

Studying Some of Biological Effects of Grape Seeds on Carbon Tetrachloride (CCl₄)-Induced Liver Damage in Rats

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Abstract: Grape seeds represent a low percentage of the fruit weight, although the antioxidant phenolic compounds present in the seeds account for 60% to 70% of the total polyphenols in the grape. These seeds are used as functional food due to its powerful antioxidant properties. The present study was planned to investigate the efficacy of dried grape seeds on hepatoprotective activities against carbon tetrachloride (CCl₄)-induced liver damage in laboratory rats. The experiment conducted for a period of 4 weeks on fifty male Sprague-Dawley rats weighing 200 ± 5 g, divided into five groups (10 rats of each group). Group 1, was injected subcutaneously with saline solution and kept as negative control group. Groups 2, 3, 4 and 5 were injected subcutaneously with CCl₄ (2 ml/kg). Group 2 was kept as positive control group (untreated rats). Other groups (3, 4 and 5) fed on supplemented diet with three different levels of grape seeds (10, 20 and 30%, respectively). The results revealed that CCl₄-induced a significant decrease in serum levels of total protein, albumin, GSH and total antioxidant; and serum activity of GPx, SOD and CAT enzymes. On the other hand, an increased serum activities of GGT, AST, ALT and ALP; levels of total bilirubin, blood urea nitrogen, uric acid, creatinine and MDA seen. These obtained serum results were confirmed by histological determination, which revealed multiple focal areas of necrotic and calcified hepatocytes encircled with fibrous connective tissue proliferation. Injected rats with CCl₄ combined with three different levels of grape seeds caused a significant improvement in all the biochemical parameters as well as liver cellular structure. The most improvements of all biochemical parameters and liver structure tended towards normal results, in treated rats with higher level of dried grape seeds. In conclusion, the present findings suggest that regular intake of grape seeds may be useful in improving liver and kidney functions and may protect against CCl₄ toxicity, exhibiting stronger antioxidant activity.

Key words: Grape Seeds • Liver Functions • Kidneys Functions • Antioxidant Enzymes

INTRODUCTION

Liver is the largest internal organ in human body. It processes and stores many of the nutrients absorbed from the intestine that are necessary for body functions. Some of these major functions include protein, carbohydrate and fat metabolism. It also

secretes bile into the intestine to help to absorb nutrients [1]. The liver is the first organ to encounter ingested nutrients, drugs and environmental toxicants that enter the hepatic portal vein from the digestive system. Liver function can detrimentally altered by injury resulting from acute or chronic exposure to toxicants [2].

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Grape seeds are a byproduct of the juice and wine-making industries and can be used for functional foods development and improve beneficial health properties [3]. Grape seeds have powerful antioxidant properties due to its rich content of polyphenol compounds. The polyphenol compounds in grape seeds are as much as that in peels and grape fruit [4]. The most abundant of polyphenol compounds founded in grape seeds are flavonoids, monomeric, flavan-3ols, catechin and epicatechin, which are interesting for pharmaceutical and food factories for its medical treatments and health beneficial [5]. Also, grape seeds were found to be rich in its content of gallic acid, catechin, epicatechin and epicatechin-3-O-gallate and a wide variety of procyanidins oligomers [6]. In addition, tocopherols, tocotrienols, carotenoids, flavonoids, phenolic acids and phytosterols are chemical components presented in grape seeds oil and have a biological importance due to its antioxidant activity [7]. The most beneficial health effects of grape seed include protection against atherosclerosis [8], modulate antioxidant enzymes [9], protect against oxidative damage in brain cells of rats [10] and anti-inflammatory effects [11]. Therefore, the present study was designed to evaluate the effect of different levels (10, 20 and 30%) of dried grape seeds. This was determined by measuring liver function and activity of antioxidant enzymes as well as study histopathological changes of rats, intoxicated with CCl₄.

MATERIALS AND METHODS

Materials

Grape Seeds: Grape seeds were purchased from a local market of Spices, Grains and Oils, Holy Makkah, KSA.

Rats and Diet: Fifty male Sprague-Dawley rats weighing 200 ± 5 g were obtained from the Laboratory Animal Colony, Medicine College, Umm Al Qura University, KSA. Basal diet constituents were purchased from Baghfar Company for Pharmaceutical and Chemical, Jedda, KSA. Basal diet consists of casein 20%, soybean oil 5%, sucrose 10%, choline chloride 0.20%, mineral mixture 4.0%, vitamin mixture 1.0%, fibers 5%, L-Cystine 0.18% and the remainder was corn starch.

