Abrogation of carbon tetrachloride-induced hepatotoxicity in Sprague-Dawley rats by Ajwa date fruit extract through ameliorating oxidative stress and apoptosis

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Abstract: Ajwa, a variety of date palme *Phoenix dactylifera L*., has long been used and considered as one of the most popular fruits in the North Africa and Middle East region. For Muslims this fruit is of religious importance and is mentioned several times in Quran. Besides being a part of the Arabian essential diet, dates have been used traditionally for number of complications. This study aimed to evaluate the possible potential of Ajwa date extract to guard against carbon tetrachloride (CCL_4)-induced liver damage in rats. Adult male Sprague-Dawley rats were given Ajwa date extract and silymarin (a standard reference drug) at doses of 300 & 50mg/kg, p.o., respectively for 2 weeks before CCl_4 (2 ml/kg, s. c., twice weekly for 8 consecutive weeks), and concomitantly administered with CCl_4 for 8 consecutive weeks. Like silymarin, Ajwa date extract produced significant decrease in serum levels of alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP), total cholesterol, triglycerides (TG) and LDL-cholesterol as well as lipid peroxides measured as malondialdehyde (MDA), hydroxyproline and caspase-3 contents of liver tissue with marked increase in serum albumin, HDL-cholesterol and reduced glutathione (GSH) content as well as enzyme activities of super oxide dismutase (SOD), catalase (CAT) and glutathione-S-transferase (GST). In conclusion, Ajwa date extract afforded significant protection against CCl_4-induced hepatocellular injury; an effect that could be attributed to its antioxidant, antiapoptotic and antifibrotic activities.

Keywords: *Phoenix dactylifera*, oxidative stress, silymarin, hepatotoxicity, carbon tetrachloride.

INTRODUCTION

Hepatotoxicity means tendency of an agent, usually a drug or another non-infectious substance, to cause liver hurts that is associated with impaired liver functions (Bahirwani and Reddy, 2014). Certain chemicals including acetaminophen, CCl_4, galactosamine and thioacetamide are often used as a toxin to cause experimental hepatocyte injury in both *in vitro* and *in vivo* models (Kim, You *et al*., 2014, Remien, Sussman *et al*., 2014). CCl_4, a widely used hepatotoxic agent, induces toxicity in rat liver which closely resembles human cirrhosis. It was reported that CCl_4 induces liver cell injury via the generation of trichloromethyl free radical ($^{\bullet}CCl_3$) by the mixed function oxidase system of the endoplasmic reticulum (Ismail, El-Megeid *et al*., 2009, Jain, Kapadia *et al*., 2012). Beside ($^{\bullet}CCl_3$), other toxic products arising from per oxidative degeneration of membrane such as trichloromethyl peroxy free radical ($CCl_3OO^{\bullet}$) and chlorine free radical (Cl^{-}) are responsible for the majority of hepatocyte destruction induced by CCl_4 (Jain, Kapadia *et al*., 2012, Song, Choi *et al*., 2011).

For centuries, *Phoenix dactylifera* (Palmae) has been used and considered one of the most popular fruits in Middle Eastern countries and North Africa (Gribaa, Dardelle *et al*., 2013). Palm date fruits at their different stages of maturity contain thirteen flavonoid glycosides of luteolin, quercetin and apeginin (Amira el, Behija *et al*., 2012, Hong, Tomas-Barberan *et al*., 2006). It has high nutritional benefits due to the sugar, protein, fat and mineral products including cupper, sulfur, iron, magnesium and fluoric acid (Al-Farsi and Lee, 2008). Also, dates are a rich source of potassium and dietary fibers which prevent LDL-cholesterol absorption in the gut. Additionally, the fibers work as a bulk laxative helping to protect the colonic mucosa by decreasing exposure time as well as binding to chemical carcinogens in the bowel (Biglari, AlKarkhi *et al*., 2008).

Date fruits are consumed in Arab areas for a long time as a part of essential diet. *Phoenix dactylifera* is a member of Arecaceae family and its leaves, barks, pits, fruits and pollens were reported to have anticancer, antioxidant, hepatoprotective, antidiabetic, antihypertensive, antiulcerative, anti-inflammatory, antiproliferative, antimutagenic, antidiarrheal, antibacterial, antifungal and antiviral potentials. Besides these, dates also increase level of estrogen, testosterone, red blood cells (RBCs), hemoglobin (Hb), packed cell volume (PCV), reticulocytes and platelet counts. It can also cure male and female infertility, and possess cerebroprotective, neuroprotective and haemopoietic activities (Chandrasekaran and Bahkali, 2013, Mallhi, Qadir *et al*., 2014). The various constituents of Ajwa date fruit are widely used in traditional medicine in the treatment of

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numerous ailments including memory disturbances, fever, inflammation, paralysis, loss of consciousness and other various disorders. It is also recommended to be used as astringent in intestinal troubles, treatment of sore throat, colds, bronchial asthma, cystitis, gonorrhea, edema, hepatic disorders and to nullify alcohol intoxication (Rahmani, Aly *et al*., 2014).

