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Prophylactic effect of date fruit extract on hepatotoxicity induced by either carbon tetrachloride or diethylnitrosamine in male rats

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Abstract: The aim of this research was to explore the protective effect of date palm fruit extract (DFE) on either carbon tetrachloride (CCl_4)- or diethylnitrosamine (DENA)-induced hepatotoxicity in male albino rats. CCl_4 and DENA were given in a single intraperitoneal (ip) dose of 3ml/kg and 150 mg/kg, respectively. Rats injected with CCl_4 or DENA showed significant increases in the serum levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), total bilirubin (TB), total protein (TP) and albumin (ALB). Moreover, there were a significant elevation in the hepatic content of malondialdehyde (MDA); and a significant inhibition in both the level of reduced glutathione (GSH) and the activities of antioxidant enzymes including superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and glutathione-s-transferase(GST)in the liver of the intoxicated rats. On the other hand, the results exhibited marked enhancement in the concentrations of total lipids (TL), total cholesterol (TC), triglycerides (TG) and low density lipoprotein-cholesterol (LDL-C); while the level of high density lipoprotein-cholesterol (HDL-C) was significantly decreased in CCl_4- or DENA-treated rats. Pretreatment with DFE (1g/kg) for 4 weeks to rats injected with a single dose of either CCl_4 (3ml/kg) or DENA (150 mg/kg) significantly alleviated the toxin-induced detrimental effects in the above mentioned biochemical parameters. Finally, it could be concluded that DFE is a hepatoprotective agent and can be protect against either CCl_4- or DENA-induced hepatotoxicity in rats.

keywords: Hepatotoxicity, Carbon tetrachloride, Diethylnitrosamine, Date palm fruit extract, Antioxidant enzymes

1.Introduction

The liver is a crucial organ for maintenance of homeostasis and body functions. It is the principal site for metabolism, and serves in detoxification of unwanted substances by various biochemical pathways such as reduction, oxidation, condensation, hydration, conjugation, hydrolysis and isomerization. Therefore, its optimal function is pivotal for body general health and disorder of any of the above mentioned processes may lead to liver injury which in turn may lead to many diseases [1]. Hepatotoxicity, however, can be produced by medicines, chemicals and dietary disorders [2]. Inflammation, immunomodulation and oxidative stress are amongst the common biological mechanisms contributing to the induction of liver damage and dysfunction [1].

A variety of drugs and chemicals are used experimentally to induce animal model of hepatotoxicity. CCl4 is a well-known hepatotoxin which has been widely used to induce an animal model of hepatotoxicity for studying the mechanisms of cytopathology and hence the possible therapeutic aspects [3]. It has been reported that CCl_4 can induce hepatotoxicity by increasing lipid peroxidation (LPO) and oxidative stress [4]. However, the toxicity of CCl_4 is due to its reactive metabolite, the trichloromethyl free radical ($CCl_3^{\bullet}$), which is mainly formed in the liver by the action of cytochrome P450 (CYP450) monooxygenase system. This reactive metabolite ($CCl_3^{\bullet}$) can cause oxidative damage to the biological vital macromolecules such as proteins, nucleic acids and lipids. Furthermore,

the $CCl_3^{\bullet}$ radical can be converted into the trichloromethylperoxy radical ($CCl_3OO^{\bullet}$) when it reacts with oxygen, and this radical is still more reactive and can initiate the process of LPO [4]. CCl_4-induced liver injury is progressed from steatosis to centrilobular necrosis, and ultimately may develop fibrosis and cirrhosis [5]. Also, CCl_4 can promote production of inflammatory cytokines and recruitment of inflammatory cells and can cause liver damage and dysfunction [6].

DENA is a well-known potent hepatocarcinogen. It is generally widespread in nature and can be found in various items such as cheddar, handled meats, mixed drinks, tobacco products and restaurants. It is confirmed to be one of the natural cancer-causing agents [7]. It can cause hepatic cancer in all animal species, as well as in humans [8]. The toxin can lead to hepatocarcinogenesis, mitotic hepatocytes and chronic inflammation [9]. DENA is also known to cause injury to many enzymes implicated in DNA repair, which could also relate to its potential to induce liver cancer in experimental animal models. On the other hand, DENA exposure has been associated with accumulation of reactive oxygen species (ROS) in hepatocytes, which may result in oxidative damage to DNA and other nucleophiles, and this mechanism could be implicated in the induction of hepatocarcinogenesis on exposure to DENA [10].

Several agents, particularly natural products, were reported to protect the liver against xenobiotics-induced toxicity by either prophylactic or therapeutic effects. Medicinal plants are representing an important alternative complimentary sources for potential hepatoprotective agents, and could consider a key source of innovative promising therapies [1]. Many of the anticancer agents used todays have been obtained from natural products of various origins, and plants are the main source [11].