Chemicals and Kits: Carbon tetrachloride (CCl₄) was purchased from El-Gomhorya Company for Chemical and Pharmaceutical, Cairo, Egypt. Formalin, diethyl ether and kits for biochemical analysis of serum gamma-glutamyl transferase (GGT), aspartate transaminase (AST), alanine

aminotransferase (ALT), alkaline phosphates (ALP), total protein (TP), albumin (Alb), total bilirubin (TBr), urea nitrogen (UN), uric acid (UA), creatinine (Cr), malondialdehyde (MDA), reduced glutathione (GSH), glutathione peroxidase (GPx), superoxide dismutase (SOD), catalase (CAT) and total antioxidant were purchased from Baghfar Company for Pharmaceuticals and Chemicals, Jedda, KSA.

Methods

Preparation of Grape Seeds: Dried grape seeds were cleaned from foreign materials and washed with tap water to remove possible potential dust. Afterwards, it was dried by cotton cloth to remove the excess liquid prior to drying. Then dried grape seeds were ground using grinder mill and sieve was used to obtain a powder particle size of less than 0.2mm.

Preparation of Basal Diet: The basal diet (AIN-93M) was prepared according to Reeves *et al.*, [12]. Diet was formulated to meet recommended nutrients levels for maintenance health rats.

Induction of Hepatotoxicity: Hepatotoxicity in rats was done according to the described method by Sundaresan and Subramanian [13]. In briefly, animals of group 2, 3, 4 and 5 were given a single subcutaneous injection of CCl₄ (2 ml/kg).

Experimental Design and Grouping of Rats: A total of 50 male Sprague-Dawley rats weighing 200 ± 5 g were used in the experiment. All animals were allowed free access to tap water, fed standard basal diet and kept under normal healthy laboratory conditions for one week for acclimatization. After one week (acclimatization period), rats were randomly divided into five groups of 10 rats each. The first group was fed only on the basal diet and served as a negative control group (normal rats). The second group was fed only on the basal diet and kept as a positive control group (untreated rats). The third, fourth and fifth groups fed on supplemented diets with 10, 20 and 30% dried grape seeds, respectively.

At the end of the experimental period (4 weeks), animals were fasted for 12-hr., except for water and then rats were sacrificed. Blood samples were collected from the posterior vena cava into dry clean centrifuge tubes and left at room temperature to clot and then centrifuged for 10 minutes at 3000 rpm for serum separation. Serum samples were carefully aspirated using a needle and transferred into dry clean test tubes and kept frozen at

-20°C for biochemical analysis. Liver of all rats were removed and washed with saline solution, air dried and then immersed in neutral buffered formalin 10% for histopathology examination.

Biochemical Analysis

Liver Function Assay: Serum activity of GGT was determined according to described method by kits (Diamond Co, Hannover, Germany) as described by Young, [14]. Serum AST, ALT activities were assayed colorimetric as described by Bergmeyer *et al.* [15] using (Diamond Co, Hannover, Germany) kits. Serum level of ALP was determined by the method of kits (Diamond Co, Hannover, Germany) as described by Roy, [16]. Serum TP, Alb and TBr levels were determined colorimetric as described by Young [14], Young [17] and Tietz [18], respectively. The biochemical indices were determined in rat serum using a UV/Vis spectrophotometer (Humastar 200, automatic biochemistry analyser, Wiesbaden Germany).

Kidney Function Assay: Serum urea nitrogen (UN) was determined using BioMerieux kits according to Patton and Crouch [19]. Serum uric acid (UA) levels were determined by enzymatic colorimetric method as described by Fossati *et al.* [20]. Serum creatinin level was determined using colorimetric kinetic as described by the Jaffe reaction [21].

Malondialdehyde: Malondialdehyde was assayed quantitatively in serum using the MDA assay kit (by a spectrophotometric method, ABCAM, UK) according to manufacturer instructions as described by Draper and Hadley [22]. The MDA in the sample reacts with thiobarbituric acid (TBA) to generate a MDA-TBA adduct. The MDA-TBA adduct is quantified colorimetrically (OD = 532 nm). This assay detects MDA levels as low as 1 nmol/well colorimetrically. The results were expressed in nmol/ml.

