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Research Article
Phoenix dactylifera L. Extract Diminished Apoptotic Effect in Cirrhotic Liver of a Rat Model

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Abstract

Background and Objective: Cirrhotic liver is one of the major end stages of chronic liver ailments. It is an irreversible process converts into cirrhosis and may ends with hepatocellular carcinoma. Liver injury is associated with production of Reactive Oxygen Species (ROS), leading to an increase in oxidative stress-mediated apoptosis. Thus, in this study the antioxidant and anti-apoptotic effects of *Phoenix dactylifera* L. extract were tested on CCl_4-induced rats. **Materials and Methods:** Four groups (untreated, treated with plant extract, CCl_4-induced and CCl_4-induced and treated groups) were enrolled in the current study (10 rats in each). All groups were treated and scarified for blood collection and histopathological investigations after 6 weeks. Circulating liver enzymes (ALT and AST) and apoptotic extrinsic signals (Fas and FasL) were measured in the blood of all groups. Moreover, histopathology and immunohistochemistry of Apoptosis Inducing Factor (AIF) in liver were observed. **Results:** It was observed that *Phoenix dactylifera* L. administration in rats significantly decreased the circulating ALT and FasL levels, as well as diminished chronic liver damage and cirrhosis as illustrated by histopathology and confirmed by AIF immunoreactivity decrease. **Conclusion:** It was concluded that *Phoenix dactylifera* L. may have protective effect against CCl_4-induced hepatotoxicity in rats. This protection effect is mediated by an anti-apoptotic strategy.

Key words: Apoptosis, apoptosis-inducing factor, fibrosis, anti-apoptotic liver enzymes, liver injury, cirrhotic liver, hepatotoxicity, *Phoenix dactylifera* L.

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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

Liver fibrosis and cirrhosis are major public health problem globally. Nowadays, management of this aliment is the scope of many researches[1]. Viral, alcoholic and nonalcoholic hepatitis are the most common origins of chronic liver injury that may ends in liver fibrosis. This disease stage is reflected a process of wound healing of chronic hepatic destruction, which associated with collagen deposition in liver. It may end in cirrhosis and finally cause hepatocellular carcinoma[1].

Liver injury is accompanying with induction of Reactive Oxygen Species (ROS) production, causing an increase in oxidative stress and imbalance in the whole redox status of the body. Oxidative stress, associated with liver damage is a vital factor which induces apoptosis[2], which plays an important role in chronic liver diseases of various etiologies[3]. Apoptosis of hepatocytes is associated with migration of Hepatic Stellate Cells (HSC) to the site of injury to phagocytize apoptotic bodies. This process leads to activation and deposition of extracelluar matrix that ends in liver fibrosis and then cirrhosis[4].

ROS-mediated apoptosis accompanied with two pathways; extrinsic (receptor-mediated) and intrinsic (mitochondrial) ways. Both of them take part in liver injury[3]. The extrinsic way is called receptor-mediated because it is dependent on binding of cell-surface receptors to extracellular death ligands. For instance, binding of the apoptotic cell-surface receptor (Fasor Apo-1 or CD95) to its ligand (FasL)[5]. By contrast, the mitochondrial way is associated with mitochondrial changes. Bcl-2 family (e.g., Bax, Bak, Bcl-2 and Bcl-xl) is known to be one of the most significant factors controlling the latter pathway. This family regulates the permeability of the mitochondrial outer membrane, resulting in the induction of apoptotic molecules including apoptosis-inducing factor (AIF)[6]. In normal cells, AIF is synthesized in the cytoplasm then transported into the mitochondrial intermembrane space. On the state of apoptotic induction, AIF translocated to the nucleus to interact with DNA, inducing chromatin condensation and large-scale DNA fragmentation in a caspase-independent manner. The binding of AIF with DNA may be linked with the presence of a strong positive electrostatic potential at the AIF surface[7].

Liver fibrosis and cirrhosis obstacle was managed using different herbal therapeutic strategies. There is a growing interest in use of herbal extracts due to their antioxidant potency. Extracts of fresh fruits are considered a perfect supplier of natural anti-oxidants that is better and safer than chemical extracts. *Phoenix dactylifera* L. (date palm) is amain nutritional fruit in North Africa and the Middle East including many countries of the Arabian Gulf Cooperation Countries (GCC). Through the last few decades, various reports studied different therapeutic options of *Phoenix dactylifera* L. due to its antioxidant potency of polyphenols and flavonoids. This antioxidant effect increased the potent ability of *Phoenix dactylifera* L. extract to suppress free radicals through different machineries[8-10].

