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The Ameliorative Effect of Phoenix Dactylifera Extract on CCl4 Hepatotoxicity in New Zealand Rabbits

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Abstract: The present study was conducted to evaluate the hepatoprotective effect of The date flesh (Phoenix dactylifera L.) against carbon tetrachloride (CCl_4) hepatotoxicity and improvement of Immune functions which affected by free radicals liberating CCL_4 in New Zealand rabbits. Fruits of the date palm (Phoenix dactylifera) are very commonly consumed in many parts of the world, and are a vital component of the diet in most of the Arabian countries. According to Prophet Mohammad (peace be upon him) says many Muslims believe that consumption of dates, particularly in the morning on an empty stomach, can reverse the actions of any toxic material that the subject may have been exposed to. Accordingly, carbon tetrachloride (CCL_4) hepatotoxicity was induced in rabbits in order to study the hepatoprotective activity of dates flesh (Phoenix dactylifera). Sixty New Zealand rabbits weighing about 1Kg were assigned to six groups, (ten/group). G1 was control, G2 received a single dose of Siwa date palm extract orally, G3 injected S/C with 1.0 ml CCL_4 solution /Kg, G4 injected S/C with 2.0 ml CCL_4 solution /Kg, G5 and G6 pretreated with a single dose a single dose of 15 ml of Siwa date palm extract orally. After 6, 12, 24 and 48 hours post-treatment blood samples were collected from the ear vein. The sera were separated and used for determining of ALT, AST and IgG, IgM and IgA and the liver homogenates for estimation of MDA and GSH as a biomarker of lipid peroxidation and antioxidative stress. The obtained results revealed that, CCL_4 caused significant increases in the levels of ALT and AST in (G3,G4) but treatment with Siwa date palm extract caused marked ameliorations of transaminase enzymes activity ALT and AST in (G5,G6). Moreover, there was a significant increase in MDA and decrease of GSH due to the oxidative stress induced by CCL_4 on membrane polyunsaturated fatty acids in rabbit's liver while Pretreatment with Siwa date palm extract, was significantly ameliorated the increased levels of MDA and decline of GSH in the liver tissue caused by CCL_4 hepatotoxicity. Meanwhile, Siwa date palm extract significantly increase in immune functions (IgG, IgM and IgA) in G2 while CCL_4 significantly decrease it specially IgG in a dose and time dependant (G3,G4). On the other hand, Pretreatment with Siwa date palm extract in G5, G6 elevated that levels near to the control. Thus, This study suggests that CCL_4-induced liver damage in rabbits can be ameliorated by administration of extract of date flesh .

Key words: Hepatoprotective; Hepatotoxicity; Phoenix Dactylifera; carbon tetrachloride.

INTRODUCTION

The date (Phoenix dactylifera L.) has been an important crop in arid and semiarid regions of the world. It has always played an important part in the economic and social lives of the people of these regions. The fruit of the date palm is well known as a staple food. It is composed of a fleshy pericarp and seed. Date palms have been cultivated in the Middle East over at least 6000 years ago [1]. In fact, Muslims believe that "*He who eats seven dates every morning will not be affected by poison or magic on the day he eats them*" [2]. Dates are a good source of energy, vitamins, and a group of elements like phosphorus, iron, potassium, and a significant amount of calcium [3]. Dates contain at least six vitamins including a small amount of vitamin C, and vitamins B_1 (thiamine), B_2 (riboflavin), nicotinic acid (niacin) and vitamin A [4]. Recent studies indicate that the aqueous extracts of dates have potent antioxidant activity [5]. The antioxidant activity is attributed to the wide range of phenolic compounds in dates including p-coumaric, ferulic, sinapic acids, flavonoids and procyanidins [6]. And higher iron chelation ability, DPPH scavenging activity, and antioxidant activities [7]. Of late, more attention has been paid to the role of natural

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antioxidants mainly phenolic compounds, which may have more antioxidant activity than vitamins C, E, β-carotene [8,9].

