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Research Article
Effect of Roasted Date Palm Rich Oil Extracts in Liver Protection and Antioxidant Restoration in CCl_4-induced Hepato Toxicity in Rats

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Abstract

Background and Objectives: Roasted date palm seeds are commercially available in the markets and used as hot-coffee drink-substitute and traditionally in the treatment of male infertility. This study aimed to evaluate the lipid profile, hepatoprotective, *in vivo* antioxidant activities of roasted date palm rich oil extracts on carbon tetrachloride (CCl_4) induced oxidative liver injury in rats. **Materials and Methods:** Rats were divided into 5 groups (n = 5), placebo (G1), rats received CCl_4 (G2), rats received CCl_4 and silymarin (G3), rats received CCl_4 and roasted date palm seed hexane extract (RDPHE) (G4) and rats received CCl_4 and roasted date palm seeds chloroform extract (RDPCE) (G5). Liver functions and lipid profile were determined by spectrophotometric assays. The superoxide dismutase (SOD) and glutathione peroxidase (GPx) activities were assayed by ELISA. **Results:** Both extracts of roasted seeds significantly decreased ALT level compared to G2 and significantly improved total cholesterol compared to both G2 and G3. The activity of SOD and GPx were significantly enhanced in G3, G4 and G5 compared to G2. Significant negative correlations of ALT with total cholesterol and HDL-C and also between ALP and LDL-C were recorded. Clear preservation of tissue architecture, intact hepatocytes, disappearance of tissue necrosis, fibrosis and marked reduction in inflammatory cellular infiltration were observed in the liver tissues of the treated groups. **Conclusion:** In conclusion, RDPHE and RDPCE enhanced the antioxidant enzymes activity and have ameliorative effects against CCl_4-induced hepatic injury in rats. The significant negative correlation was observed between liver enzymes (ALT, ALP) and lipid profile.

Key words: *Phoenix dactylifera*, roasted date palm seeds, hepatoprotective, superoxide dismutase, glutathione peroxidase, ALT, AST, lipid profile

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Plants and plant derived products were used to improve the human quality of life since prehistoric civilizations and still occupy a big part of the human use either as a food or as medications[1-6]. One of the worldwide commonly used plants is the date palm (*Phoenix dactylifera* Linn family Arecaceae "Nakhla") which particularly consumed as a food in most of the countries and used as a remediation for several diseases in their growing areas[7,8]. Date palm fruits and seeds are used in the commercial production of edible oil[9]. In addition, date palm is a rich source of sugars (glucose and fructose), vitamins, fibers, minerals and phenolic compounds with antioxidant, antibacterial, anti-inflammatory and fertility enhancer activities[10,11]. Date palms contain large amount of carotenoids e.g., lutein, β-carotene and neoxanthin which represent a class of natural fat soluble pigments and impart bright coloration to the plants. These carotenoids are a precursor for vitamin A and protect the cell from the oxidative stress of free radicals by acting as antioxidants[12]. Moreover, date palm roasted crushed seeds are widely used in Saudi Arabia as an coffee substitute and traditionally used in the treatment of male infertility by local Bedouins[13]. Roasted date palm is also used as an animal feed in the gulf countries[13,14]. Moreover, potential content of phenolic and flavonoid secondary metabolites were detected in roasted date palm extracts[13]. Because of highly important economic and health-associated values related to the roasted date palm seeds in Saudi Arabia, it was designated the current study to evaluate the hepatoprotective activity of the oil rich extracts (nonpolar extracts) of the roasted date palm seeds including their potential antioxidant activity in the experimental animals. Date palm seeds from the dates growing in Egypt were previously examined for its protective effect against CCl_4- induced hepatotoxicity[15]. However, the previous study investigated the aqueous extract (polar extract) of the date palm seeds while the current work concerning oil rich extracts (nonpolar extracts) of the roasted date palm seeds as a medicinally important and edible plant product in Saudi Arabia.