When summarize prior studies about the administration of *Phoenix dactylifera* L. for treatment of liver injury and fibrosis, some ascribed this effect to the antioxidant activity[11,12], suppression of Hematopoietic Stem Cells (HSC) activity[13], decreasing the inflammatory, angiogenic and the levels of fibrotic factors, as well as increasing the fibrolytic potentiality[13-15]. However, no previous works in the literature studied the impact of *Phoenix dactylifera* L. on apoptosis. Thus, the current study specifically addresses insufficient consideration of the topic by studying the apoptotic signals and relates them with the effect of *Phoenix dactylifera* L. extract against liver cirrhosis.

Carbon tetrachloride (CCl_4) is usually used in induction of fibrotic and cirrhotic liver in experimental rat models. It induces liver injury through free radicals production. This chemical was found to create liver pathology closely similar to that in human suffered from liver fibrosis[15].

Overall, the current study aimed at exploring the role of *Phoenix dactylifera* L. extract in ameliorating hepatotoxicity induced by CCl_4 in rats through tracking some apoptotic markers *in vivo*.

MATERIALS AND METHODS

Duration of the experiment: The total period of this research is 6.5 months from October, 2017 to April, 2018 divided as follow: 6 weeks for sample preparation and analysis, 6 weeks for animal experiment, 4 weeks for liver enzymes and apoptotic signals examination, 2 weeks for histopathological examination, 2 weeks for Apoptosis Inducing Factor (AIF) immunohistochemical examination, 2 weeks for statistical analysis and 4 weeks for writing the manuscript.

Sample preparation and analysis: *Phoenix dactylifera* L. was grown in Al-Madinah Al-Munawarah and samples used in this study were collected from a local market. The aqueous extract sample of *Phoenix dactylifera* L. was prepared and tested its chemical composition[8].

Experimental animals: Forty adult male Wister albino rats (weighed 150-160 g) were purchased from King Abd Al Aziz University Animal House, Jeddah, KSA, at 6 weeks of age. The animals were housed in a specific pathogen free barrier area in a room with controlled temperature (22±1°C) and humidity (50±10%) and a 12 h light/dark cycle. The rats were allowed *ad libitum* access to water and standard laboratory diet consisting of casein 10%, salts mixture 4%, vitamins mixture 1%, corn oil 10% and cellulose 5% completed to 100 g with corn starch[16]. The experiment was carried out in accordance to the protocol approved by the Ethical Committee of Faculty of Applied Medical Sciences Research, Taibah University, KSA (Serial number: MLT 201502).

Experimental design: Animals were divided randomly into 4 groups; 10 rats in each group. Group I: It was served as normal control that was orally and daily administered the equivalent amount of the vehicle (distilled water) for 6 weeks. Group II: It was received 2 g kg^{-1}/day of *Phoenix dactylifera* L. extract dissolved in 10 mL distilled water via gastric tube. Group III: It was injected subcutaneously with 1.0 mL carbon tetrachloride solution (CCl_4 was dissolved in corn oil at 1:1 percent/kg rats three times a week). Group IV: It was administered 2 g kg^{-1}/day of *Phoenix dactylifera* L. extract orally plus 1.0 mL carbon tetrachloride solution/three times a week/kg rats. After 6 weeks, rats were anaesthetized with 1.8 mg kg^{-1} urethane and retro-orbital plexus blood samples were collected for determination of the circulating liver enzymes (ALT and AST) and apoptotic extrinsic signals (Fas and FasL). Then, rats were scarified and liver of all animals were taken for histopathological and immuno-histochemistry examinations.

Liver enzymes and apoptotic signals examination: The collected blood samples were centrifuged at 3000 rpm for 5 min to obtain serum for estimating liver enzymes (ALT and AST) and the levels of Fas and FasL as receptor-ligand connection for apoptotic mediation.