Reduced Glutathione Assay: Serum reduced glutathione (GSH) levels were determined as described by Beutler *et al.* [23], using Autoanalyzer (Roche-Hitachi, Japan).

Glutathione Peroxidase Assay: Activity glutathione peroxidase enzyme was assayed quantitatively in serum using the GPX assay kit (by a spectrophotometric method, BioAssay Systems, USA) according to the method of Hissin and Hiff [24].

Superoxide Dismutase Assay: Serum activity of superoxide dismutase was assayed quantitatively in serum using the SOD assay kit (by a spectrophotometric method BioAssay Systems, USA) as described by Kakkor *et al.*, [25]. In the assay, superoxide (O_2^-) is provided by xanthine oxidase (XO) catalyzed reaction. O_2^- reacts with a WST-1 dye to form a colored product. SOD scavenges the O_2^- thus less O_2^- is available for the chromogenic reaction. The color intensity (OD = 440nm) is used to determine the SOD activity in a sample. The results were expressed in U SOD/ml.

Catalase Assay: Serum activity of catalase was assayed quantitatively using the catalase assay kit (by a spectrophotometric method, Bio-Assay Systems, USA) according to method of Sinha [26], measuring catalase degradation of H_2O_2 was using a redox dye. The change in color intensity at 570nm is directly proportional to the catalase activity in the sample. The procedure involves adding a Substrate to sample, incubation for 30 min, followed by a Detection Reagent and reading the optical density. The results were expressed in U Catalase/Liter. *Unit definition:* one unit is the amount of catalase that decomposes 1 μ mole of H_2O_2 per min at pH 7.0 and room temperature.

Total Antioxidant Assay: Total Antioxidant Capacity was assayed quantitatively in serum using the catalase assay kit (by a spectrophotometric method, BioAssay Systems, USA) as described by Woodford and Whitehead [27]. In the assay, Cu_2^+ is reduced by antioxidant to Cu^+ . The resulting Cu^+ specifically forms a colored complex with dye reagent. The color intensity at 570nm is proportional to TAC in the sample. The results were expressed in μ M Trolox Equivalents.

Histopatological Examination: Liver of each animal was carefully washed in an isotonic solution. The fixed specimens were then trimmed, washed and dehydrated in ascending grades of alcohol. Specimens were then cleared in xylol, embedded in paraffin, sectioned at 4-6 microns thickness, stained with Heamtoxylin and Eosin stain for histopathological examination as described by Bancroft and Gamble [28].

Statistical Analysis: The results were expressed as means \pm standard deviations in each group. Differences between groups were assessed using computerized statistical package of social sciences (SPSS) program (SPSS, 20 software version) with one-way analysis of variance

(ANOVA) followed by Duncan's multiple range tests. $p < 0.05$ values were considered to be statistically significant.

RESULTS

Results in Table 1 represents the serum GGT, AST, ALT and ALP activities of normal rats, CCl₄-intoxicated rats and those treated with grape seeds. It showed that injected CCl₄ significantly elevated the serum activity of GGT, AST, ALT and ALP, compared with that of normal rats. However, all different levels of grape seeds in CCl₄-intoxicated rats caused significant improvement of serum GGT, AST, ALT and ALP activities, compared with that of untreated ones (positive rats).

Data in Table 2 showed significant reduction in serum TP and Alb and elevation in TBr levels, compared with that of normal rats. Treated rats with CCl₄ with different levels of dried grape seeds induced significant elevation in serum TP and Alb and reduction in TBr levels, compared with that of untreated CCl₄-intoxicated rats.

Recorded data in Table 3 revealed that there is significant increase of serum UA, BUN and Cr levels in CCl₄-intoxicated rats (positive rats), compared with that of normal rats. The significant reduction in serum UA, BUN and Cr levels was observed in CCl₄-treated rats with the different levels of dried grape seeds, compared with that of untreated rats.

Tables 4 shows serum malondialdehyd (MDA) and reduced glutathione (GSH) concentrations in normal inject

rats with CCl₄ and treated CCl₄ - intoxicated rats with grape seeds. In comparison to the normal control rats, CCl₄ caused a significant increase of serum MDA and decrease GSH levels. Administration of different levels of grape seeds caused significant reversal of serum MDA and GSH levels toward the normal levels.