Liver is one of the main organs generating free radicals in different pathological conditions [10]. Molecules most exposed to damage by free radicals are polyunsaturated fatty acids (PUFA) of biological membranes. Deprived of one electron they undergo lipid peroxidation, which can lead to cellular membrane damage [11]. This is the one of mechanisms of hepatocytes injury [12]. Liver diseases are mainly caused by toxic chemicals, excessive consumption of alcohol, infections and autoimmune disorders. Most of the hepatotoxic chemicals damage liver cells mainly by inducing lipid peroxidation and other oxidative damages [13].

Carbon tetrachloride (CCL_4) has been used extensively to study liver injury induced by free radicals in an animal model system. CCL_4-treated rats are widely used to study liver damage where it was reported that CCL_4 induced not only necrosis but also apoptosis in rat liver [14,15]. Although the mechanism by which CCL_4 causes liver damage is unclear, several lines of evidence suggest that the liver damage can be caused by free radical metabolites [16]. CCL_4 is converted to the trichloromethyl radical by cytochrome *P*-450 through a 1-electron reduction. A fatty acid radical is generated by the reaction between trichloromethyl radical and unsaturated fatty acids, and lipid peroxidation follows.

Immune functions (IgG, IgM and IgA), IgG comprise approximately 80% of the serum antibody. IgG is responsible for most antibacterial, antiviral and antitoxic activity. IgA is the second most common immunoglobulin in serum (after IgG) and is the predominant immunoglobulin found in mucosal secretion. Secretory IgA can neutralize viruses, bind toxins, agglutinate bacteria, prevent bacteria binding to mucosal epithelial cells and bind to various food antigens. IgM is the most primitive and largest immunoglobulin of the five classes (IgG, IgA, IgM, IgD and IgE). It has an important function in complement-dependent bacteriolysis [17].

The aim of the present study was conducted to investigate the hepatoprotective effect of Siwa date palm against carbon tetrachloride hepatotoxicity and improvement of Immune functions which affected by free radicals liberating CCL4.

MATERIAL AND METHODS

Experimental Animals: Sixty New Zealand rabbits weighing about 1Kg were used for the study. The animals were acclimatized for a period of two weeks in the new environment before initiation of experiment. Rabbits were maintained on adequate stable commercial balanced diet and water "ad libitum"

Preparation of Plant Extracts: Siwa date palm extract Date fruits were obtained from Siwa Date Factory in Egypt. The flesh was manually separated from the pits and soaked in cold distilled water [1:3 ratio, weight to volume] and kept for 48 hours at a temperature of 4°C. The water extract was prepared freshly and given to the animals orally by stomach tube [18].

Induction of Hepatotoxicity: The animals were divided into control, carbontetrachloride (CCL_4) and test groups (CCL4 + Siwa date palm extract) for in vivo carbon tetra chloride hepatotoxicity animals received a single dose of CCL_4 solution (50% v/v) in paraffin oil for administration subcutaneously [19].

Experimental Design: A total number of sixty New Zealand rabbits weighing about 1Kg were distributed randomly into six groups (ten/group).

Group 1: Normal control that was orally and daily administered the equivalent amount of the vehicle (distilled water) for the same period and used as a control.

Group 2: Received only a single dose of 15 ml of Siwa date palm extract orally.

Group 3: Injected S/C with 1.0 ml CCL_4 solution /Kg rabbits.

Group 4: Injected S/C with 2.0 ml CCL_4 solution /Kg rabbits.

Group 5: Pretreated with a single dose of 15 ml of Siwa date palm extract orally + 1.0 ml CCL_4 solution /Kg rabbits S/C.

Group 6: Pretreated with a single dose of 15 ml of Siwa date palm extract orally + 2.0 ml CCL_4 solution /Kg rabbits S/C.