The tests for liver enzymes were performed using the clinical chemistry automated machine Dimension×P and, Siemens Healthcare Diagnostics Ltd. Frimley, Camberley, UK. Kits, sampling, reagent delivery, mixing, processing and printing of results were automatically performed by the Dimension® System. The rest of the samples were kept at -20 for apoptotic signal (Fas and FasL) analysis. Fas and FasL were determined using enzyme-linked immuno-sorbent assay (ELISA) kit (Sigma-Aldrich, Saint Louis, U.S.A.) with a minimum detectable dose of 5 and 2 pg mL^{-1} in serum respectively. All procedures were performed at room temperature; according to the manufacturer's instructions. Each sample and standard protein was assayed in triplicate. Optical density at 450 nm for FasL and Fas was measured with a spectrophotometric microtiter plate reader (LabsystemsiEMS Reader, Helsinki, Finland). A standard curve obtained with FasL or Fas samples provided with the kit was used to determine the FasL and Fas in each sample.

Histopathological examination: At the end of the experiment liver tissues were taken and fixed immediately in 10% buffered formalin. Tissues were processed by conventional technique. Paraffin sections were stained with Harris's haematoxylin and eosin (H and E) and Masson trichrome. Sections were examined blinded to the different treated groups. Pathological changes were assessed according to the scoring for non-alcoholic steatohepatitis (NASH) used by Brunt and Tiniakos[17]. In which necro-inflammation was scored depending on the degree of steatosis (0-3), ballooning degeneration of hepatocytes (0-2) and lobular inflammation (0-3) with total score 0-8. While, fibrosis was staged from 1-4 as following: Stage 1: Zone 3 perivenular, perisinusoidal/ pericellular fibrosis, focal or extensive, stage 2: As above with focal or extensive periportal fibrosis, stage 3: Bridging fibrosis, focal or extensive and stage 4: Cirrhosis[18].

Apoptosis Inducing Factor (AIF) immuno-histochemical examination: Paraffin sections of 5 µm thickness were mounted on charged slides. Immunostaining was performed using the Avidin-Biotin complex (ABC) (ImmunoCruz™ rabbit ABC Staining System; Santa Cruz Biotechnology, Catalog Number sc-2018) according to the manufacturer's guidelines. Briefly, sections were incubated in 1% hydrogen peroxide. Then, antigen retrieval was performed by microwaving sections in 10 mM citrate buffer pH 6.0 for 15 min. Blocking serum was added to avoid non-specific staining. Followed by anti-AIF antibody (E-1) dilution 1:50 (a mouse monoclonal antibody, Santa Cruz Biotechnology, Catalog Number sc-13116) at 4°C overnight, then secondary antibody and DAB chromogen. Sections were counter stained with Mayer's hematoxylin. Each run breast carcinoma known to be positive for AIF was used as positive control and Phosphate Buffered Saline (PBS) was used instead of the primary antibody as a negative control. Positive cells showed nuclear immunostaining. Positive cells were counted in 10 high-power fields(×400) and quantified as a percentage[19].

Statistical analysis: All the obtained data were subjected to statistical analysis using SPSS for windows (version 20, SPSS, Chicago, IL, USA). Data were presented as means±standard

deviations. Student's t test was used for comparison between means of different groups. A $p<0.05$ was considered statistically significant.

RESULTS

***Phoenix dactylifera* L. diminished liver enzyme levels in cirrhotic rats:** The data in Table 1 summarized the effect of 2 g kg^{-1}/day of *Phoenix dactylifera* L. aqueous extract administration on different studied parameters in CCl_4-induced liver cirrhosis. In the current study, the present results predicted no statistical differences between GI and GII regarding the levels of ALT (9.72+2.75 and 10.88+2.14, respectively), while there were high statistical increase ($p<0.05$) in the levels of the same enzyme when comparing either GIII or GIV (38.02+5.14 and 28.71+4.6, respectively) compared to GI and GII. However, ALT levels were significantly decreased ($p = 0.001$) in GIV compared with GPIII. Regarding AST levels, there was significant increase in their levels in GIII (315.68+81.94) and GIV (249.42+49.28) when compared to GI (124.78+8.59) and GII (127.22+9.6). On the contrary, there was no significant difference between GIII and GIV.