Dates contain healthy polyphenolic antioxidants called tannins which have been reported to exhibit antiinfective, antiinflammatory and antihemorrhagic potentials (Habib, Platat *et al*., 2014). It has antioxidant flavonoids such as beta carotene, lutein and zea-xanthin which have precautionary potentials against detrimental effects of oxygen free radicals towards body cells and structures (Shams Ardekani, Khanavi *et al*., 2010). Those eating dates were found to have some protection from colon, prostate, breast, endometrial, lung, oral cavity and pancreatic cancers (Abdelrahman, Fathalla *et al*., 2012). Moreover, dates are good source of vitamin A which has extensively been reported to exert antioxidant activity and preemptive anticancer potency beside an essential role in vision and maintenance of healthy skin and mucous membranes (El Arem, Saafi *et al*., 2014).

We, as Muslims, believe in the Prophet Muhammad (peace be upon him) who said: "If somebody eats seven Ajwa dates in the morning, neither magic nor poison will hurt him that day". Accordingly, the current study aimed at assessing the ability of Ajwa date fruit total extract to prevent CCl_4-induced hepatotoxcisty in rats.

MATERIALS AND METHODS

Materials

The fruit of Ajwa dates (*Phoenix dactylifera*) was obtained from Al-Madinah dates Co. "Tomoor" (Al-Madinah Al-Munawarah, Kingdom of Saudi Arabia). High-grade chemicals including silymarin, CCl_4, absolute ethanol, Ellman's reagent, thiobarbituric acid (TBA), reduced glutathione (GSH), 1,1´,3,3´-tetraethoxypropane, bovine serum albumin (BSA), pyrogallol and trichloroacetic acid (TCA) were purchased from Sigma-Aldrich Inc. (St. Louis, MO, USA). All other chemicals, reagents, and solvents were of analytical grade that obtained from standard suppliers and were used without further purification.

Extraction of Ajwa dates

The obtained Ajwa date fruits (*Phoenix dactylifera*) were identified and authenticated by Dr. Albaraa Elsaied (Lecturer of Plant Ecology, Department of Botany, Faculty of Science, Al-Azhar University, Cairo, Egypt), and voucher specimens were deposited in the Regional Center for Mycology and Biotechnology, Al-Azhar University, Cairo, Egypt. The total Ajwa date fruit extract was obtained by homogenizing 100g dates using a blender. The fruit homogenate was soaked in 500 ml of a mixture of absolute ethanol and distilled water (1:1). This ethanol water mixture was chosen as extraction solvents to maximally extract the phenolic compounds in the date fruit. The mixture was macerated for 48 hr at room temperature after being vigorously shacked for 10 minutes and filtered using a funnel. After filtration, the funnel was rinsed several times with 10 ml aliquots of extraction solvent. Subsequently, the filtrate was evaporated to dryness in vacuum at 40 °C using Soxlet evaporator and concentrated solution was measure and calculated for rat dose.

Identification and characterization of plant constituents

Analysis of phenolic compounds in the obtained total extract was performed by HPLC (Agilent 1000) on a reverse phase Zorbax Eclipse XDB-C18 column (4.6 x 150mm, 5µm), using a gradient program with two solvents system [A: 0.5% CH_3COOH in CH_3CN : H_2O (1: 1), B: 2% CH_3COOH in H_2O] at a constant solvent flow rate of 1.2ml/minute and injection volume of 20 µl. Detection of signals was achieved at 280 nm using UV-VIS detector through comparison with standard authentic phenolic samples (purity >99.0%) which were particularly purchased from Sigma-Alderich for HPLC analysis.

The obtained HPLC analysis reports indicated that this dates fruit extract contains some phenolic acids including coumaric acid, ferulic acid, gallic acid, hydroxybenzic acid, and hydroxycinnamic acid as well as caratonoids (lutein, β- carotene and neoxanthin) in addition to some vitamins (A, B1, B2, B3, B5, B6, C, E, K, and folic acid). It was also found to contain proteins, sterols, triterpenes, carbohydrates, essential and non-essential amino acids besides several essential oils as well as many minerals, including Ca^{2+}, Fe^{3+}, Mg^{2+}, P^{3+}, K^{+}, Na^{+} and Zn^{2+} (table 1).