Date palms (*Phoenix dactylifera* L.) are widely grown in the hot arid regions mainly in the Middle East and North Africa. Date fruits have very sweet taste and fragrant, and contain very high nutritional value because of its components such as carbohydrates, vitamins and minerals [12]. It is an ancient plant that used for the treatment of many diseases and disorders in folk medicine [13]. Some of the date fruit's biological benefits have been recorded as an anti-inflammatory, antitumor, antidiabetic, nephroprotective, hepatoprotective, antioxidant and fertility improving [14]. So, Dates provide an appropriate alternative therapy for various illnesses [13]. In previous study, [15] reported the ability of Ajwa dates to protect the liver against chronic exposure to CCl_4 in rats. Moreover, in recent study, administration of aqueous extract of Ajwa dates in rats treated with DENA restored biochemical parameters of toxin-induced liver injury towards normal [16]. On basis of these information, the present study was conducted to explore the prophylactic effect of DFE against hepatotoxicity induced by acute exposure to either CCl_4 or DENA in adult male rats.

2. Materials and Methods

1. Experimental animals

In this experiment, adult male albino rats weighing about 140g have been used. They were obtained from Helwan Animal Farm, Helwan, Egypt. Rats were kept in a well-ventilated room in stainless steel cages and fed on commercially standard diet. Drinking water was allowed *ad libitum*. Care and handling of the rats was carried out under guidance rules of the Animal Ethics Committee of Mansoura University, Egypt.

2. Chemicals

Diethylnitrosamine (DENA) and carbon tetrachloride (CCl_4) were obtained from local suppliers for Sigma-Aldrich Co Chemicals. DENA was dissolved in 0.9% NaCl normal saline and injected ip as a single dose of 150 mg/kg [17]. CCl_4 was diluted in corn oil (1:1) and injected in a single ip dose of 3 ml/kg [18].

3. Preparation of DFE

The flesh of date fruits was separated manually and soaked in distilled water (w/v) for 48 hours 4◦C. Date fruit solution then was homogenized till a uniform suspension was formed [19]. The suspension was then kept at -4°C to avoid microbial and fungal contamination. Aqueous extract of date's fruit flesh was chosen because most of

antioxidants and active ingredients are extracted in water [20].

4. Experimental animal groups

After the acclimatization period (14 days), male rats were divided randomly into six groups (6 animals/group) as follows:

i. **Control rats:** Animals didn't receive any treatment.

ii. **DFE-treated rats:** Animals received DFE in an oral dose of 1g/kg daily for 4 weeks.

iii. **CCl_4-treated rats:** Animals received a single dose of CCl_4 (3ml/kg, ip).

iv. **DFE and CCl_4-treated rats:** Animals received DFE for 4 weeks, as described in group ii, and at the end of this period, they received a single dose of CCl_4, as described in group iii.

v. **DENA-treated rats:** Animals received a single dose of DENA (150 mg/kg, ip).

vi. **DENA and DFE-treated rats:** Animals received DFE for 4 weeks, as described in group ii, and at the end of this period, they received a single dose of DENA, as described in group v.

5. Blood and liver tissue sampling

Animals were fasted overnight at the end of the experimental period. They were then sacrificed under anesthesia and the blood samples drained from jugular vein were gathered in centrifugation tubes. Collected blood was applied to centrifugation at 3000 rpm for 15 minutes. Using clean pipette, the serum of each sample was separated, labeled and kept at -20°C for subsequent biochemical analyses. In the meantime, rats were dissected quickly and the livers were removed carefully, washed with saline, cleaned and dried using lint free tissue. A known portion of the liver tissue was cut with scissor, weighed accurately, homogenized in cold distilled water to form 10% (w/v) and finally kept at -20°C for assaying suggested parameters.

6. Biochemical analyses

Colorimetric methods of [21, 22, 23] were used for the estimation of serum TP, ALB and TB contents, respectively. Enzymatic activities of serum AST and ALT were determined by the method described by [24], the procedure of [25] was applied to determine the ALP activity. The level of hepatic MDA was estimated according to the procedure of [26]. Liver content of GSH was evaluated by the colorimetric method of [27]. The hepatic activities of SOD, CAT, GPx and GST were assessed according to the methods of [28, 29, 30, 31], respectively. The concentrations of serum TL, TC, TG and HDL-C were estimated using the method of [32, 33, 34, 35], respectively. While, serum LDL-C level was calculated mathematically by using the formula of [36], as follows:

LDL-C (mg/dl) = TC – HDL-C – TG/5.

7. Statistical analyses

All the grouped data were statistically evaluated with Graph Pad prism software. Differences among groups were evaluated by one-way analysis of variance (ANOVA) followed by Tukey as a post test. *P* value equal or less than 0.05 was considered to be significant. All values were expressed as mean ± SE of six rats per group. Percentage of change from control was calculated for each treated group.