***Phoenix dactylifera* L. induced anti-apoptotic activity in cirrhotic rats:** Some apoptotic markers were tested in all groups to investigate the effect of *Phoenix dactylifera* L. extract on apoptosis and illustrated in Fig. 1.The levels of sera Fas in GIII (35.55+4.21) showed statistical significant increase when compared with GI (28.74+4.86) and GII (29.78+1.93). While, there were no detected significant differences between other groups. On the other hand, the concentration of Fas receptor's ligand (FasL) was significantly increased in GII, GIII and GIV compared with GI (118.89+8.32, 163.98+12.21, 142.4+12.58 and 94.27+11.8, respectively). In addition, their levels were significantly increased in GIII and GIV compared to GII. Moreover, the levels of FasL in GIV were significantly decreased compared to their levels in GIII.

Regarding apoptosis inducing factor (AIF) as shown in Fig. 2, both GI and GII were relatively immuno-negative (approximately 1%; Fig. 3). Only few cells showed cytoplasmic immune-reaction. While, GIII and GIV showed AIF nuclear translocation with significant increase in immunoreactivity of AIF (6.4±2.22 and 4.1±1.45, respectively) compared with GI and GII. The current results also revealed significant decrease of AIF in GIV (approximately 38%) compared to GIII (approximately 60%) (Fig. 3).

***Phoenix dactylifera* L. decreased the fibrotic score in cirrhotic rats:** The current study revealed normal microscopic structure of liver of the two control groups (GI and GII); treated with the vehicle and with only *Phoenix dactylifera* L. (Fig. 4, 5a, b). On the other hand, treated liver with CCl_4 (GIII) showed extensive steatosis, ballooning degeneration of hepatocytes and inflammatory infiltrate (Fig. 4c), with a mean NASH score 6.7±0.48 (Table 1). While, treated liver with *Phoenix dactylifera* L. and CCl_4 (GIV) showed less hepatic injury (Fig. 4d) with mean NASH score 4.2±1.55 (Table 1). The difference was statistically significant ($p> 0.05$). Intriguingly, all CCl_4-treated rats showed liver cirrhosis (mean score 4.00±0.0), while this cirrhotic score was decreased significantly

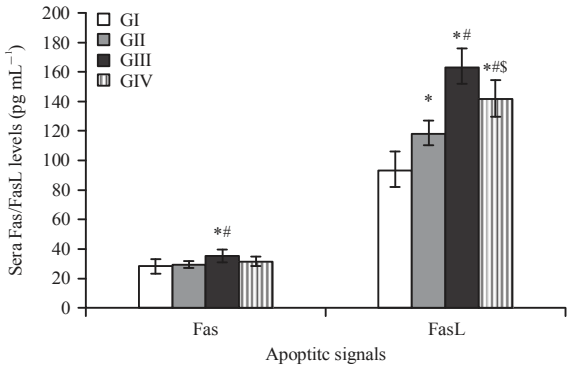

Fig. 1: Apoptotic signals (Fas and FasL) variations across groups

Graphical data were expressed as Mean±SD, n = 10 in each group, Fas: Apoptotic cell-surface receptor (pg mL^{-1}), FasL: Fas ligand (pg mL^{-1}), AIF: Apoptosis-inducing factor, *p<0.05: Statistical significance compared with GI, #p<0.05: Statistical significance compared with GII, $p<0.05: Statistical significance compared with GIII

Table 1: Effect of *Phoenix dactylifera* L. extract administration on NASH, fibrotic scores and liver enzymes in CCl_4-induced liver cirrhosis

	Mean±SD			
Variables	GI (H_2O)	GII (PDL)	GIII (CCl_4)	GIV (CCl_4 and PDL)
	0.00±0.00	0.00±0.00	6.70±0.48*#	4.20±1.55*#$
NASH score	0.00±0.00	0.00±0.00	6.70±0.48*#	4.20±1.55*#$
Fibrosis	0.00±0.00	0.00±0.00	4.00±0.00*#	3.30±.48*#$
ALT	9.72±2.75	10.88±2.14	38.02±5.14*#	28.71±4.6*#$
AST	124.78±8.59	127.22±9.6	315.68±81.94*#	249.42±49.28*#

Data were expressed as Mean±SD, n = 10 in each group, PDL: Phoenix dactylifera L., CCl_4: Carbon tetrachloride, NASH: Non-alcoholic steatohepatitis, ALT: Alanine aminotransferase (U L^{-1}), AST: Aspartate aminotransferase (U L^{-1}), *p<0.05: Statistical significance compared with GI, #p<0.05: Statistical significance compared with GII, $p<0.05: Statistical significance compared with GIII