Animals and experimental design

The study was performed according to the Institutional Animal Care and Use Committee of Faculty of Pharmacy, Al-Azhar University, Egypt. Male Sprague-Dawley rats weighing 160 ± 5g were purchased from the Laboratory of Animal Colony, Ministry of Health and Population, Helwan, Cairo, Egypt. Under standard laboratory conditions, animals were maintained and fed with basal diet pellets, supplied with water *ad libitum* in a temperature-controlled environment (20-22°C) and 40-60% relative humidity and an alternating 12 hr light-dark cycle. A total of 70 rats were indiscriminately divided into seven groups, i.e., 10 animals each. In group 1, rats were given normal saline orally every day for 10 consecutive weeks. In group 2, rats were injected subcutaneously with corn oil (2 ml/kg) twice a week for 8 successive weeks. In group 3, rats were injected subcutaneously with CCl_4 (2 ml/kg) diluted in corn oil (50% v/v) twice weekly for 8 sequential weeks (Edfawy, Hassan *et al*., 2012). In group

4, rats were given silymarin as a reference standard hepatoprotective agent in a daily oral dose of 50 mg/kg dissolved in normal saline for 10 consecutive weeks (Pradeep, Mohan *et al*., 2007). Rats in group 5 were given Ajwa date fruit extract in a daily oral dose of 300 mg/kg for 10 sequent weeks (Pujari, Vyawahare *et al*., 2011). This dose of Ajwa dates extract is related to the recommended daily human dose (seven dates per day at the early morning). Rats in group 6 were pretreated orally with silymarin for 2 weeks, then given CCl_4 and silymarin for 8 weeks using the same dose schedules mentioned above. In group 7, rats were pretreated orally with Ajwa date fruit extract for 2 weeks, then given CCl_4 and dates extract for 8 weeks using the same dose schedules mentioned above.

Serum and tissue preparation

After 10 weeks, blood samples were collected from retro-orbital venous plexus under light ether anesthesia in non-heparinized tubes. Blood samples were centrifuged for 20 minutes at 4000 rpm and sera were carefully separated into clean dry Eppendorf tubes using a Pasteur pipette and stored at −20°C. The animals were sacrificed and each resected liver was divided into three sections; the first part was fixed in formalin 10% and embedded in paraffin for histopathological examinations. The second part of each was homogenized in ice-cold 0.15 M KCL (w/v) using Sonicator homogenizer (4710 Ultrasonic homogenizer, Cole-Parmer instrument Company, USA) to prepare 10% (w/v) homogenate. The liver homogenate was centrifuged at 5000 rpm for 10 minutes and the resulting supernatant was obtained and used for determination of total protein content of hepatic tissue using BSA as standard protein (Lowry, Rosebrough *et al*., 1951). Thereafter, tissue homogenate was divided into aliquots and was used for the determination of liver contents of MDA, GSH, hydroxyproline and caspase-3 as well as enzymatic activities of SOD, CAT, and GST. The third part was preserved frozen at −80°C for further studies if required.

Biochemical assays

Serum ALT, AST and albumin were assayed by colorimetric procedures using kits provided from Human Diagnostic (Germany). ALP, HDL- and LDL-cholesterol were assayed by colorimetric procedures using kits provided from Biomed Diagnostic Egy. Chem. (Egypt) according to the method of Tietz (Tietz, 1979). Serum total cholesterol was estimated using enzymatic colorimetric method using kits provided from Biomed Diagnostic Egy. Chem. (Egypt) (Richmond, 1973). Serum triglycerides were determined by colorimetric procedures using kits provided from Spectrum Diagnostics (Egypt) according to the method of Bucolo and David (Bucolo and David, 1973).

Lipid peroxides were estimated as MDA contents in hepatic tissue in accordance to the method of Ohkawa *et al* (Ohkawa, Ohishi *et al*., 1979). GSH was assayed by the method of Moron *et al* (Moron, Depierre *et al*., 1979). CAT activity was assayed by the method of Sinha (Sinha, 1972). SOD was assayed according to the method of Marklund and Marklund (Marklund and Marklund, 1974). According to Habig *et al*, the activities of GST in liver homogenates were assayed (Habig, Pabst *et al*., 1974). Quantitative estimation of hydroxyproline content was carried out using rat specific hydroxyproline ELISA kit supplied by Cusabio® Biotech (Wuhan, China) according to manufacturer's instructions. Quantitative determination of caspase-3 content was achieved by a double-antibody sandwich ELISA kit supplied by bioassay technology laboratory (Shanghai, China) according to manufacturer's instructions.