Annually, liver diseases accounted for about 2 million deaths worldwide[16]. Liver has an essential role in detoxification and elimination of numerous endogenous and exogenous compounds and thus, any hepatic injury leads to many health complications[17]. It also plays a crucial role in the metabolism of plasma lipids and lipoproteins, therefore, irregular lipid profile in patients with severe liver illness is potentially expected[18]. Additionally, hepatic damage is associated with oxidative stress, triggering depletion of reduced glutathione levels and cellular necrosis[19]. Carbon tetrachloride (CCl_4) is used as a model to study hepatotoxic effects through numerous mechanisms[20]. The ability of oil rich extracts of roasted date palm seeds to protect liver cells from oxidative damage of CCl_4, improve the lipid profile and stimulate the antioxidant enzymes in the experimental rats is a good marker for such edible plant as a health promoting agent.

MATERIALS AND METHODS

This study was carried out at Pharmacology and Medicinal Chemistry Departments, College of Pharmacy, Qassim University, KSA, from February, 2019-January, 2020.

Chemicals: Carbon tetrachloride was provided from Merck Company, Germany (BDH Chemicals, Poole, UK). Silymarin was obtained from MADAUS GmbH, 51101 Koin Company, Germany and roasted date palm seeds cake was purchased from local market in Medina, Saudi Arabia.

Plant materials and extraction method: Accurately, 1 kg of the crushed roasted date palm seeds were extracted with n-hexane and chloroform in sequences by cold maceration method[21]. The extracts were shaken for 24 h on an electronic shaker before filtration. The filtrated extracts were evaporated to dryness under vacuum at 30°C and the mother liquor extracts (oil rich extracts) were then stored in -20°C fridge for further experimental work.

Animals: In total, 25 Wistar rats weighing about 200-240 g were housed in a controlled environment with 25±2°C temperature, 35-75% relative humidity and 12 h light/dark cycles. They were given a standard diet and water *ad libitum*. All experiments were performed in compliance with the guidelines for animal's studies which issued by the Ethical Committee of College of Pharmacy, Qassim University, KSA.

Animal groups: The rats were randomly allocated into five equals groups (5 per group), Group 1 (G1): rats served as a placebo control group, received olive oil (0.5 mL/rat; IP) twice a week, Group 2 (G2): rats received CCl_4 (10% in olive oil, 0.5 mL/rat, IP) twice a week for 4 weeks for inducing liver injury[15], Group 3 (G3): rats received CCl_4 (10% in olive oil, 0.5 mL/rat, IP) twice a week for 4 weeks simultaneously with silymarin (25 mg kg^{-1}) as standard hepatic support drug which was administered orally in once daily manner[22], Group 4 (G4): rats received CCl_4 (10% in olive oil, 0.5 mL/rat, IP) twice a week for 4 weeks simultaneously with Roasted Date Palm Seeds Hexane Extract (RDPHE) of phoenix dactylifera

seeds (1.0 g kg^{-1}) which was administered orally in a daily manner[15], Group 5 (G5): rats received CCl_4 (10% in olive oil, 0.5 mL/rat, IP) twice a week for 4 weeks simultaneously with Roasted Date Palm Seeds Chloroform Extract (RDPCE) of phoenix dactylifera seeds (1.0 g kg^{-1}) which was administered orally in a daily manner.

Serum sampling: At the end of the experimental study, blood samples were obtained from the rats by retro- orbital puncture after mild anesthesia by diethyl ether. Sera were separated from all groups and stored at -80°C until analyses. Prior to assay, samples were thawed at room temperature.

Determination of liver function tests: The serum level of ALT, AST and ALP were assayed by kinetic method while the total protein was analyzed by colorimetric method. All used kits were provided by the Crescent Diagnostics Test (KSA) according to the method of Thefeld *et al*[23] for ALT and AST, whereas, ALP level was established by using the method of Belfield and Goldberg[24] and total protein was assayed according to the method of Weichselbaum[25].

Determination of lipid profile: Assay kits, Crescent Diagnostics Test (KSA) were used to measure the serum level of total cholesterol and triglycerides (TG) by following the colorimetric methods proposed by Trinder[26,27], respectively; while, High Density Lipoprotein (HDL) level was established using the colorimetric method of Ramadan *et al*[28]. The Low Density Lipoprotein (LDL) concentration was calculated according to the equation of Friedewald *et al.*[29]:

$$\text{LDL-C (mg dL}^{-1}\text{)} = \text{Total cholesterol-VLDL-HDL-C}$$

Determination of superoxide dismutase (SOD) activity: Superoxide dismutase (SOD) was determined according to instructions of kit manual provided by Cloud-Clone Corn company with serial number: 47A9824D88. The microplate was measured by ELISA reader at 450 nm and the results of SOD enzyme activity were expressed as ng mL^{-1} of serum.