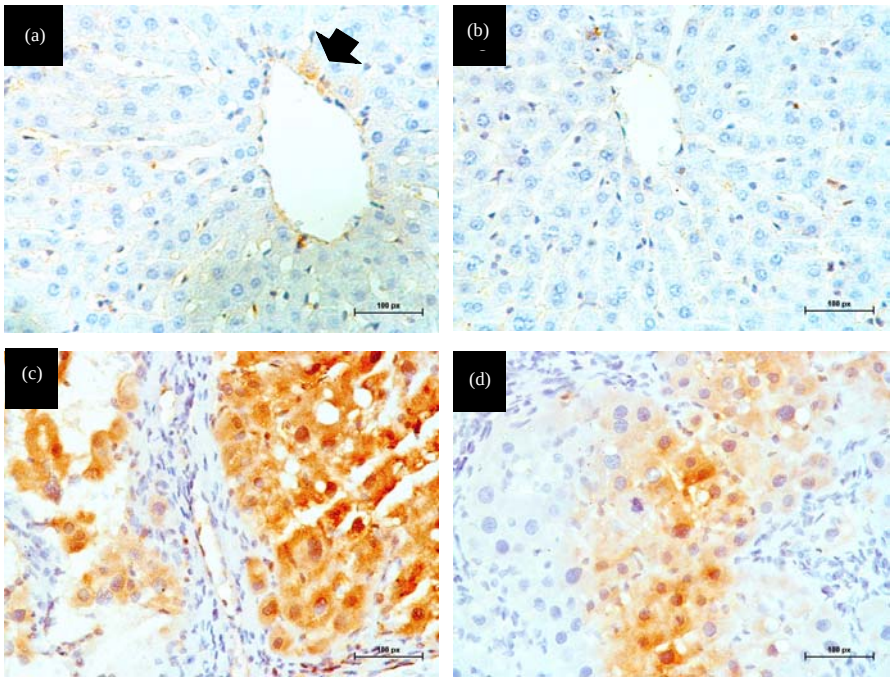

Fig. 2(a-d): Immunohistochemical staining of AIF (DAB×400). Only cells with nuclear immunostaining were considered positive for AIF, (a-b) Liver of group I and II rats with negative immunostaining. Few cells in group I showed cytoplasmic staining (arrow), (c) Liver of CCl_4 treated rat showing many immunoreactive cells and (d) Liver of CCl_4 and date palm treated rat with few immunoreactive cells (arrow)

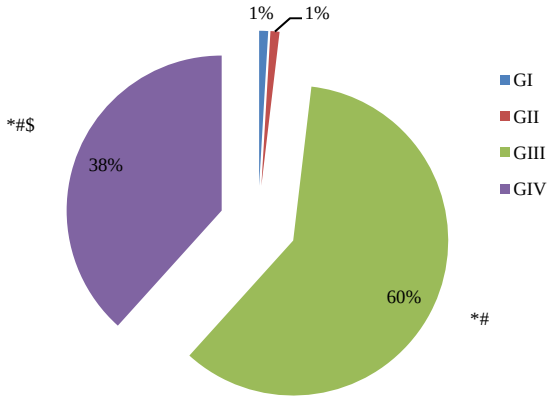

Fig. 3: Apoptosis Inducing Factor (AIF) determination across groups
*p<0.05: Statistical significance compared with GI, #p<0.05: Statistical significance compared with GII, $p<0.05: Statistical significance compared with GIII

(3.30±.48) after *Phoenix dactylifera* L. extract administration in GIV (Fig. 5c, d). The difference was also statistically significant (p>0.05).