Sampling and Analysis: Blood samples were collected from the ear vein of rabbits into a centrifuge tubes without anticoagulant after 6, 12, 24 and 48 hours post-treatment. The serum was separated by centrifugation of blood samples at 4000 r.p.m for 15 minutes and kept frozen at -20 °C till assayed. The clear sera were used for determining of ALT, AST [20] and IgG, IgM and IgA [21]. Two animals from each group were slaughtered after 6, 12, 24 and 48 hours post-treatment and the liver was excised, washed in saline and homogenized for estimation of MDA [22] and GSH [23] as a biomarker of lipid peroxidation and antioxidative stress respectively.

Composition of the experimental diets were shown in the following table :

Ingredient	[g/kg]
Yellow Corn	62.2
Soybean meal, 44%	223.3
Wheat bran	233.3
Barley	150.0
Alfalfa hay	301.2
Ground limestone	10.0
Dicalcium phosphate	12.0
Common salt	5.0
Vit.+Min. Premix†	3.0
Total	1000 gm

†: Each 3 Kg premix contains: Vit. A, 12,000,000 IU; Vit. D_3, 3,000,000 IU; Vit. E, 10,0 mg; Vit. K_3, 3,0 mg; Vit. B_1, 200 mg; Vit. B_2, 5,0 mg; Vit. B_6, 3,0 mg; Vit. B_{12}, 15.0 mg; Biotin, 50.0 mg; Folic acid 1,0 mg; Nicotinic acid 35,0 mg; Pantothenic acid 10,0 mg; Mn 80 g; Cu 8.8 g; Zn 70 g; Fe 35 g; I 1 g; Co 0.15 g and Se 0.3 g.

RESULTS AND DISCUSSION

The present study evaluated the hepatoprotective activities of Siwa date palm extract against CCL_4 induced liver toxicity. It is established that hepatotoxicity induced by CCL_4 depends on the cleavage of the carbon chlorine bond to generate trichloromethyl free radical (CC L_3) that reacts rapidly with oxygen to form a trichloromethyl peroxy radical (CC L_3O_2). This metabolite possibly attack membrane polyunsaturated fatty acids thereby causing lipid peroxidation leading to impairment of membrane function and liver injury [24]. The result of hepatic injury is leakage of cellular enzyme into plasma [25]. When the liver cell plasma membrane is damaged, a variety of enzymes normally located in the cytosol are released into blood stream. Their estimation in the serum is a useful quantitative marker for the extent and type of hepatocellular damage [26]. Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) are the most often used and most specific indicators of hepatic injury and represent markers of hepatocellular necrosis.

CCL_4 caused significant increases in the levels of (AST) aspartate aminotransferase, alanine aminotransferase (ALT) enzymes in (G3,G4) and that is in agreement with those of other authors[19]..On the other hand, our study demonstrated that the treatment with Siwa date palm extract caused marked ameliorations of transaminase enzymes activity ALT and AST in (G5,G6). Our results are in accordance with results of other autherswho showed the effect of extracts from dates (Phoenix dactylifera L.) on carbon tetrachloride-induced hepatotoxicity in rats [18].

The oxidative stress induced by CCL_4 on membrane polyunsaturated fatty acids in rabbit's liver thereby causing lipid peroxidation leading to highly significant rise of lipid peroxidation product (MDA) and a significant decline of endogenous antioxidants GSH. However, Pretreatment with natural antioxidant Siwa date palm extract, was significantly ameliorated the increased levels of MDA and decline of GSH in the liver tissue caused by CCL_4 hepatotoxicity. These findings are in agreement with many authers who reported that the oral administration of date palm extract produced a very marked improvement of the altered hepatic MDA, and GSH content of the cirrhotic rats [27]. These results were also in accordance with the results of many authers who noted that aqueous date extract was found to inhibit significantly the lipid peroxidation by inhibition of TBARS formation [28].