Histopathological examination of the liver

Liver tissue samples that obtained at autopsy from rats in different groups were fixed in 10% neutral buffered formalin for 24 hr and decalcificated in formic acid. Washing was performed with tap water, and then serial dilutions of alcohol (methyl, ethyl and absolute ethyl) were used for dehydration. Specimens were cleared in xylene and embedded in paraffin at 56°C in hot air oven for 24 hr. Paraffin bees wax tissue blocks were prepared for sectioning at 4 microns thickness by sledge microtome and the obtained tissue sections were placed on glass slides, deparaffinized, stained by hematoxyline & eosin stain, and then examined under light microscope (Banchroft, Stevens *et al*., 1996).

STATISTICAL ANALYSIS

Statistical analysis of the data was carried out using Graphpad prism version 5 (Graphpad, San Diego, CA). Analysis of group differences was performed using one-way analysis of variance ANOVA followed by Tukey-kramer for multiple comparison tests. The level of significance was accepted with $p \leq 0.05$ and all relevant results were graphically displayed as mean ± SEM.

RESULTS

Table 2 shows that CCl_4 administration produced significant decrease in body weight by about 29% and increase in liver body-weight ratio by about 44%, as compared with the control corn oil group. Both pretreated groups with silymarin and Ajwa date fruit extract restored body weight to normal while they decreased liver-body weight ratio to 30% and 28%, respectively, as compared with CCl_4-treated group.

Table 3 shows that subcutaneous injection of CCl_4 twice weekly for 8 weeks in a dose of 2ml/kg revealed significant increase in serum levels of ALT (668%), AST (656%), ALP (239%), total cholesterol (156%), TG (221%) and LDL-cholesterol (302%). It also induced significant decreases in serum albumin (54%) and HDL-cholesterol (40%) compared to the control group.

Table 1: Phenolic acids and flavonoids in Ajwa date fruit extract.

Phenolic acids	Content (mg %)	Flavonoids	Content (µg/100 g)
Coumaric acid	14.12	Quercetin	28.08
Ferulic acid	11.08	Catechin	16.08
Gallic acid	6.01	Lutein	11.89
Hydroxybenzic acid	3.18	Epicatechin	9.08
Hydroxycinnamic acid	0.121	Apigenin	5.01

Table 2: Effect of pretreatment with silymarin and Ajwa date fruit extract on CCl_4-induced changes in the final body weight, liver weight and liver-body weight ratio

Parameters Groups	Initial body weight (g)	Final body weight (g)	Increase in body weight (g)	liver weight (g)	Liver-body weight ratio (100x)
Control saline	162.0±1.398	318.4±4.420	156.4±3.156	10.4±0.291	3.26±0.083
Control corn oil	162.1±1.538	317.7±3.953	155.6±4.214	10.4±0.192	3.29±0.076
CCl_4	161.1±1.479	225.2±3.738[a]	64.1±3.132[a]	10.7±0.574	4.76±0.195[a]
Silymarin	161.3±1.283	318.9±3.656[b]	157.6±2.621[b]	9.5±0.256	3.00±0.083[b]
Dates extract	161.5±1.550	322.7±4.277[b]	161.2±2.871[b]	9.9±0.225	3.08±0.101[b]
Silymarin + CCl_4	161.0±1.317	302.9±2.510[b]	141.9±2.373[a,b]	10.1±0.301	3.34±0.117[b]
Dates + CCl_4	161.4±1.318	302.3±3.176[b]	140.9±2.618[a,b]	10.3±0.298	3.42±0.131[b]

Table 3: Effect of pretreatment with silymarin and Ajwa date fruit extract on CCl_4-induced changes in ALT, AST, ALP, albumin, total cholesterol, TG, LDL-cholesterol and HDL-cholesterol.