DISCUSSION

Nowadays, there is an extreme growing interest to use natural[20] and plant extracts[21,22] instead of chemical synthetic products[23-26] for different therapeutic purposes related to liver aliments. One of these plant extracts is *Phoenix dactylifera* L. extract that it used in the current study for cirrhotic liver therapy. The rationale of the selection of that plant came after the complete chemical analysis of it in the authors' previous study[8], which indicated that *Phoenix dactylifera* L. was rich in sugar (71.2-81.4% dry weight), while ash represented 1.68-3.94%; they contained low concentrations of protein and lipid (1.72-4.73% and 0.12-0.72%, respectively). The predominant mineral was potassium and the main

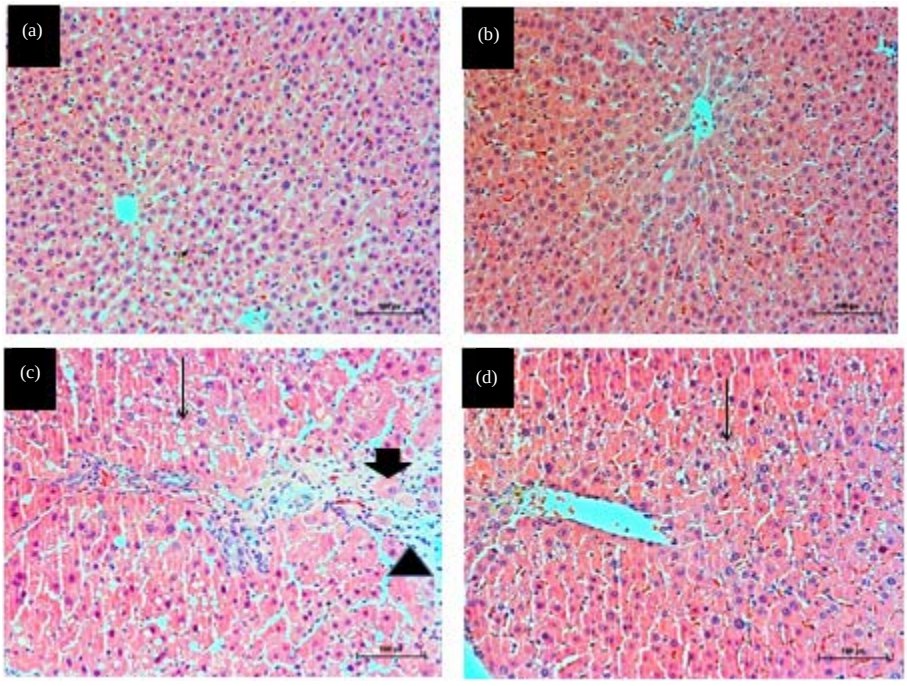

Fig. 4(a-d): Photomicrographs of liver sections from different studied groups (H and E×200), (a-b) Normal liver of groups I and II rats (control), (c) Liver of CCl_4 treated rat showing extensive steatosis (thin arrow), ballooning degeneration of hepatocytes (thick arrow) and inflammatory infiltrate (arrow head) and (d) Liver of CCl_4 and date palm treated rat showing moderate steatosis (thin arrow)

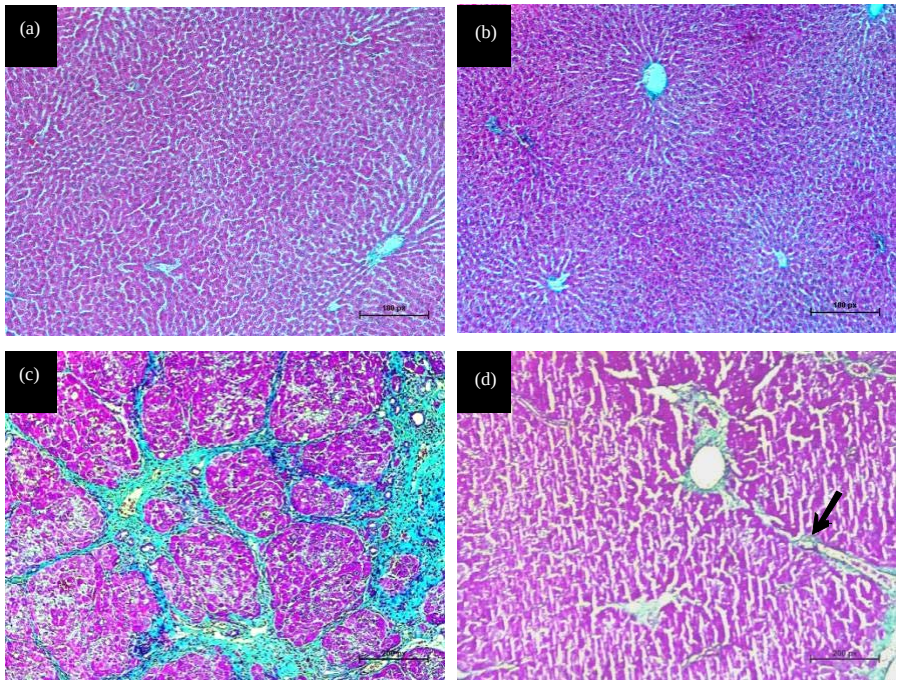

Fig. 5(a-d): Masson trichrome stained liver sections (×100), (a-b) Normal liver of groups I and II showed fine collagen fibers surrounding the central vein and the sinusoids, (c) Liver cirrhosis in a CCl_4 treated rat and (d) Liver of CCl_4 and date palm treated rat showing bridging fibrosis (arrow)