Determination of glutathione peroxidase (GPx) activity: Glutathione peroxidase (GPx) activity was determined according to instructions of kit manual provided by Cloud-Clone Corn company with serial number: 32C904FD45. The microplate was measured by ELISA reader at 450 nm and the results of GPx were displayed as pg mL^{-1} serum.

Histopathological examination: The liver from all rats were sliced and fixed in 10% buffered neutral formalin. The formalin-fixed tissue was processed using automated tissue processor machine (Leica TP1020) and paraffin imbedded blocks was formed. Serial 3-5 µm sections were prepared by using microtome (Leica RM2245) and stained by hematoxylin and eosin stain and examined by using light microscope (Olympus BX41), digital image camera (5 MP Binocular Microscope Electronic Eyepiece USB Video CMOS Camera for Image Capture) and Toup View image analyzer.

Statistical analysis: The statistical analysis was conducted by using statistical package for the social science (SPSS, V. 21.0). Results of study were expressed as Mean±Standard Deviation (SD). Comparisons between different studied groups were carried out by one-way analysis of variance (ANOVA) followed by *post hoc* Bonferroni tests to compare between individual groups. Person correlation (r) was carried out to evaluate the correlations between different parameters. The level of statistical significance was set at $p<0.05$.

RESULTS

Effect of RDPHE and RDPCE on serum hepatic biomarkers: The liver function in the placebo, injured and treated animals was measured by determining the liver enzymes ALT, AST and ALP as well as total protein levels. The data presented in Table 1 revealed that the ALT level was significantly increased in the CCl_4 injured group (G2) compared to the placebo (G1). On the other hand, the ALT level was significantly decreased in RDPHE and RDPCE (G4 and G5) compared to the CCl_4 group (G2). Also, the level of ALT in G4 was significantly lower than its level in silymarin treated group (G3). However, no effect on the level of AST and ALP were observed in the treated groups compared to either placebo or injured group. The measured total protein level showed a significant decrease in CCl_4 group compared to G1. While, its level was significantly increased in treated rats with chloroform extract of date palm oil group as well as silymarin group compared to CCl_4 group (Table 1).

Effect of RDPHE and RDPCE on lipid profile: The measured lipid profile was total cholesterol, TG, HDL-C and LDL-C. The data showed in Table 2 revealed that total cholesterol, TG and HDL-C levels were significantly decreased in the CCl_4 group in comparison to G1. On the other hand, the serum level of TG and HDL-C levels were significantly increased in silymarin, RDPHE and RDPCE groups compared to G2. Furthermore, there was a significant increase in total cholesterol level in

Table 1: Hepatic biomarkers of different studied groups (n = 25)

Parameters	Control group	CCl_4 group	CCl_4+silymarin group	CCl_4+RDPHE group	CCl_4+RDPCE group
ALT (U L^{-1})	037.26±01.90	054.84±13.42[a]	048.93±02.02	35.22±02.02[bc]	037.97±05.27[b]
AST (U L^{-1})	143.43±51.20	158.75±34.73	131.32±32.29	153.02±12.40	145.04±27.82
ALP (U L^{-1})	167.71±55.07	185.28±32.93	176.24±28.98	174.61±25.37	129.95±56.84
Total protein (g dL^{-1})	007.61±00.27	005.62±01.09[a]	006.91±00.50[b]	006.16±00.51[a]	007.13±00.46[b]

Results are expressed as Mean±SD, RDPHE: Roasted date palm seeds n-hexane extract, RDPCE: Roasted date palm seeds chloroform extract, [a]Significant difference from control group, [b]Significant difference from CCl_4 group, [c]Significant difference from silymarin group, p<0.05 was considered significant, ALT: Alanine aminotransferase, AST: Aspartate aminotransferase, ALP: Alkaline phosphatase