Parameters Groups	ALT(U/L)	AST(U/L)	ALP(U/L)	Albumin (g/dl)	Total cholesterol (mg/dl)	TG (mg/dl)	LDL-cholesterol (mg/dl)	HDL-cholesterol (mg/dl)
Control saline	33.7± 2.84	34.5± 2.55	91.3± 5.70	4.0± 0.16	116.4± 3.99	59.3± 4.16	58.6± 4.75	46± 3.12
Control corn oil	33.6± 2.81	34.3± 2.18	92.9± 5.81	4.0± 0.18	118.0± 2.94	59.8± 3.26	58.9± 1.48	45.3± 1.99
CCl_4	258.7± 13.60[a]	259.4± 18.20[a]	315± 9.97[a]	1.8± 0.13[a]	302.1± 17.30[a]	192.1± 17.30[a]	236.6± 18.80[a]	27.2± 2.53[a]
Silymarin	31.1± 2.02[b]	31.6± 2.03[b]	90.5± 5.90[b]	4.2± 0.17[b]	106.8± 7.16[b]	59.5± 5.23[b]	55.1± 9.63[b]	39.4± 2.86[b]
Dates extract	31.7± 2.28[b]	32.3± 3.69[b]	89.1± 7.49[b]	4.3± 0.17[b]	123.8± 9.74[b]	65.1± 6.11[b]	63.4± 8.71[b]	47.3± 4.34[b]
Silymarin+ CCl_4	67.4± 5.30[a,b]	71.6± 5.65[a,b]	158.7± 8.10[a,b]	3.7± 0.21[b]	178.6± 12.90[a,b]	87.5± 9.140[b]	94.4± 12.75[b]	64.6± 4.84[b]
Dates + CCl_4	68.6± 5.08[a,b]	71.3± 6.73[a,b]	155.4± 9.80[a,b]	3.6± 0.19[b]	168.8± 8.38[a,b]	86.2± 12.33[b]	92.4± 11.70[b]	59.0± 7.96[b]

Data are presented as mean ± SEM (n = 10). Multiple comparisons were accomplished using one way ANOVA followed by Tukey-kramer's test. [a] and [b] indicate significant change from control and CCl_4 groups respectively at $p \leq 0.05$.

Moreover, administration of CCl_4 produced significant increases in the hepatic tissue contents of MDA (267%), hydroxyproline (152%) and caspase-3 (149%) as compared with the control group. Furthermore, it produced significant decreases in normal tissue content of GSH, SOD, CAT and GST by about 47%, 55%, 82% and 74%, respectively, compared to the control group (table 4).

In contrast, daily administration of silymarin (50 mg/kg) for 2 weeks prior to CCl_4 and concomitantly for 8 weeks during CCl_4 administration significantly reduced the elevated serum levels of ALT (74%), AST (72%), ALP (50%), total cholesterol (41%), TG (54%) and LDL-cholesterol (60%) and significantly increased the serum albumin level (98%) and HDL-cholesterol (138%) in comparison to CCl_4-treated group (table 3). However, it lowered hepatic tissue contents of MDA (64%), hydroxyproline (54%) and caspase-3 (52%) and restored GSH, SOD, CAT and GST activities by about 64%, 130%, 485% and 394%, respectively in comparison to CCl_4-trated group (table 4). In like manner, treatment of animals with Ajwa date fruit extract for 2 weeks before CCl_4 and for 8 weeks synchronously with CCl_4 significantly minimized the elevated serum levels of ALT (73%), AST (72%), ALP (51%), total cholesterol (44%),

Table 4: Effect of pretreatment with silymarin and Ajwa date fruit extract on CCl_4-induced changes in MDA, GSH, CAT, SOD, GST, hydroxyproline and caspase-3 contents.

Parameters Groups	MDA (nmol/g tissue)	GSH (µmol/g tissue)	CAT (U/mg protein)	SOD (U/mg protein)	GST (U/mg protein)	Hydroxyproline (µg/g tissue)	Caspase-3 (µg/g issue)
Control saline	6.67± 0.41	28.6± 1.50	0.0183 ±0.001	0.0613 ±0.004	0.552 ±0.041	152.5± 12.47	4.39± 0.35
Control corn oil	6.66± 0.44	28.6± 1.40	0.0182 ±0.001	0.0615 ±0.004	0.551 ±0.042	150.8± 12.61	4.39± 0.37
CCl_4	24.43± 1.23[a]	15.3± 0.88[a]	0.0081 ±0.002[a]	0.0109 ±0.001[a]	0.146± 0.015[a]	380.7± 15.75[a]	10.95± 0.35[a]
Silymarin	5.91± 0.41[b]	43.8± 2.80[a,b]	0.0250 ±0.001[a,b]	0.1220 ±0.009[a,b]	0.884± 0.051[a,b]	140.0± 12.69[b]	4.21± 0.20[b]
Dates extract	5.87± 0.41[b]	43.1± 2.90[a,b]	0.0231 ±0.001[b]	0.1153 ±0.011[a,b]	0.872± 0.054[a,b]	139.0± 11.85[b]	4.26± 0.23[b]
Silmarin + CCl_4	8.79± 0.37[b]	25.1± 1.50[b]	0.0186 ±0.001[b]	0.0638 ±0.005[b]	0.721± 0.050[b]	174.9± 13.09[b]	5.29± 0.37[b]
Dates + CCl_4	9.00± 0.37[b]	25.7± 1.50[b]	0.0178 ±0.001[b]	0.0640 ±0.005[b]	0.692± 0.043[b]	174.8± 13.05[b]	5.17± 0.43[b]

Data are presented as mean ± SEM (n = 10). Multiple comparisons were accomplished using one way ANOVA followed by Tukey-kramer's test. [a] and [b] indicate significant change from control and CCl_4 groups respectively at $p \leq 0.05$.