sugars were glucose and fructose. They contained high concentrations of aspartic acid, proline, alanine, glycine, valine and leucine; low concentrations of threonine, serine, isoleucine, tyrosine, arginine, phenylalanine and lysine and very low concentrations of methionine and histidine. These observations confirmed that *Phoenix dactylifera* L. can play a major role in human nutrition and health obstacles prevention[8].

This research article was hypothesized to test the apoptotic impact of aqueous extraction of *Phoenix dactylifera* L. on hepatotoxicity in rats induced by CCl_4. In this study, liver injury and cirrhosis were successfully induced by CCl_4 as manifested by a significant increase in the levels of ALT and AST, increased NASH score and induction of cirrhosis in rats administered to CCl_4 when compared with control group. Mechanistically-wise, this can be explained by increased oxidative stress and lipid peroxidation caused by CCl_4 that destroy the membranes of the organelles and cause necrosis of liver cells with sustained release of cytosolic enzymes like ALT and AST into the blood. The aminotransferases enzymes are taken as indicators of hepatocyte injury[27,28].

The increment in the levels of the ALT aminotransferase showed significant decreases after administration of the aqueous extract of *Phoenix dactylifera* L. in the group of rats treated with *Phoenix dactylifera* L. after CCl_4-liver cirrhosis induction (GIV) when compared with the positive control group which treated with CCl_4 only (GIII). The same results were obtained for AST but the difference was about the statistical significant, where the difference in case of ALT was statistically significant but in case of AST was not. These results are in agreement with other previous studies indicated the capability of the *Phoenix dactylifera* L. extract on restoring the CCl_4 hepatotoxicity in rats through retrieve the ordinary function of the damaged liver cells[12-14].

Phoenix dactylifera L. is identified to have high content of antioxidants. A lot of researches elucidate the antioxidant and antimutagenic effects of the aqueous extract of *Phoenix dactylifera* L. These effects are due to the high amount of phenolic compounds (flavonoids, sinapic acids, p-coumaric, procyanidins and ferulic), trace elements (selenium, copper, zinc and manganese) and also to the presence of vitamin C in the *Phoenix dactylifera* L. fruit[9,10,14,29,30]. The mechanism by which the aqueous extract of *Phoenix dactylifera* L. exerts its anti-oxidant and anti-mutagenic effects may be through scavenging free radicals as hydroxyl and superoxide radicals and suppression of protein oxidation and lipid peroxidation. Others proved that the anti-oxidant effect of *Phoenix dactylifera* L. is through decreasing the level of malonyldialdehyde (MDA), the initiator of lipid peroxidation[12], as indicated previously indicated that the antioxidant effect of selenium may be through modulating the MDA levels[25].

The protective effect of *Phoenix dactylifera* L. extract is also reflected by the significant decrease of NASH score in rats treated with both *Phoenix dactylifera* L. and CCl_4 compared with those treated with CCl_4 alone. The NASH score reflects the degree of inflammation, necrosis, steatosis and ballooning degeneration of hepatocytes. In addition, there was a significant decrease in the degree of fibrosis after *Phoenix dactylifera* L. extract administration. This result may be related to the antioxidant effect of *Phoenix dactylifera* L. extract. Also it was found that this extract has anti-inflammatory effect through suppressing the production of tumor necrosis factor-a (TNF-a), interleukin-6, interleukin-1β, nuclear factor-κB and cyclooxygenase-2. Furthermore, *Phoenix dactylifera* L. may have antifibrotic effect in liver through suppression of HSC, decreasing α-smooth muscle actin, TGF-β1, collagen III and inhibitor of metalloproteinases-1[13,14] and 2. All of these observations confirmed the current results regarding the therapeutic effect of *Phoenix dactylifera* L. extract against liver cirrhosis.