Table 2: Lipid profile of different studied groups (n = 25)

Parameters (mg dL^{-1})	Control group	CCl_4 group	CCl_4+silymarin group	CCl_4+RDPHE group	CCl_4+RDPCE group
Total cholesterol	127.09±17.89	45.81±04.94[a]	64.76±09.58[a]	69.76±04.29[abc]	97.76±13.003[abc]
Triglycerides	110.61±14.24	42.73±10.82[a]	77.70±13.23[ab]	69.47±11.50[ab]	90.52±07.731[b]
HDL-C	051.50±09.75	24.60±01.56[a]	34.96±01.66[ab]	43.84±03.75[b]	44.34±16.055[b]
LDL-C	017.87±11.85	12.66±04.29	15.54±05.08	14.06±03.48	18.17±06.278

Results are expressed as Mean±SD, RDPHE: Roasted date palm seeds n-hexane extract, RDPCE: Roasted date palm seeds chloroform extract, [a]Significant difference from control group, [b]Significant difference from CCl_4 group, [c]Significant difference from silymarin group, p<0.05 was considered significant, HDL-C: High density lipoprotein-cholesterol LDL-C: Low density lipoprotein-cholesterol

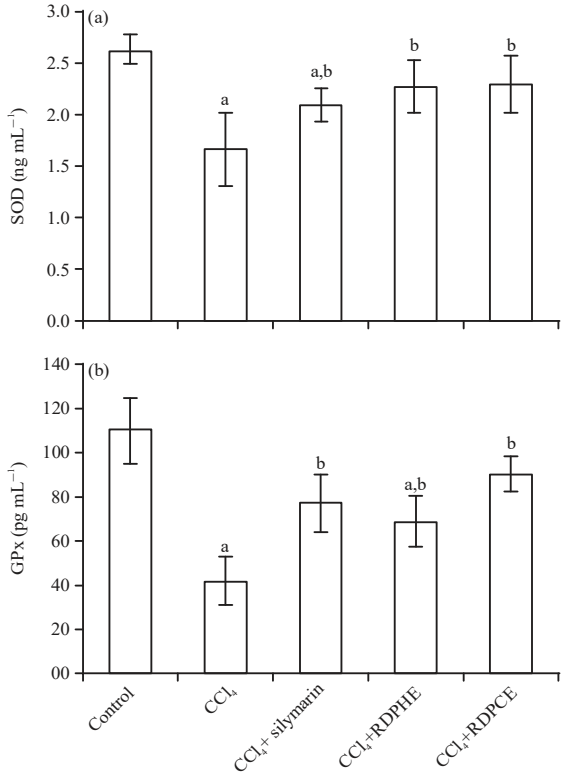

Fig. 1(a-b): Antioxidant enzymes of different studied groups (n = 25), (a) Superoxide dismutase (SOD) and (b) Glutathione peroxidase (GPx)

Result are expressed as Mean±SD, RDPHE: Roasted date palm seeds n-hexane extract, RDPCE: Roasted date palm seeds chloroform extract, [a]Significant difference from control group, [b]Significant difference from CCl_4 group, p<0.05 was considered significant

RDPHE and RDPCE (G4 and G5) compared to CCl_4 and silymarin groups. Regarding to LDL serum level, there was no significant difference among different studied groups.

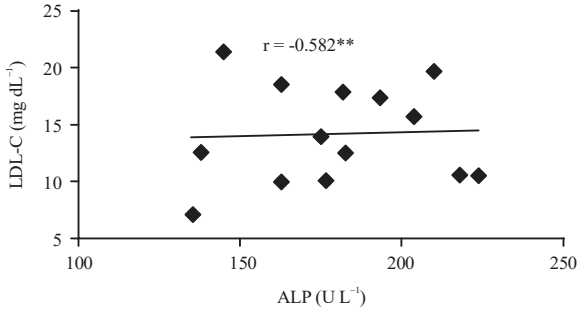

Fig. 2: Correlation between ALP and LDL-C

Results are expressed as correlation coefficient (r), **p<0.01, ALP: Alkaline phosphatase, LDL-C: Low density lipoprotein-cholesterol