3 Results

1. Hepatocellular injury biomarkers

Table 1 shows that, injection of rats with a single ip dose of either CCl_4 (3ml/kg) or DENA (150 mg/kg) markedly increased serum activity of ALT, AST, ALP and TB content, when compared with control results. Meanwhile, serum TP and ALB contents were decreased in rats exposed to the same treatment with mentioned toxins, as compared to control group. In animals treated with DFE (1g/kg) for 4 weeks followed by injection of a single dose of either CCl_4 (3ml/kg) or DENA (150 mg/kg), a significant reduction in serum levels of liver mentioned enzymes and TB, accompanied with markedly raised serum contents of TP and ALB were observed, in comparison with groups treated with either DENA alone or CCl_4 alone. However, serum activities of ALT, AST and ALP in the pretreated groups were still significantly high, in comparison with the results of control group. Interestingly, serum levels of TB, TP and ALB in the pretreated groups returned to near the control values.

Table 1. Effect of DFE on either CCl_4- or DENA-induced adverse changes in the levels of serum biomarkers of liver function in rats.

	Con	DFE	CCl_4	DFE+CCl_4	DENA	DFE+DENA
TP (g/dl)	9.48± 0.29	9.59±30	6.17± 0.15^{a}	8.39±0.34^{b}	7.69± 0.15^{a}	8.85± 0.25^{c}
Alb (g/dl)	3.88± 0.03	3.97±0.06	2.59± 0.03^{a}	3.62±0.11^{b}	2.97± 0.07^{a}	3.76± 0.11^{c}
TB (mg/dl)	0.24± 0.034	0.27±0.032	0.52± 0.034^{a}	0.27± 0.011^{b}	0.51± 0.032^{a}	0.30± 0.028^{c}
ALT (U/l)	19.67±0.47	18.00±1.55	94.87± 2.42^{a}	62.23± 5.90^{ab}	83.00± 0.97^{a}	65.77± 5.07^{ac}
AST (U/l)	19.93± 1.46	20.70±1.44	49.60± 0.55^{a}	32.80± 2.01^{ab}	48.62± 0.52^{a}	35.52± 2.14^{ac}
ALP (IU/L)	126.6± 6.67	114.3±12.97	220.2± 4.35^{a}	164.1± 9.18^{ab}	305.2± 5.50^{a}	243.7±10.39^{ac}

- Con = Control, DFE = Date fruit extract, CCl_4 = Carbon tetrachloride, DENA = Diethylnitrosamine.
- Values were expressed as mean ± SE (n = 6 for each group).
- **a, b** and **c** = Significant difference at $p \leq 0.05$ comparing to control, CCl_4 and DENA groups, respectively.

Oxidative stress and antioxidant biomarkers

As described in table 2, injection of either DENA (150 mg/kg, ip) or CCl4 (3ml/kg, ip) significantly increased the levels of MDA, accompanied with marked decreases in SOD, CAT, GST and GPx activity and level of GSH content in the liver, when compared to the results of control group. On the other hand, treatment of rats with DFE (1g/kg) for 4 weeks followed by injection of a single dose of either DENA (150 mg/kg) or CCl4 (3ml/kg) significantly attenuated the adverse changes in mentioned parameters of oxidative stress, as compared to the results of groups treated with either DENA or CCl4 alone. However, the differences in the hepatic levels of MDA, SOD, CAT, GPx, GST and GSH in the pretreated groups were statistically significant in comparison with the results of control group.

Table 2. Effect of DFE on either CCl_4- or DENA-induced adverse changes in the hepaticoxidative stress biomarkers in rats

	Con	DFE	CCl4	DFE+CCl4	DENA	DFE+DENA
MDA(nmol/g)	69.7± 5.96	89.1± 3.8	139±12.31^{a}	98.6± 4.58^{ab}	139.6±5.49^{a}	105.4± 3.81^{ac}
GSH(mg/g)	9.62±0.75	8.05± 0.53	2.77± 0.24^{a}	4.88± 0.49^{ab}	3.94± 0.13^{a}	6.56± 0.33^{ac}
SOD(U/g)	618.7 ± 28.6	645.5± 27.3	257.5±34.6^{a}	488.1± 22.2^{ab}	274.2± 18.6^{a}	441.0±19.3^{ac}
CAT(U/g)	1.99± 0.004	1.98± 0.007	1.58±0.039^{a}	1.88± 0.016^{ab}	1.59± 0.027^{a}	1.81± 0.030^{ac}
GPx(U/g)	22.52± 2.10	23.96± 1.66	7.81±0.19^{a}	13.30± 0.31^{ab}	7.89± 0.42^{a}	12.84± 0.36^{ac}
GST(U/g)	5.81± 0.11	6.18± 0.21	3.89±0.17^{a}	5.11± 0.098^{ab}	3.03± 0.18^{a}	3.91± 0.08^{ac}

- Con= Control, DFE= Date fruit extract, CCl_4= Carbon tetrachloride, DENA= Diethylnitrosamine.

Values were expressed as mean ± SE (n = 6 for each group)

a, b and **c** = Significant difference at p≤ 0.05 comparing to control, CCl_4 and DENA groups, respectively.