Phoenix dactylifera L. extract is also associated with decreasing angiogenesis is reflected by decreased[13,14] VEGF, VEGFR-1 and CD31. Many previous studies revealed that *Phoenix dactylifera* L. can ameliorate liver tissue injury and fibrosis induced[11-14] by CCl_4. These results came in parallel with the current findings. However, all these studies were subjective and did not use definite fibrotic scoring system for assessing the grade of liver injury.

Regarding apoptosis, the programmed cell death in terms of apoptotic signals (Fas and FasL) and AIF immunoreactivity were significantly increased in the group of rats treated with CCl_4 (GIII) when compared with the control groups (GI and GII). This remarkable increase could be due the fact that CCl_4 is an initiator of apoptosis through fashioning reactive free radicals. The produced free radicals have functions in liver injury directed to apoptosis, necrosis and extracellular matrix deposition. The free radicals could initiate oxidative damage to mitochondria which lead to death of hepatocyte or enhances the pro-apoptotic action[31] of TNF-α. Increased secretion of FasL after CCl_4 was previously reported by Sato's team[32]. Additionally, the hepatocyte apoptosis induced after CCl_4 was confirmed previously by light and electron microscopy, the *in situ* immuno-histochemical labeling of nuclear DNA fragmentation, flow cytometry and DNA gel electrophoresis[25]. These mechanistic approaches precisely

confirmed the current finding of that apoptosis signals (Fas, FasL and AIF) were increased after CCl_4 treatment.

The present study revealed that *Phoenix dactylifera* L. ameliorates apoptosis induced by CCl_4 administration. There was significant decrease of FasL level in rats treated with *Phoenix dactylifera* L. after CCl_4-liver cirrhosis induction compared with those treated with CCl_4 alone (i.e., positive control). The same output was also obtained with AIF. Normally, AIF is located in the mitochondrial intermembrane space. On induction of apoptosis, AIF translocates to the nucleus. So, in this study only cells with nuclear immunostaining were considered positive. The nuclear translocation of AIF in apoptotic cells has been demonstrated in many studies of different tissue injury such as myocardial infarction[33,34], neuronal cell death in traumatic brain[35] and Alzheimer's disease[36]. Regarding liver, it was found that AIF has a role in acute liver injury. In a mice model of acetaminophen-induced acute liver failure, perisinusoidal injured hepatocytes showed AIF nuclear translocation. This translocation is ameliorated by administration of melatonin that has antioxidant effect[37]. In the current study, it found increased AIF nuclear immune-reactivity of hepatocytes in CCl_4 treated rats. This immune-reactivity is significantly decreased with administration of *Phoenix dactylifera* L. For future studies, using the extract of *Phoenix dactylifera* L. in nano-capsulation plat-forms may ameliorate its therapeutic effect by increasing its targetability and biocompatibility, reflecting better apoptotic modulation in liver disease treatment as approached previously in different natural products and ectracts[38-41].

CONCLUSION

Current study revealed that the aqueous extract of *Phoenix dactylifera* L. can ameliorate the hepatotoxicity, liver damage and cirrhosis caused by CCl_4 administration in rat. This effect may be related to its ability to abrogate apoptosis. The present observations indicated that *Phoenix dactylifera* L. affect apoptosis through both the extrinsic receptor-mediated pathway as reflected by decreasing FasL level and the intrinsic mitochondria-dependent pathway reflected by decreasing AIF nuclear immune-reactivity.

SIGNIFICANCE STATEMENT

This study discovers the possible apoptotic effects in the form of apoptotic extrinsic signals (Fas and FasL) and apoptosis inducing factor (AIF) after *Phoenix dactylifera* L. administration in rats that can be beneficial for treatment of cirrhosis. This study will help the researcher to uncover the critical area of hepatic apoptosis-mediated cell death through *Phoenix dactylifera* L. that many researchers were not able to explore. The previous studies did not explore apoptosis after *Phoenix dactylifera* L. administration but genotoxicity, metalloproteinases, nuclear factor-kappa B pathway, inflammatory cytokines, growth factor, cytochrome P450 2E1 and heme oxygenase-1. Thus, a new theory on the unique components of the current regional plant found in Saudi Arabia may be arrived at.

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