Effect of RDPHE and RDPCE on antioxidant enzymes: To investigate the ability of enhancing the antioxidant activity of RDPHE and RDPCE, serum superoxide dismutase (SOD) and glutathione peroxidase (GPx) levels were measured. The current results revealed that 4 weeks of CCl_4 treatment induced significant decline in serum SOD and GPx enzymes activities compared with the placebo group. The RDPHE and RDPCE of *Phoenix dactylifera* seeds as well as silymarin groups demonstrated significant enhancement in the serum levels of both enzymes compared with the CCl_4 group (G2) (Fig. 1 a, b)

Correlations between liver enzymes and lipid profile: Negative significant correlations were detected between ALT and HDL (r = 0.702, p = 0.01), ALT and total cholesterol (r = 0.485, p<0.05) (Table 3). In addition, ALP showed negative significant correlation with LDL-C (r -0.582, p<0.01) (Fig. 2).

Histopathological evaluation: The histological studies of the liver from control group showed normal tissue architecture with distinct intact hepatocytes and well-arranged sinusoidal

Table 3: Correlation between liver enzymes and lipid profile

Biomarkers (U L^{-1})	Total cholesterol (mg dL^{-1})	Triglycerides (mg dL^{-1})	HDL-C (mg dL^{-1})
ALT	-0.485*	-0.411	-0.702**
AST	-0.047	-0.180	-0.117
ALP	-0.149	-0.154	-0.179

Results are expressed as correlation coefficient (r), *p<0.05, **p<0.01, ALT: Alanine aminotransferase, AST: Aspartate aminotransferase, ALP: Alkaline phosphatase