Lipid profile

Table 3 illustrates that, injection of either DENA (150 mg/kg, ip) or CCl_4 (3ml/kg, ip) produced marked elevations in the serum TL, TC, TG and LDL-C levels, accompanied with marked decrease in HDL-C. Meanwhile, DFE treatment (1g/kg) for 4 weeks followed by injection of used single dose of either DENA or CCl_4 significantly ameliorated the TL and lipid fractions when compared to rat groups treated with either DENA or CCl_4. However, serum TL, TC, TG and LDL-C contents in the pretreated groups appeared significantly high, as compared to the results obtained in the control groups. Also, the HDL-C content in the pretreated groups was still markedly low, in comparison with the result of control group.

Concerning the effect of daily treatment of rats with DFE (1g/kg) alone for 4 weeks, obtained results in tables 1-3 displayed insignificant changes in all measured biochemical parameters, as compared to the control group

Table3. Effect of DFE on either CCl_4- or DENA-induced adverse changes in the serum lipid profile in rats.

	Con	DFE	CCl_4	DFE+CC_4	DENA	DFE+DENA
TL(mg/dl)	212.3± 4.19	219.9±4.98	327.6± 10.34^{a}	271.3±2.63^{ab}	377± 8.57^{a}	305.3±3.76^{ac}
TC(mg/dl)	63.5± 1.53	69.8± 5.85	100.3±1.84^{a}	77.7± 2.12^{ab}	103±3.84^{a}	78.6±2.18^{ac}
TG(mg/dl)	52.90± 3.77	58.56± 4.47	103.9± 1.92^{a}	82.47± 4.04^{ab}	96.25± 1.14^{a}	77.05± 2.66^{ac}
LDL-C(mg/dl)	14.35± 0.82	15.70±1.58	66.22± 1.40^{a}	39.74± 0.90^{ab}	72.34± 2.38^{a}	40.84± 1.16^{ac}
HDL-C (mg/dl)	38.64± 1.09	42.82± 3.62	11.72± 0.82^{a}	21.42± 0.86^{ab}	11.34± 1.26^{a}	21.10± 0.91^{ac}

- Con= Control, DFE= Date palm fruit extract, CCl_4= Carbon tetrachloride, DENA= Diethylnitrosamine.
- Values were expressed as mean ± SE (n = 6 for each group).Values in parentheses represent % of changes from control.

a, b and **c** = Significant difference at $p \leq 0.05$ comparing to control, CCl_4 and DENA groups, respectively

IV. Discussion

Liver is the principal site of metabolism of almost all drugs and toxins [37, 38]. This is required a sufficient anti-oxidant and detoxification materials including enzymatic and non-enzymatic substances. So, depletion of this hepatic defense system could represent a major risk factor which could be associated with liver injury, because the liver cannot be sufficiently cleared xenobiotics during metabolism [39]. Actually, potential hepatotoxicity in association with the exposure to drugs and toxic chemicals is a matter of a great concern and needs further and ongoing investigation to find out an appropriate solve. Nowadays, the use of natural products particularly from medicinal plants in the treatment of diseases and in the protection against chemicals-induced hepatotoxicity is highly accepted by many researchers all over the world. In this trend, the present work was designed to explore the potential prophylactic impact of DFE on acute hepatotoxicity induced by either CCl_4 or DENA in male adult rats.

1. CCl_4-induced hepatotoxicity

Obtained results of the current study showed that, exposure of adult albino rats to a single dose of CCl_4 (3ml/kg) produced marked elevations in the serum levels of ALT, AST, ALP and TB, suggesting incidence of hepatocellular injury. In addition, the results exhibited decline in the serum contents of TP and ALB following administration of mentioned toxin, indicating impairment of liver function. This result provided an additional support that CCl_4 is a hepatotoxic agent and can induce adverse effect on the hepatocytes structure and function. CCl_4-induced hepatocellular injury could involve damage of the plasma membrane which leads to alteration of membrane permeability causing increased leakage of different hepatic enzymes such as ALT, AST and ALP into the bloodstream [40]. CCl_4 is a well-known hepatotoxin, and is commonly used as a model of both acute and chronic liver injury in the rodents [41]. Many previous animal studies demonstrated the hepatotoxicity of CCl_4. Of these studies, [42]; and [43] showed significantly elevated liver enzyme activities in the serum after CCl_4 treatment in rats. Also, [44] reported that single dose administration of CCl_4 (0.5 ml/kg) to rats resulted in liver injury as evidenced by a marked increase in serum AST, ALT and ALP. The same results were recorded by [18, 45] who confirmed that administration of CCl_4 elicited elevation in the levels of liver marker enzymes (AST, ALT and ALP) and TB; together with a decline in serum concentration of ALB, suggesting liver injury and impaired function. In recent studies, [46, 47] recorded similar increases in the serum levels of hepatic enzymes ALT, AST and ALP, as well as TB in rats intoxicated with CCl_4.