Table 5: Effect of pretreatment with silymarin or total extract of Ajwa date fruit on CCl_4-induced histopathological alterations.

Histopathological Alterations	Control	CCl_4	Silymarin	Dates	Silymarin + CCl_4	Dates + CCl_4
Centrolobuler fatty change	0	++++	0	0	++	+
Portal inflammatory changes	0	+++	0	0	0	0
Necrosis	0	+++	0	0	0	0
Other degenerative changes	0	++	0	0	+	++
Congestion in portal vein and central vein	0	++	0	0	+	0
Kupffer cells proliferation	0	0	0	0	0	0

0 =None; +=Mild; ++ =Moderate; +++=Severe; ++++=Very severe

TG (55%) and LDL-cholesterol (61%) and significantly heightened serum albumin level (93%) and HDL-cholesterol (117%) compared with CCl_4-treated group (table 3). Over and above, it decreased the hepatic tissue contents of MDA (63%), hydroxyproline (54%) and caspase-3 (53%) and restored GSH, SOD, CAT and GST activities by about 68%, 120%, 487% and 374%, respectively compared with CCl_4-treated group (table 4).

Histopathological findings, as illustrated in fig. 1 and table 5, showed no pathological alterations in the liver sections from the control group with normal histological structure of the central vein (cv) and surrounding hepatocytes (h) see fig. 1A. On the other hand, examination of the liver sections from group 3 revealed that CCl_4 administration induced fatty changes (f) as observed in most of the hepatocytes associated with necrosis (n) in some others as well as inflammatory cells infiltration (m) in the portal area and dilatation in the portal vein (p) see fig. 1B. Examination of the liver sections of group 6 illustrated that pre- and co-administration of silymarin was able to buffer some harmful effects of CCl_4 with dilatation in the central vein (cv) associated with hydropic degeneration (d) in most of the hepatocytes and fatty change (f) in other few- see fig. 1C. Lastly, examination of the liver sections of group 7 revealed that the prior and concomitant administration of Ajwa date fruit extract with CCl_4 was able to buffer some deleterious effects of the later with minor hydropic degeneration (d) which was noticed in some hepatocytes while other few cells had fatty change (f) - see fig. 1D. Likewise the control group, histopathological examinations of liver tissues from group 2 (corn oil control), group 4 (silymarin control) and group 5 (dates fruit extract control) showed intact hepatic tissue architectures with no histopathological alterations (data not shown).

DISCUSSION

The present study was designed to evaluate the capability of Ajwa date fruit extract in the protection against CCl_4-induced hepatotoxicity and oxidative stress. In general, oxidative stress is explained as the situation where there is

no balance between reactive oxygen species (ROS) production and antioxidant defenses. As an example, the metabolism of CCl_4 begins with the trichloromethyl free radical ($\bullet CCl_3$) by the action of the mixed function of the cytochrome P450 oxygenase system. This free radical reacts very rapidly with oxygen to yield a highly reactive trichloromethyl peroxy free radical ($CCl_3OO^{\bullet}$). Both radicals have the ability to bind to proteins or lipids, thus initiating lipid peroxidation that can lead eventually to per oxidative tissue damage and inflammation (Gosselin, Smith *et al*., 1984).

Accumulated evidence has shown that increased level of lipid per oxidation products plays a major role in hepatotoxicity (Fouad, Al-Mulhim *et al*., 2013). In this context, similar findings are obtained in the current work where CCl_4 injection produced significant elevation in serum levels of ALT, AST, ALP, total cholesterol, TG, and LDL-cholesterol. It also induced significant decrease in serum albumin and HDL-cholesterol. Moreover, injection of CCl_4 produced significant increases in MDA, hydroxyproline and caspase-3 contents in liver tissues. It also induced a significant decrease in liver tissue content of GSH as well as SOD, CAT and GST enzyme activities. These results are in agreement with those obtained by several researchers who demonstrated that administration of CCl_4 significantly increases serum hepatic enzymes and decreases serum albumin (Gangarapu, Gujjala *et al*., 2013, Yacout, Elguindy *et al*., 2012). Also, our data are in consistence with Al-Assaf (Al-Assaf, 2013) and Ahmed *et al* (Ahmed, Gulfraz *et al*., 2014) who found that rats treated with CCl_4 show increases in serum total cholesterol, LDL-cholesterol and TG as well as a decrease in serum HDL-cholesterol. Moreover, our results are parallel with other studies which concluded that CCl_4 administration reveals significant elevation in MDA and reduction of both GSH content & activity of antioxidant enzymes SOD, CAT & GST in hepatic tissue (Gangarapu, Gujjala *et al*., 2013, Najappaiah and Hugar, 2012). Furthermore, our results were in harmony with Yacout *et al*, who demonstrate that CCl_4 induced liver fibrosis as marked by significant increase in hydroxyproline content (Yacout, Elguindy *et al*., 2012). These findings are strengthened by our subsequent corroboratory histopathological observations that showed abnormal histological profile of the liver tissues in CCl_4 treated animals.