The present results in Table 5, shows significant decrease in serum activity of glutathione peroxidase (GPx) superoxide dismutase (SOD) and catalase (CAT) enzymes and total antioxidant level in CCl₄ - intoxicated rats, compared with that of normal rats. In comparison to the untreated intoxicated rats with CCl₄; the administration of different level of grape seeds to CCl₄- intoxicated rats caused a significant increase of serum GPx, SOD and CAT activities and total antioxidant level.

Histopathological Examination: Photomicrograph of liver sections from normal control rats showed normal structure with healthy hepatic histological findings (Figure 1). Whereas, in CCl₄-intoxicated rats (positive control rats), examination of sections of the liver revealed multiple focal areas of necrosis and calcified hepatocytes encircled with fibrous connective tissue proliferation (Figure 2).

The photomicrograph of liver sections of injected rats with CCl₄ combined with 10% grape seeds appeared focal area of mononuclear cells infiltration (Figure 3). In addition, liver sections of CCl₄-intoxicated rats and treated with 20% grape seeds revealed congested central vein (Figure 4). Furthermore, animals that received CCl₄ together with 30% grape seeds regained a healthy hepatic histological structure (Figure 5).

Table 1: Effect of Grape seed on serum activities of liver enzymes in intoxicated rats with CCl₄

Groups	Parameters as Mean ± SD			
	GGT (U/L)	AST (U/L)	ALT (U/L)	ALP (U/L)
G 1: Normal control rats	19.32±0.49 ^d	44.35±0.48 ^d	64.28±0.48 ^d	45.05±0.48 ^d
G 2: Positive control rats (+ CCl ₄)	81.48±0.62 ^a	112.32±0.79 ^a	123.91±0.98 ^a	79.47±1.27 ^a
G 3: 10% GS + CCl ₄	61.03±0.18 ^b	86.01±0.53 ^b	86.62±0.53 ^b	69.25±1.09 ^b
G 4: 20% GS + CCl ₄	30.23±0.37 ^c	70.17±0.86 ^c	73.93±0.86 ^c	60.12±0.72 ^c
G 5: 30% GS + CCl ₄	19.15±0.52 ^d	44.87±0.63 ^d	61.76±1.03 ^d	45.76±1.22 ^d

Means with different letters in each column are significantly different at $p < 0.05$.

GS: Grape seeds, GGT: gamma-glutamyl transferase, AST: aspartate transaminase, ALT: alanine aminotransferase, ALP: alkaline phosphates

Table 2: Effect of Grape seed on status of liver and kidney function in intoxicated rats with CCl₄

Groups	Parameters as Mean ± SD		
	TP (g/L)	Alb (g/L)	TBr (mg/dl)
G 1: Normal control rats	67.51±1.58 ^a	42.18±0.71 ^a	0.34±0.70 ^d
G 2: Positive control rats (+ CCl ₄)	52.19±3.08 ^d	22.17±0.81 ^e	2.23±0.17 ^a
G 3: 10% GS + CCl ₄	56.41±2.41 ^c	22.24±1.30 ^d	1.56±0.17 ^b
G 4: 20% GS + CCl ₄	60.60±1.97 ^b	35.15±1.49 ^c	0.80±0.09 ^c
G 5: 30% GS + CCl ₄	67.19±0.63 ^a	42.74±0.85 ^b	0.40±0.08 ^d

Means with different letters in each column are significantly different at $p < 0.05$. GS: Grape seeds.

TP: total protein, Alb: albumin, TBr: total bilirubin

Table 3: Effect of Grape seed on renal function in intoxicated rats with CCl₄

Groups	Parameters as Mean ± SD		
	UA (mg/dl)	BUN (mg/dl)	Cr (mg/dl)
G 1: Normal control rats	3.73 ± 0.10 ^e	32.80 ± 0.91 ^e	0.54±0.17 ^d
G 2: Positive control rats (+ CCl ₄)	6.93 ± 0.15 ^a	61.84 ± 0.61 ^a	2.77±0.25 ^a
G 3: 10% GS + CCl ₄	5.79 ± 0.17 ^b	52.91 ± 0.40 ^b	1.75±0.22 ^b
G 4: 20% GS + CCl ₄	5.11 ± 0.15 ^b	45.11 ± 0.48 ^c	0.99±0.28 ^c
G 5: 30% GS + CCl ₄	4.51 ± 0.20 ^d	40.09 ± 0.81 ^d	0.45±0.77 ^d

Means with different letters in each column are significantly different at p< 0.05. GS: Grape seeds.