Many studies have demonstrated that CCl_4 can cause acute and chronic hepatic injuries [48]. It has been revealed that, Cl_4 can cause liver injury through the production of ROS with an increase in the oxidative stress, which can damage the plasma membranes of hepatocytes leading to the leakage of liver enzymes into the blood stream [49, 50]. However, in order to assess the mechanism by which CCl_4 induced liver injury and hence elevation of hepatic enzymes activities in the serum in the present study, biochemical indices of oxidative stress were evaluated in the hepatic tissue. Obtained results showed adverse changes in the oxidative stress markers as indicated by elevated hepatic content of LPO product (MDA), accompanied with a decrease in the hepatic antioxidant parameters including SOD, CAT, GPx, GST and GSH. This finding added support that LPO and oxidative stress are implicated in the mechanisms of CCl_4-induced hepatocellular injury in rats. In this regard, numerous studies showed the ability of CCl_4 to induce imbalance between production of ROS and endogenous antioxidants, which leads ultimately to induction of oxidative stress and cell injury. One of the studies, [51] reported that, exposure to CCl_4 in rats was found to induce hepatic injury by increasing ROS and LPO, and suppression of the antioxidant

enzyme activities in the liver of rats. Also, [52, 46] recorded a marked rise in the hepatic content of MDA, the marker of LPO, which was accompanied with significant decline in both GSH and CAT after intoxication with CCl_4 in rats. Recently, chronic exposure to CCl_4 (orally administered for 8 weeks, 3 times/week) was also found to decrease the levels of hepatic GSH, GPx, CAT and SOD in rats, while it increased the hepatic content of MDA [53].

CCl_4-mediated hepatotoxicity and hepatopathy is attributed to its biotransformation by hepatic cytochrome P450 2E1 to the free radical $CCl_3^{\bullet}$, which is further converted to a highly reactive species $CCl_3OO^{\bullet}$ by the reaction with oxygen. The peroxy radical $CCl_3OO^{\bullet}$ binds covalently to cellular macromolecules, particularly lipids, leading to a chain reaction of polyunsaturated fatty acids in the membrane phospholipids, causing LPO, with subsequent functional and morphological changes to the cell membrane. LPO is a harmful process as it is an autocatalytic, irreversible reaction which can alter the membrane permeability and fluidity of lipid bilayer leading, finally, to membrane damage and release of intracellular enzymes and other components to the extracellular fluid causing inflammatory reactions and cell necrosis [54, 55].

In the present study, increased oxidative stress in the liver of rats after injection of CCl_4 was found to be accompanied with marked suppression in the antioxidant defense system. The observed decrease in GSH level and activities of antioxidant enzymes such as SOD, CAT, GPx and GST in the liver of CCl_4-intoxicatedrats might be due to increased scavenging of reactive substances that were produced as a result of the toxin metabolism, degenerative hepatocytes which might be associated with inflammatory reactions and possibly decreased hepatic production of antioxidants [56]. This led to loss of balance between ROS production and antioxidant defense with subsequent production of oxidative stress and tissue damage.

It has been reported that, GSH is a highly effective cellular non-enzymatic antioxidants in the body. It contributes to two main defense processes in the cell: i- it offers a reducing agent for oxidant molecules (such as, H_2O_2 and hydroperoxides) as it donates hydrogen and converted to the oxidized form (GSSG), and this process is catalyzed mainly by GPx enzyme; ii- GSH is used in detoxification of xenobiotics since it used as a substrate for conjugation reactions in phase II metabolism which are catalyzed by GST enzyme. Consumption and consequently depletion of cellular GSH under the conditions of oxidative stress is therefore expected. So, for restoration of the normal concentration of GSH inside the cell, an important antioxidant enzyme called glutathione reductase (GRd) is necessary for this biochemical process since it catalyzes the reduction of GSSG [57, 58].

It is clearly appeared that, GSH and its related enzymes (GPx, GRd and GST) represent a large part of antioxidant defense system and can catalyze the reduction of oxidized reactive molecules into non-toxic products and then can end the chain reaction of LPO process [59].The decline in activities of the GSH-related enzymes together with the level of GSH itself is therefore used as a marker for oxidative stress and cytotoxicity. Beside GSH and its related enzymes, the antioxidant enzymes of SOD and CAT were found to exert an effective role in the defense mechanism against reactive molecules-induced cell injury. They represent the first line used for cytoprotection against ROS. Considering SOD, it plays a major role in catalyzing the dismutation of superoxide radicals ($O_2^{\bullet -}$) into H_2O_2 [60]. While, CAT catalyzes the conversion of H_2O_2 into H_2O and O_2 [61].