The mechanism by which the date flesh induces its hepatoprotective activity is not certain. However, it is possible that β-sitosterol, a constituent of Phoenix dactylifera, is at least partly responsible for the protective activity against CCl4 hepatotoxicity [29]. An additional and important factor in the hepatoprotective activity of any drug is the ability of its constituents to inhibit the aromatase activity of cytochrome P-450, thereby favoring liver regeneration. On that basis, it is suggested that flavonoids in Phoenix dactylifera could be a factor contributing to its hepatoprotective ability through inhibition of cytochrome P-450 aromatase [30]. In addition, the recorded content of vitamin C in the date flesh (0.179%) may also play a role in hepatoprotection. Liver cytochrome P-450 is significantly reduced in ascorbic acid-deficient guinea pigs [31].

Concerning the effect of the oral administration of Siwa date palm extract on immune functions (IgG, IgM and IgA) (Table 2) it was cleared that Siwa date palm extract elicited significant increase the IgG, IgM and IgA in (G2) while CCL4 significantly decrease it specially IgG in a dose and time dependant (G3,G4) in comparison with control group (G1). On the other hand, pretreatment with Siwa date palm extract in (G5,G6) elevate the level of IgM, IgG, IgA near to the control level. This could be attributed to the potent antioxidant activity of Siwa date palm extract [5]. These results were nearly correlated with that of other authers who stated that there is a lot of information about the role of free radicals in the immune defense mechanism where the involvement of Free radicals leading to weakness of immunity [32]. Also, these findings were coincided with others who concluded that the supplementation with the antioxidant protected immune responses in individuals exposed to certain environmental sources of free radicals [33].

Table 1: The mean values of ALT, AST in G1, G2, G3, G4, G5 and G6.

	Serum							
	ALT (U/L)				AST (U/L)			
	6 H	12 H	24 H	48 H	6 H	12 H	24 H	48 H
Control	55.70±4.52c	55.01±3.12c	56.03±2.45d	55.89±4.05d	41.00±1.35c	41.30±0.75d	41.09±1.70d	41.70±0.87d
Group2	56.80±3.55c	57.90±3.90c	56.40±2.69d	54.50±2.49d	38.30±1.13c	41.30±1.27d	39.40±1.40d	37.50±0.43e
Group3	81.56±6.44b	80.50±4.02b	82.34±4.35b	87.35±3.11b	49.02±2.61b	55.10±2.03b	57.93±1.35b	60.11±1.71b
Group4	93.98±4.31a	94.90±2.78a	96.02±3.47a	98.30±3.78a	58.09±0.95a	61.20±1.80a	68.98±1.49a	79.12±2.50a
Group5	58.10±3.12c	58.30±2.37c	59.70±2.14d	60.02±2.60d	42.80±0.85c	43.80±0.69c	44.20±0.73d	46.71±0.90c
Group6	73.60±5.01b	72.80±3.49b	71.70±2.19c	68.80±3.20c	52.80±0.35b	46.50±0.75c	48.30±0.42c	44.10±0.36cd

Means within the same column carrying different letters are significantly different (P<0.05)

Table 2: The mean values of MDA and GSH in G1, G2, G3, G4, G5 and G6.

	Liver homogenate							
	MDA (ug/mg protein)				GSH (umol/mg protein)			
	6 H	12 H	24 H	48 H	6 H	12 H	24 H	48 H
Control	1.31±0.02d	1.30±0.05d	1.29±0.02d	1.38±0.01d	70.90±2.11b	71.34±3.19b	70.84±4.50b	69.98±2.30b
Group2	0.87±0.03f	0.85±0.02f	0.88±0.02f	0.86±0.03f	98.52±4.51a	99.13±3.78a	101.68±3.78a	101.49±4.44a
Group3	1.47±0.02c	1.49±0.01c	1.48±0.05c	1.50±0.02c	65.89±3.23bc	66.85±2.65b	64.24±2.11c	63.33±3.45cd
Group4	2.48±0.01a	2.47±0.03a	2.46±0.02a	2.49±0.05a	60.98±3.77c	59.51±2.11c	60.05±2.65c	59.03±1.25d
Group5	1.15±0.03e	1.18±0.04e	1.12±0.03e	1.16±0.02e	67.76±2.51bc	67.64±3.40b	68.48±1.37b	69.41±1.30b
Group6	1.75±0.03b	1.74±0.02b	1.68±0.04b	1.69±0.03b	64.00±2.58bc	65.88±3.12bc	64.49±1.92c	65.15±1.40c