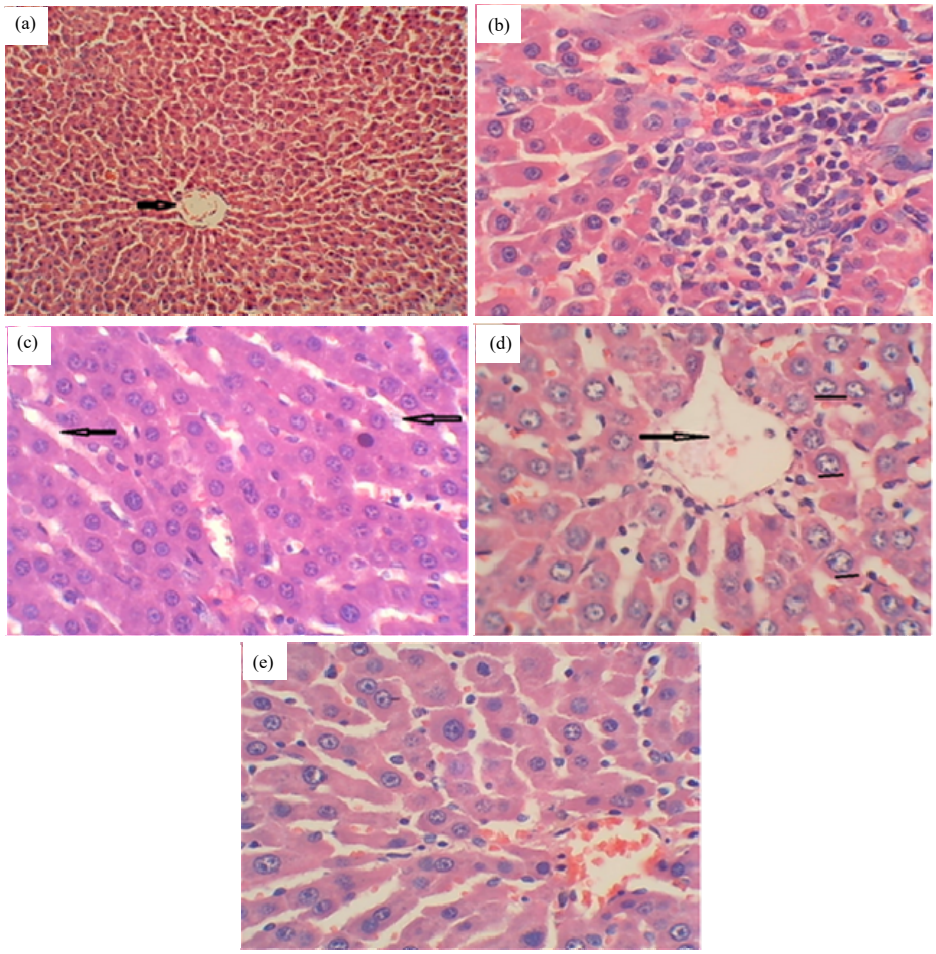

Fig. 3(a-e): Histological section of the liver stained with H and E stain, (a) Control group of rats showing normal tissue architecture with distinct intact hepatocytes and well-arranged sinusoidal spaces around a central vein (arrow), (b) Rats intoxicated with CCl_4 showing disarrangement of normal hepatic architecture with intense inflammatory cellular infiltration, fibrosis and vacuolar degeneration, (c) Rats treated with silymarin showing preservation of tissue architecture with intact sinusoids (arrow) and marked reduction of inflammatory cellular infiltration, (d) Rats treated with roasted date palm seeds n-hexane extract (RDPHE) showing intact liver cells around the central vein (arrow) with intact nucleus (line). With mild degree of inflammatory cells infiltration and (e) Rats treated with roasted date palm seeds chloroform extract (RDPCE) showing preserved tissue architecture with intact hepatocytes and mild degree of inflammatory cellular infiltration, the hepatocytes also showing decreased fatty vacuole

spaces. The central vein and the portal tract were arranged in a regular and well organized pattern (Fig. 3a). The liver section of rats intoxicated with CCl_4 showed dis-arrangement and degeneration of normal hepatic cells with intense inflammatory cellular infiltration with centrilobular necrosis, fibrosis, cloudy swelling and fatty degeneration of hepatocytes (Fig. 3b). In silymarin treated group, marked reduction in the inflammatory cellular infiltration was noted with preservation of the hepatocytes, tissue architecture and sinusoidal arrangement. The fatty vacuoles within hepatocytes were also

reduced (Fig. 3c). In rats treated with RDPHE of *Phoenix dactylifera* seeds, the liver sections showed hepatocytes with intact nucleus and arrangement. The central vein, portal tract and sinusoids were preserved. There was mild inflammatory cellular infiltration (Fig. 3d). In rats treated with RDPCE of *Phoenix dactylifera* seeds, the tissue architecture was also preserved with well-arranged hepatocytes and intact nucleus. There was a mild degree of inflammatory cellular infiltration and no centrilobular necrosis seen (Fig. 3e).

DISCUSSION

The current study revealed a significant increase ($p<0.05$) in serum ALT level as well as significant declines ($p<0.05$) in SOD and GPx enzyme activities in addition to serum total protein level in CCl_4 group (G2) compared to the placebo group (G1) which received olive oil. The toxic effect of CCl_4 on the liver cells was also confirmed by the histopathological examination of liver tissues which showed a major damage manifested as disarrangement and degeneration of normal hepatic cells with intense inflammatory cellular infiltration, centrilobular necrosis, cloudy swelling and fatty degeneration of hepatocytes. These observations come in consistent with Shankar *et al.*[30], who stated that CCl_4 induces liver cell necrosis with inflammatory cellular collections and fibrosis. Hepatic oxidative stress persuaded by CCl_4 intoxication and the reactive intermediate products generated during its bio-activation could cause a decline in the activities of SOD and GPx[31].

The current work showed significant decrease in the serum level of total cholesterol, TG and HDL-C in CCl_4 group (G2) compared to the placebo group (G1). These results are integrated with negative significant correlations between ALT with total cholesterol and HDL-C, as well as between ALP and LDL-C. The present result comes in agreement with Ishikawa *et al.*[32] observations. The current results could be explained by the toxic injury of CCl_4 which decreases the ability of liver cells to produce VLDL and HDL and the variations in their composition in apolipoprotein which resulted in decrease of the triglycerides and cholesterol esters secretion[33]. Inversely, our findings come in contrarily with the work of Mahmoodzadeh *et al.*[34], who stated that CCl_4 causes a significant increase in the triglycerides, total cholesterol and LDL-C levels as a result of stimulation of the transfer of acetate into liver cells and enhances lipid esterification[20].