2. DENA-induced hepatotoxicity

Diethylnitrosamine (DENA) is a well-known hepatocarcinogen. It presents in tobacco smoke, water, cured and fried meals, cheddar cheese, agriculture chemicals and cosmetics and pharmaceutical products [62]. DENA is experimentally used to induce hepatocellular carcinoma, as well as it used to study the mechanisms of cytotoxicity. This leads the scientists to make a lot of researches to solve the problem of cancer therapy. It has been reported that, biotransformation of DENA is mainly occurred in the liver by the catalysis of CYP 450 dependent enzymes. Some of the

byproducts and reactive metabolites produced during these reactions react covalently with cellular components leading to cellular necrosis, mutation and cancer [63].

In the present study, treatment of rats with a single dose of DENA (150 mg/kg, ip) produced significant increase in the serum ALT, AST and ALP activity, suggesting hepatocellular injury and destruction of cell membrane. In consistence with the present finding, several studies reported similar elevations in the activities of the liver enzymes following DENA administration. For examples: [64, 65, 66, 67].

The current study also recorded elevations in serum levels of ALP and TB after exposure to DENA in rats, which suggested hepatobiliary injury [86].This finding is similar to the results obtained by [66, 67] who recorded elevation in serum level of ALP and TB following ip injection of DENA (single dose of 200 mg/kg). Such increase in the levels of ALP and TB in the blood of rats treated with DENA could be attributed to injury of biliary ducts, due to toxicity of DENA. In other words, loss of functional integrity and damage of hepatic cell membranes (pathologic alterations) induced by DENA might lead to release of TB and the membranous enzyme of ALP into the blood [69, 70]. It has been known that, bilirubin is a bile pigment, which is formed during the process of heme breakdown, a part of hemoglobin which presents in the erythrocytes [71]. Formed bilirubin is then received by the liver, exposed to a conjugation process and then included in the bile constituents [68]. Therefore, inability of the liver to form conjugated bilirubin on exposure to DENA is an indicative of impaired liver function, and can be manifested by the elevation in the serum level of unconjugated bilirubin [72].

DENA-induced hepatocellular injury can also cause disturbance in synthetic function of the liver. In the current study, acute exposure of rats to DENA was found to produce a reduction in the serum contents of both TP and ALB. It has been known that, the majority of plasma proteins including ALB is primarily produced by the liver, thus obtained reduced TP and ALB in the DENA-treated rats suggested impairment of the liver function. Similarly, both [73, 67] reported decreases in TP and ALB in rats administered DENA. It is well known that, normal DNA structure and function is necessary for normal cellular protein synthesis, thus ability of DENA, as a genotoxic agent [74], to induce DNA damage in the hepatic cells could explain its lowering effect on serum levels of TP and ALB in the treated rats.

Potentially, obtained disturbances of serum biochemical markers of liver status (ALT, AST, ALP, TB, TP and ALB) by DENA injection may be considered as a secondary event came after oxidative tissue damage and hepatopathy induced by DENA. In support of this mechanism, current finding demonstrated that exposure of rats to a single toxic dose of DENA can cause hepatic oxidative stress, as reflected by significantly increased the hepatic content of pro-oxidant parameters such as MDA (the marker of LPO), accompanied with markedly decreased the hepatic levels of antioxidant indices including GSH, SOD, CAT, GPx and GSH in DENA-treated rats. The implication of oxidative stress in the mechanisms of DENA-induced hepatotoxicity has been previously reported by several animal studies. [75] reported marked increases in both LPO and total nitrate/nitrite (NOx), and decreases in the antioxidant parameters (GSH, GPx, CAT and GST) in the liver tissue of rats treated with DENA. The authors suggested that reactive oxygen and nitrogen species induced by DENA play an important role in the toxin-induced liver damage and carcinogenesis. Also, [66] found that, injection of DENA to rats led to increase in the hepatic content of LPO and decrease in CAT, SOD, GPx and GSH levels. In recent study, chronic exposure to DENA (100 mg/l, in drinking water for 8 weeks) significantly increased MDA and NO levels, which was accompanied with marked decline in CAT and GPx activities [76]. DENA-induced oxidative tissue damage has also been evidenced by previously published histopathological alterations in the liver of rats [75, 66, 67]. Present results of DENA-induced oxidative stress as reflected by redox imbalance (increased LPO and decreased antioxidant parameters) could add an additional support that DENA is a hepatotoxic agent and hence can initiate hepatocarcinogenesis. However, production of ROS and reactive metabolites during DENA bioactivation in the liver can

explain its ability to induce oxidative stress and several pathologic effects such as necrosis, DNA adducts, DNA-strand breaks, which in turn cause hepatocellular carcinoma [77, 78, 79].