In consistent with observations of several other studies, notable deviant histoarchitectures of the liver tissues from CCl_4-treated rats included several focal necrosis, fatty degeneration of hepatocytes and inflammation in addition to signs of fibrosis (Najappaiah and Hugar, 2012, Yacout, Elguindy *et al*., 2012). In the last decades, many efforts have been made to find out a possible strategy to calm down the always undesirable oxidative stress revolutions and to eliminate the free radicals by using different types of antioxidants. As an example, several plants that contain polyphenols, such as Ginkgo biloba and Turmeric, have been reported to exert significant antioxidant potentials and were relatively able to extinguish the majority of free radicals-induced injuries (Daba, Abdel-Aziz *et al*., 2002). The marked antioxidant potential of these polyphenols seems to be due to their ability to release electrons that can buffer the free radicals by converting them into more stable products and thus terminate the free radical chain reactions (Singh, Chidambara Murthy *et al*., 2002). As well, many natural compounds were reported to exert beneficial effects against CCl_4-mediated liver injury, either via decreasing production of CCl_4-derived oxidative stressors or through antioxidant activity of the protective agent themselves (Yeh, Hsieh *et al*., 2013).

In the current study, pretreatment with Ajwa date fruit extract ameliorates CCl_4-induced alterations in serum levels of ALT, AST, ALP, total cholesterol, triglycerides, LDL-cholesterol, HDL-cholesterol and albumin as well as body weight and liver-body weight ratio. In another confirmation for its serviceable modulatory actions, Ajwa date fruit extract was efficiently able to mitigate the lipid per oxidation, hydroxyproline and caspase-3 contents in the rats livers caused by CCl_4 as manifested by decreased MDA and increased GSH hepatic contents as well as enhanced SOD, CAT, and GST enzymes activities. These observations are in harmonization with several other studies that reported similar results and claimed that *Phoenix dactylifera* seeds improved CCl_4-induced alterations in liver function parameters and remarkably attenuated the accompanied oxidative stress revolution (Abdelaziz and Ali, 2014, Al-Qarawi, Mousa *et al*., 2004, Azadbakht, Siahpoosh *et al*., 2012, Naskar, Islam *et al*., 2010, Sangi, El-feky *et al*., 2014, Sheikh, Elsaed *et al*., 2014). Of note that our results demonstrated, for the first time, that the elevations of hydroxyproline and caspase-3 hepatic contents induced by CCl_4 were significantly abrogated close to normal values in animals pretreated with Ajwa date fruit extract indicating that the extract could inhibit apoptosis and fibrosis in hepatocytes.

Histological examinations of liver tissues from rats subjected to CCl_4 with prior and concomitant administration of Ajwa date fruit extract presented another strong testimony for Ajwa date fruit potential to ameliorate the CCl_4-induced liver poisoning. It was obvious that pre- and co-administration of Ajwa date fruit extract significantly attenuated the incidence of liver lesions (including hydropic degeneration, fatty changes, vacuolization and fibroblast proliferation) triggered by CCl_4 intoxication. Similar observations made by others give credence to the present notion (Abdelaziz and Ali, 2014, Sheikh, Elsaed *et al*., 2014).

In the light of the current observations as well as the above cited reports, it is pertinent to presume that Ajwa date fruit, being antioxidants, effectively reduce the formation of CCl_4-induced free radicals which are known