BUN: blood urea nitrogen, UA: uric acid, Cr: creatinine

Table 4: Effect of Grape seed on markers of oxidative stress in intoxicated rats with CCl₄

Groups	Parameters as Mean ± SD	
	MDA (nmol/l)	GSH (nmol/l)
G 1: Normal control rats	36.46±0.99 ^d	173.55±1.86 ^a
G 2: Positive control rats (+ CCl ₄)	80.95±0.58 ^a	53.84±0.79 ^e
G 3: 10% GS + CCl ₄	61.95±0.24 ^b	86.93±0.60 ^d
G 4: 20% GS + CCl ₄	51.44±0.53 ^c	134.31±0.51 ^c
G 5: 30% GS + CCl ₄	34.46±0.72 ^c	166.90±0.97 ^b

Means with different letters in each column are significantly different at p< 0.05. GS: Grape seeds.

MDA: malondialdehyde, GSH: reduced glutathione

Table 5: Effect of Grape seed on antioxidant enzymes and total antioxidant in CCl₄-intoxicated rats

Groups	Parameters as Mean ± SD			
	GPx (mmol/dL)	SOD (U/ml)	CAT (mmol/dl)	Total antioxidant (mmol trolox/liter)
G 1: Normal control rats	20.61±0.57 ^a	4.54±0.08 ^a	65.39±0.73 ^a	3.28±0.06 ^a
G 2: Positive control rats (+ CCl ₄)	9.93±0.23 ^d	2.90±0.05 ^e	43.38±0.53 ^d	2.67±0.03 ^d
G 3: 10% GS + CCl ₄	12.64±0.29 ^c	3.07±0.17 ^d	48.36±0.66 ^c	2.87±0.08 ^c
G 4: 20% GS + CCl ₄	16.99±0.37 ^b	3.70±0.07 ^c	55.62±0.77 ^b	3.25±0.06 ^b
G 5: 30% GS + CCl ₄	20.61±0.32 ^a	4.35±0.03 ^b	64.91±0.63 ^a	3.79±0.04 ^a

Means with different letters in each column are significantly different at p< 0.05. GS: Grape seeds.

GPx: glutathione peroxidase, SOD: superoxide dismutase, CAT: catalase

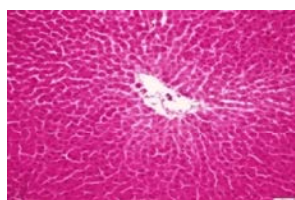


Fig. 1: Photomicrograph of liver sections from the normal control rats showing apparently healthy hepatic histological findings (H&E X 400)

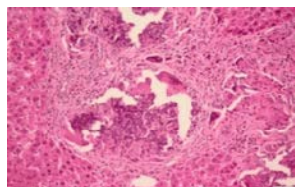


Fig. 2: Photomicrograph of liver sections from positive rats showing multiple focal areas of necrotic and calcified hepatocytes encircled with fibrous connective tissue proliferation (H&E X 400)

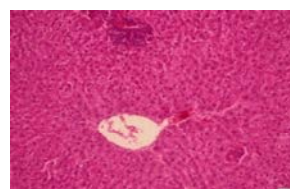


Fig. 3: Photomicrograph of liver sections from treated CCl₄-intoxicated rats with 10% grape seeds showing focal area of mononuclear cells infiltration (H&E X 200)

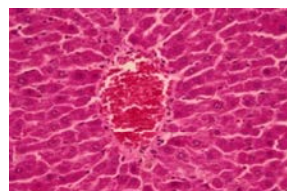


Fig. 4: Photomicrograph of liver sections from treated CCl₄-intoxicated with 20% grape seeds showing congested central vein (H&E X 400)

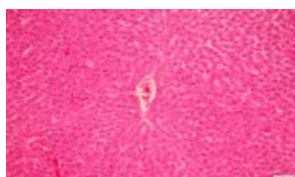


Fig. 5: Photomicrograph of liver sections from treated CCl_4 -intoxicated with 30% grape seeds showing apparently healthy hepatic histological structure (H&E X 400).