3. Effect of either CCl_4 or DENA on lipid profile

Current findings demonstrated that, acute exposure to CCl_4 in rats caused significant increases in the serum contents of TC, TG and LDL-C, while it produced a significant decrease in serum level of HDL-C. This result is in accordance with the published study of [52] who reported that CCl_4 induced deleterious changes in lipid profile including increases in the levels of TC, TG and LDL-C, accompanied with a marked decrease in level of HDL-C in the serum of rats. Also, [80] showed a significant increase in the level of TC, TG and LDL-C, while no difference in serum level of HDL-C level in rats treated with CCl_4. It has been reported that, increased serum levels of TC may cause liver damage, and chronically may cause atherosclerosis and coronary artery disease. Also, elevated level of TG in the serum of rats treated with CCl_4 may result in deposition of lipid droplets in the liver leading to fatty liver [81]. The disturbance in serum levels of lipid profile in rats intoxicated by CCl_4 could be attributed, in part, to toxin-induced oxidative tissue damage in the liver, which led to structural and functional alterations in the hepatocyte membrane. It has been known that, the liver has receptors on the cell membrane called LDL receptors, which chiefly mediated the clearance of LDL-C particles from the blood circulation. In addition, liver LDL receptors are considering a main regulator of cholesterol metabolism since they have a vital role in hepatic uptake and clearance of plasma cholesterol [82]. Therefore, with abnormal LDL receptors structure and function, as in case of intoxication by CCl_4, serum levels of LDL-C as well as TC could elevate [83].

Potential adverse changes in serum lipid profile in DENA-treated rats were examined in the current study. Obtained results showed that, the levels of serum TC, TG and LDL-C were significantly elevated while, the value of serum HDL-C was reduced in DENA-exposed rats. This result was parallel to previous finding of [84] which demonstrated elevation in the serum concentrations of TC, TG and LDL-C; and decrease in serum level of HDL-C in rats injected with DENA (150 mg/kg). In addition, [85, 67] showed elevated levels of TC and TG and reduced values of serum HDL-C in DENA-exposed rats. Potentially, disturbances in serum lipid profile in DENA-treated rats could be attributed to impairment of liver metabolic function, which may include suppressed activities of lipid metabolizing enzymes, due to the cytotoxic effect of acute exposure to DENA.

4. Beneficial effects of DFE on either CCl_4- or DENA-induced hepatotoxicity

Phytochemicals products from herb and plant have been shown to play a significant role in the management of various liver diseases. The Prophet Mohammed (peace be upon him) recommended various medicinal herbs and plants for cure of various diseases [86]. Phoenix dactylifera(date palm) fruits are considered the most important medicinal and nutritional plants which was recommended by Prophet Mohammed (peace be upon him) to protect against various agents-induced body organs toxicity. According to a "*Hadith*", the sayings of Holy Prophet Muhammad (peace be upon him), "*he who eats seven Ajwa dates every morning will not be affected by poison or magicon the day he eats them*" [87]. Therefore, date palm fruit is widely consumed in the Arab world, particularly Islamic populations.

The results of the present study showed that pretreatment of rats with DFE for 4 weeks before injection of a single acute dose of either CCl_4 or DENA significantly lowered serum levels of ALT, AST, ALP and TB, when compared to groups treated with mentioned toxins alone. Moreover, the same treatment regimen markedly raised the lowered serum concentrations of both TP and ALB, due to exposure to either CCl_4 or DENA. This result clearly suggested the ability of DFE to protect hepatocytes structure and function against either CCl_4- or DENA-induced cellular injury. In accordance, it has been reported that treatment with aqueous extract of date flesh significantly reduced CCl_4-induced elevation in the liver enzymes (ALT, AST and ALP) and level of TB in plasma of the treated rats [19].

Also, [88] reported that pretreatment with Siwa DFE markedly ameliorated the elevated activities of serum ALT and AST in rabbits treated with CCl_4. Co-treatment of adult male rats with CCl_4 in a dose of 1.2 ml/ kg (3 times/week) and Ajwa date extract in a dose of 1g/kg (5 days/week) for 4 and 12 weeks resulted in a significant reduction in the serum activity of hepatic marker enzymes (AST and ALT), accompanied with marked increase in the serum concentration of ALB [15]. Similar finding was reported by [45] who observed significant improvement in the activities of ALT, AST and ALP; and levels of ALB in the serum of rats exposed to combined treatment with DFE and CCl_4. In another study, treatment with Ajwa date extract for 2 weeks before injection of CCl_4, produced significant decrease in ALT, AST and ALP activity; and marked increase in the level of ALB in the serum, as compared to group treated with CCl_4 alone [89].

Few studies have been published regarding the protective effect of DFE against DENA-induced hepatotoxicity. In one study, [16] reported that treatment with DFE reduced DENA-induced elevation in serum activities of hepatic diagnostic enzymes (ALT, AST and ALP) in rats, and the authors suggested the ability of DFE to protect against DENA-induced hepatocellular injury.