Means within the same column carrying different letters are significantly different (P<0.05)

Table 3: The mean values of IgG in G1, G2, G3, G4, G5 and G6.

	IgG (mg/dl)			
	6 H	12 H	24 H	48 H
control	131.00±6.20b	133.60±3.20a	139.70±4.15b	137.10±5.01a
Group 2	148.60±4.14a	139.80±2.23a	151.11±5.74a	147.60±6.32a
Group 3	93.17±2.55e	102.11±3.78c	73.16±3.36d	98.19±3.25b
Group 4	81.00±5.76f	93.70±4.46d	77.10±4.45d	89.11±4.15b
Group 5	112.70±4.61d	99.81±2.74cd	118.00±3.12c	100.00±5.05b
Group 6	103.15±4.13cd	111.70±3.33b	119.75±2.87c	98.18±4.85b

Means within the same column carrying different letters are significantly different (P<0.05)

Table 4: The mean values of IgM in G1, G2, G3, G4, G5 and G6.

	IgM (mg/dl)			
	6 H	12 H	24 H	48 H
control	15.00±0.78a	15.81±0.13b	15.80±0.23b	14.89±0.21c
Group 2	18.00±1.51a	17.11±0.48a	18.7±0.15a	21.60±0.30a
Group 3	11.01±1.45b	10.30±0.15c	13.2±0.29c	11.20±0.17d
Group 4	10.00±1.33b	9.81±0.11c	11.10±0.20d	9.18±0.14e
Group 5	13.11±0.40b	18.1±0.16a	18.9±0.23a	15.80±0.11c
Group 6	10.00±0.98b	17.20±0.20a	12.88±0.24c	17.91±0.25b

Means within the same column carrying different letters are significantly different (P<0.05)

Table 5: The mean values of IgA in G1, G2, G3, G4, G5 and G6.

	IgA (mg/dl)			
	6 H	12 H	24 H	48 H
control	73.80±7.01b	77.16±4.17b	73.00±3.81b	75.11±4.25bc
Group 2	89.15±5.32a	103.12±6.17a	93.71±4.07a	98.11±2.50a
Group 3	70.18±6.31b	73.11±6.25b	68.60±3.21bc	61.31±4.01d
Group 4	70.00±4.12b	63.80±5.01b	61.60±3.37bc	53.80±3.25d
Group 5	67.81±4.71b	67.10±4.20b	68.82±4.17bc	78.00±2.74b
Group 6	68.11±5.17b	68.80±5.80b	67.11±3.23c	68.16±3.79cd

Means within the same column carrying different letters are significantly different (P<0.05).

Many of the protective functions of immune cells depend on the fluidity of the membranes of the cell. As the concentration of polyunsaturated fatty acids in the membranes is increased, the potential for membrane lipid peroxidation mediated by free radicals also is increased. Lipid peroxidation decreases membrane fluidity, which adversely affects immune responses. Mice fed oxidized lipids show marked atrophy of the thymus and T-cell dysfunction. Loss of membrane fluidity has been related directly to the decreased ability of lymphocytes to respond to challenges to the immune system [34].

Although our bodies can manufacture antioxidant enzymes, we also need a good intake of dietary antioxidants to boost our immunity and protect us from the harmful effects of free radicals.

It could be concluded from this study that CCL4 induced liver damage in rabbits can be ameliorated by administration of extract of date flesh.

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