DISCUSSION

Liver is one of the vital organs in the body and it is responsible for detoxification of toxic chemicals and drugs. Thus it is the target organ for all toxic chemicals. Carbon tetrachloride (CCl_4) is one of the most commonly used hepatotoxins in the experimental study of liver disease [29]. It is widely used to induce liver damage because it is metabolized in hepatocytes by cytochrome P450, generating a highly reactive carbon-centered (trichloromethyl) radical, leading to lipid peroxidation and thereby causing liver fibrosis [30]. The findings of the present study revealed ability of CCl_4 to induce general disturbances in the normal physiological function of the liver. It showed significant decrease of serum total protein and albumin levels and increase in GGT, AST, ALT and ALP activities and total bilirubin level. These results were confirmed with histopathological study, which revealed multiple focal areas of necrotic and calcified hepatocytes encircled with fibrous connective tissue proliferation in injected rats with CCl_4 , compared with that of normal control rats. These results were in accordance with Naik and Panda [31] and Mahmood and Reza [32], who mentioned that increased serum levels of AST, ALT and ALP in CCl_4 -treated animals is an indicator of liver damage. These enzymes leak out from liver into the blood at the instance of tissue damage, which is always associated with hepatonecrosis. Also, Mahmood and Reza [32] showed that CCl_4 caused significant increase in serum activities of AST, ALT, ALP enzymes and, levels of total protein, total and direct bilirubin in male rats. In addition, Stephen *et al.*, [33] indicated that CCl_4 treatment significantly decrease ($p < 0.05$) the serum albumin levels compared with the normal control group of rats. In this study, histopathological investigation was used to confirm the effect of the treatment with grape seed, on liver tissues changes. Several changes observed in liver tissues as indicator of liver damage induced by CCl_4 injection in positive control rats. These finding agreed with previously report that CCl_4 causes liver

cirrhosis [34], fibrosis and mononuclear cell infiltration [35], necrosis and apoptosis [36], steatosis, degeneration of hepatocytes and increase in mitotic activity [37].

Kidneys are particularly susceptible to the effect of toxic agents that can cause renal damage and even renal failure. Biochemical tests such as serum creatinine, urea and uric acid helps in the diagnosis of kidney diseases. The present study revealed significant increase in serum urea nitrogen, uric acid and creatinine levels in CCl_4 -intoxicated rats (positive control group), compared with normal rats. The elevation in serum urea, uric acid and creatinine, indicate impairment in renal functions, which may result from intrinsic renal lesions, decreased perfusion of the kidney, obstruction of lower urinary tract or due to deranged metabolic process caused by CCl_4 . The effect of CCl_4 on renal function could be attributed to alterations in the antioxidant defensive system, resulting in kidney injury. CCl_4 also causes disorders in kidneys by generating free radicals [38]. Findings by Ogeturk *et al.* [39] suggested that exposure to CCl_4 causes acute and chronic renal injuries. Also, Stephen *et al.* [33] reported that administration of CCl_4 significantly increased ($p < 0.05$) serum creatinine and BUN levels as compared with the control group of rats.

The result of the present study clearly showed significant increase in serum MDA level and decrease in serum GSH and total antioxidant levels and activities of GPx, SOD and CAT enzymes in rats treated with CCl_4 , compared to normal rats. The observed increase of lipid peroxidation (MDA), the decrease in circulating antioxidant enzymes and decrease of serum total antioxidants level confirm that the CCl_4 induced depletion of antioxidants system. These results also found by Ruidong *et al.* [40], who demonstrated that MDA levels in the CCl_4 -treated group as indicators of lipid peroxidation were significantly higher than that in the normal control group. In addition, CCl_4 treatment resulted in the depletion of antioxidant enzymes as indicated by the depletion in the activities of SOD, CAT and GSH-Px in hepatotoxic rats.

Regarding the physiological and histopathological effect of grape seeds on CCl_4 -intoxicated rats, the most important result drawn from the current study is the powerful ability of grape seeds in inducing significant amelioration in liver and kidneys functions and antioxidant defense. As well as, the improvement in histopathological structure of liver, compared with untreated CCl_4 -intoxicated rats (positive group). These amendments were more detectable in treated rats with higher level (30%) of grape seeds. These results were in