In the current study, DFE-induced a significant improvement of the hepatocytes integrity and function as reflected by reduced activities of liver enzymes and levels of TB, and raised contents of TP and ALB in the serum could suggest the ability of the date fruit to protect the liver cells against either CCl_4- or DENA-induced oxidative tissue damage. This suggestion was confirmed by obtained results of the current study which showed that treatment of DFE (1g/kg) to rats before injection of a single dose of either CCl_4 (3ml/kg) or DENA (150 mg/kg) significantly attenuated CCl_4- or DENA-increased production of MDA, the marker of LPO, in the liver, when compared to the results of rats treated with CCl_4 or DENA alone. Also, the same treatment regimen (DFE plus CCl_4 or DFE plus DENA) exhibited marked increase in hepatic SOD, CAT, GST and GPx activity; in addition to the level of non-enzymatic antioxidant GSH, as compared to groups treated with either CCl_4 or DENA alone. These results clearly provided an evidence that DFE can afford a protective impact against either CCl_4- or DENA-induced oxidative hepatic tissue damage, possibly because of its potent anti-oxidative activity [90].

Considering the inhibitory effect of DFE on CCl_4-induced hepatic oxidative stress, previous studies similarly demonstrated that pretreatment with Siwa DFE significantly ameliorated the levels of MDA, by decreasing its concentration, and GSH, by increasing its content, in the liver tissue of rabbits intoxicated with CCl_4 [88]. In addition, administration of DFE significantly improved the hepatic oxidative stress parameters in CCl_4-intoxicated rats as manifested by markedly lowered levels of MDA together with significantly increased levels of GSH, GPx and SOD [45]. Recently, Ajwa date extract simultaneously administered with CCl_4 for 8 consecutive weeks in rats resulted in significant decrease in MDA content; and restored levels of GSH; and activities of SOD, CAT and GST in the hepatic tissue [89].

Few literatures are available regarding the protective impact of DFE against DENA-induced hepatic oxidative tissue damage. In long-term study, [16] investigated the hepatocarcinogenic effect of DENA and the protection afforded by post-treatment with DFE for 10 weeks. The authors recorded improvement of the hepatic activity of antioxidant enzymes in rats treated with both DENA and DFE as indicated by observed significant increases in the activities of SOD, GPx and GRd. In parallel, marker of LPO (MDA) was markedly lowered in the hepatic tissue, suggesting suppression of DENA-induced oxidative tissue damage and hence carcinogenesis. Moreover, in recent study, [91] found that, long-term administration of DFE (1 g/kg, for 23 weeks) in rats suppressed the hepatic oxidative stress induced by intoxication with DENA in rats as revealed by decreased MDA and increased GSH contents in the liver.

5. Beneficial effects of DFE on either CCl_4- or DENA-induced changes in lipid profile

Present study documented that 4 weeks pretreatment with DFE ameliorated lipid profile

in the serum of rats exposed to a single dose of either CCl_4 or DENA, as indicated by lowering the levels of TL, TC, TG and LDL-C and increasing the concentration of HDL-C, in comparison with intoxicated groups by either CCl_4 or DENA. In accordance, there are published evidence which confirmed the lipids lowering effects of DFE. [92] reported the anti-hyperlipidemic effect of native date fruit and showed that, after 14 days of treatment with date fruit suspension (300 mg/kg), TC, TGs, LDL-C and VLDL-C along with HDL-C and LDL-C/HDL-C ratio were significantly decreased in hyperlipidemia-induced albino rats. In another study, [89] demonstrated that Ajwa date extract produced significant decrease in the serum levels of TC, TG and LDL-C accompanied with marked increase in HDL-C. Recently, long-term administration of DFE (1g/kg) in rats treated with DENA was found to modulate the serum parameters of lipid as reflected by lowered the levels of TC, TG and LDL-C and increased the concentration of HDL-C, as compared to the DENA-treated group [91].

Free radicals were reported to impair liver functions and can cause a defect in the perfect harmony of the hepatic metabolic reactions, which may lead to induce, for example, hyperlipidemia through multiple effects on lipid metabolism, including increased synthesis of TC, TG and LDL-C [93]. Therefore, it could be suggested that free radicals and oxidative stress produced by intoxication with DENA or CCl_4 might represent the main risk factor which adversely altered hepatic lipid metabolism in the treated rats [94, 91]. Interestingly, the ability of DFE to reduce the unwanted changes in lipid-investigated parameters produced by intoxication with CCl_4 or DENA, in the present study, might be due to the antioxidant potential of DFE and its possessing of free radicals scavenging activity, which lead to prevention of oxidative stress [13, 90]. It could be say that DFE can protect the hepatic tissue against CCl_4- or DENA-induced oxidative tissue injury, which in turn led to restoration of hepatocytes integrity and function, for example, improvement of the liver handling and metabolism of lipids and the key regulatory enzymes involved in these processes.

V. Conclusion

1. Present study added support that exposure to either CCl_4 or DENA can induce hepatotoxicity in adult male rats.

2. Long-term pretreatment with DFE showed prophylactic effect against either CCl_4- or DENA-induced hepatotoxicity, as manifested by reducing the deleterious changes in the markers of liver function, oxidative stress and lipid profile in the treated rats.

3- The hepatoprotective effect of DFE against liver injury induced by either CCl_4 or DENA could be attributed to its biological components that possess antioxidant activity and free radicals scavenging ability.

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