The findings observed in RDPHE and RDPCE treated animal groups showed significant decrease in the ALT activity concomitant with significant increase in the serum level of total protein compared to an injured group of animals (G2). Furthermore, the activity of antioxidant enzyme (SOD and GPx) was also significantly enhanced ($p<0.05$) in the RDPHE and RDPCE treated rats (G4 and G5) compared to CCl_4 group (G2). The same results for improvement of liver function and antioxidant enzyme activity were observed in silymarin treated group (G3).

Regarding the effect of RDPHE and RDPCE on lipid profile, the results confirmed significant improvement in the level of serum total cholesterol, TG and HDL-C compared to CCl_4 group (G2). Our observations were supported with significant negative correlation between ALT and total cholesterol as well as HDL-C. In addition ALP was significantly negatively correlated with LDL-C. These findings were similar to the results of the silymarin treated group (G3) on the lipid profile in rats.

This study revealed that both RDPHE and RDPCE of *Phoenix dactylifera* seeds showed ameliorative effects against CCl_4 induced hepatic injury in experimental rats as the liver of rats treated with these two extracts showed a clear preservation of tissue architecture with intact hepatocytes, central vein, portal tract and sinusoidal arrangement. Moreover, disappearance of tissue necrosis and fibrosis with marked reduction in inflammatory cellular infiltration were also observed. Furthermore, in this study silymarin showed a clear hepatoprotective effect against CCl_4 induced liver damage as far as it reduced the damaging effects of CCl_4 with preservation of the hepatocytes, tissue architecture and sinusoidal arrangement. The liver support action of both RDPHE and RDPCE in the present work is similar to that of silymarin which is used as a standard liver support drug.

These findings are consistent with a previous study on rabbits, which exhibited that treatment with the extract of Ajwa date seed amplified the activities of SOD and GPX[35]. Date palm oil is a natural antioxidant rich in phenolic compounds, carotenoids, vitamins (A, C and B complex) and many other active ingredients. Recently, these phenolic compounds showed several biological activities such as; antioxidant, anticancer and hepatoprotective activities and improve in lipid profile[36]. The impact of both RDPHE and RDPCE against lipid peroxidation and protein oxidation as factors modifying membrane organization might be related to its capacity to scavenge the oxidation-initiating agents[37]. Accordingly, the hepatoprotective effect of both RDPHE and RDPCE might be attributed to their antioxidant activity of their phenolic and other compounds content, which is able to donate a hydrogen atom to the free radicals, stopping the deleterious reaction processes and subsequently significantly improves the lipid profile significantly (TG and HDL-C)[38]. Moreover, date seeds contain quercetin (flavonoid) with potential antioxidant

activity equal to alpha tocopherol in inhibiting free radicals mediated lipid peroxidation[39]. In conclusion, both RDPHE and RDPCE *Phoenix dactylifera* have a hepatoprotective effect against CCl_4 induced hepatic injury in experimental rats by adjustment of antioxidant enzymes (SOD and GPx) and improvement in lipids and protein production. Therefore, the current study emphasizes the ameliorating role of the roasted date palm seed in hepatic diseases. The protective impact of the studied extracts against hepatic injury in the animal model could be preliminary work for further pre-clinical studies. Further studies are needed to establish the antioxidant role of *Phoenix dactylifera* roasted seeds as adjunctive therapy of hepatic and other diseases. However, undetermined main constituents of RDPHE and RDPCE *Phoenix dactylifera* which trigger the antioxidant effect are considered a limitation of the present study.

CONCLUSION

The present study ensures the potential beneficial effect of the roasted date palm seeds as a hepatoprotective supplement and suggests the use of RDPHE and RDPCE as adjunctive therapy with medication for different types of oxidative liver diseases due to its impact on biomarkers of hepatic disease and improvement in liver function. This may be attributed to their antioxidant phytochemical like flavonoids and phenolic acid.

SIGNIFICANCE STATEMENT

This study discovers the antioxidant role of the roasted date palm seed extracts that can be beneficial for ameliorating the hepatic damage and regulation the redox system. Thus, a regular use of natural food products such as roasted date palm seeds can improve hepatic functions and restore the antioxidant capacity in liver injured patients.

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