeds extract improved liver and kidney serum biomarkers as well as reduced their apoptotic changes mediated by caspase-3 expression.

Conflict of Interest

The authors have no conflict to disclose.

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