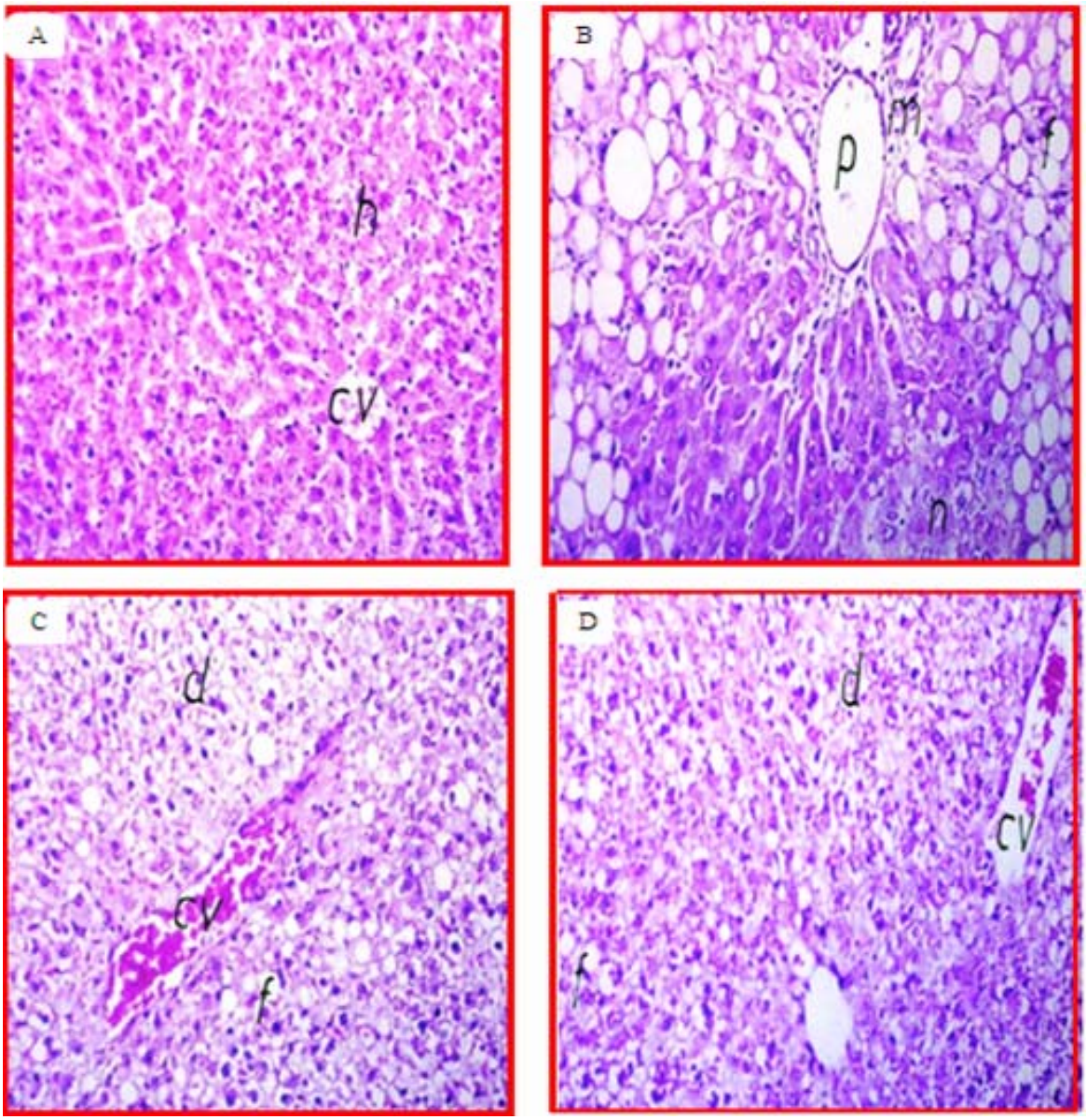

Fig. 1: Effect of pretreatment with silymarin or total extract of Ajwa date fruit on CCl_4-induced histopathological alterations in hepatic tissues. Histology of liver samples of the control group (A) showed normal histological structure (h). Conversely, liver tissues in CCl_4-group showed fatty change in hepatocytes associated with necrosis (n) and dilatation in the portal vein (p). Liver tissues in Silymarin + CCl_4 group showed relatively milder histopathological alterations as compared qualitatively to CCl_4 group. Dilatation in the central vein (cv) was detected in association with hydropic degeneration in most of the hepatocytes (d) and fatty change in other few (f). Liver tissues in Ajwa date + CCl_4 group showed relatively intact normal histological structure of the hepatic tissues with minor hydropic degeneration that was noticed in some hepatocytes (d) while other few had fatty change (f). Hematoxylin & eosin staining, magnification of x 40.

to trigger a cascade of inflammatory responses culminating in moderate to severe liver injury, and hence, could be used as effective inhibitors of the CCl_4-induced hepatotoxicity.

CONCLUSIONS

Ajwa date fruit extract has a promising hepatoprotective effect; this action could be attributed to antioxidant, antifibrotic and antiapoptotic activities. This may constitute a novel target for hepatoprotective therapeutic modality utilizing natural products.

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