accordance with several results reported that grape seed extract exhibit multi-organ protective properties against drug and chemical-induced toxicity and long-term safety [41] and may be favorable as a therapeutic option in RTx-induced oxidative stress in the rat liver [42]. Grape seed extract is a useful herbal remedy, especially for controlling oxidative damages and is considered as a potent protective agent against hepatotoxicity [43]. Several other lines of evidence revealed that, grape seed proanthocyanidins exhibited *in vivo* hepatoprotective and anti-fibrogenic effects against liver injury and acts as free radicals scavengers for protective liver damage [44] and significantly reduces serum AST, ALT and ALP activities and bilirubin level and increased serum total protein and albumin levels in dimethylnitrosamine-induced hepatic cirrhosis rats [45]. Grape seeds produce significant hepatoprotective effects by decreasing serum ALT, AST and ALP activities, serum bilirubin and MDA levels and increase albumin level and liver SOD, CAT and GPx activities with amelioration of structural changes induced in liver of diabetic rats [46]. Oral treatment with grape seeds extract significantly ameliorates indices of hepatotoxicity, nephrotoxicity and lipid peroxidation induced by benzene [47]. The beneficial effect of grape seeds on renal function in the present results were in accordance with that obtained by Rodrigo and Bosco [48], who demonstrated that grape seed extract have a beneficial effect on renal function as a result of its physiological effect, at least partially. They presented its antioxidant and anti-inflammatory properties after a six-month-period of treatment through neutralization of plasma lipoperoxidation and increased plasma CAT and SOD activities. Other researchers found that 50 mg of grape seeds extracts each day for two weeks may reduce kidney function disturbances following tissue damage caused by ischemia Ashtiyani *et al.* [49]. Grape seed extract improve glomerular filtration rate and proteinuria, increase plasma catalase and superoxide dismutase, lower lipoperoxidation and carbonylation and ameliorated inflammation by decreasing glomerular filtration rate [50]. The usefulness of grape seed is related to its content of polyphenols in the improvement of renal dysfunction [51].

The present results showed that grape seeds have the ability to improve antioxidant defense through increasing the activity of antioxidant enzyme and total antioxidant and decreased lipid peroxidation as indicated by serum level of MDA. These results confirm the reason for the improvement in liver and kidneys functions and protect from damage caused by lead toxicity as well as the improvement in the level of sex hormones. The action

mechanism of grape seeds may be related to its antioxidant and anti-inflammatory properties [50] and its powerful free radical scavenger [52]. These results are in agreement with Sehirli *et al.* [53], who reported that grape seeds extract could reduce organ injury through its ability to balance the oxidant-antioxidant status and to regulate the release of inflammatory mediators. Grape seeds are rich source of proanthocyanidins, which are mainly composed of dimers, trimers and oligomers of monomeric catechins [54]. Proanthocyanidins are potent natural antioxidants of various polyphenolic components [3]. These compounds possess a broad spectrum of antioxidative properties with greater potency than vitamin E and C, that protects the organs against free radicals and oxidative stress, both *in vitro* and *in vivo* [55]. Many data have shown that the ability of grape seed proanthocyanidins to improve antioxidant defenses for protecting the main organ function, such as preventing liver injury in the carbon tetrachloride-induced and ischemia/reperfusion -induced [56], alleviating arsenic-induced oxidative reproductive toxicity [57] and protecting the renal function from Cisplatin-induced nephrotoxicity [58]. In addition, grape seed proanthocyanidin significantly increase antioxidant enzymes activity such as SOD, GPx and CAT and ameliorate biochemical abnormalities and antioxidant system status in streptozotocin-induced diabetic rats [59]. Thus, grape seeds could prevent toxicity induced by CCl₄ via scavenge free ROS and/or by induction of cellular antioxidant enzymes. This means that grape seeds could protect against the harmful effect of ROS through its antioxidant activity and by this mean it may be beneficial in protecting the organism against oxidative stress. This is confirmed by findings of Ahmed *et al.* [60] who showed that grape seed extract has antioxidant, anti-inflammatory and antitumor activities and to mediate resistance to free radicals. Maier *et al.* [30] stated that oil produced from grape seeds is considered a rich source of polyphenolics with strong antioxidant activity.

CONCLUSION

In conclusion, the present study confirmed that rats treated with grape seeds exhibited improvement in liver and kidneys functions against injury induced by CCl₄. Grape seeds have the ability in reducing oxidative stress and augmenting antioxidants in CCl₄-intoxicated rats. Hence, regular intake of grape seeds or using it for enriching food product may help functional foods to improve health status.

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