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Original Article

Protective effect of a natural herb (Rosmarinus officinalis) against hepatotoxicity in male albino rats

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Abstract

The present study was conducted to study the chemoprotective effect of Egyptian rosemary extract (RE) against CCl_4-induced hepatotoxicity. Eight experimental groups were used as follow: (1) control; (2&3) RE alone (440 mg/kgb.w) for 4 and 8 weeks, respectively; (4) CCl_4 (1ml/kg b.w, twice a week, i.p) for 4 weeks; (5&7) RE (220&440 mg/kg) for 4 weeks before CCl_4 for a similar period; and (6&8) RE (220&440 mg/kg) for four weeks before CCl_4 in combination with RE another four weeks. The sequential CCl_4 treatment induced significant changes in serum biochemical parameters accompanied by sever histological and histochemical changes of the liver tissue, while administration of 440 mg/kg/day for either 4 or 8 weeks did not induce any changes. Administration of RE before or during the treatment with CCl_4 improved all biochemical parameters and histological picture of the liver, in a dose and duration-dependant manner. It could be concluded that RE has a protective effects against hepatotoxicity.

Key Words: Rosemary extract, hepatotoxicity, carbon tetrachloride, liver function

Efeito protetor de erva natural *(Rosmarinus officinalis)* contra hepatotoxicidade em ratos albinos machos

Resumo

O presente estudo foi realizado para verificar o efeito quimioprotetor do extrato de alecrim egípcio (RE) contra toxidade hepática induzida por CCl4-. Oito grupos experimentais foram utilizados, como segue: (1) controle; (2&3) apenas RE (440 mg/kgb.w) por 4 e 8 semanas, respectivamente; (4) CCl4 (1ml/kg b.w, duas vezes por semana, i.p) durante 4 semanas; (5&7) RE (220&440 mg/kg) por quatro semanas antes de CCl4 por um período similar; e(6&8) RE (220&440 mg/kg) por quatro semanas antes de CCl4 combinado com RE por outras quatro semanas. O tratamento seqüencial de CCl4 induziu mudanças significativas nos parâmetros bioquímicos do soro, acompanhadas por severas mudanças histológicas e histoquímicas no tecido do fígado, enquanto que a administração de 440 mg/kg/dia durante 4 ou 8 semanas não induziu nenhuma mudança. A administração de RE antes ou durante o tratamento com CCl4 aumentou os parâmetros bioquímicos e histológicos do fígado, dependendo da dose e de sua duração. Pode-se concluir que RE apresenta efeito de proteção contra a toxidade hepática.

Palavras-chave: extrato de alecrim, toxidade hepática, tetraclorido de carbono, função hepática

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Introduction

Liver diseases are common and represent the major cause of human mortality in the world. They are characterized by a progressive evolution from steatosis to chronic hepatitis, fibrosis, cirrhosis, and hepatocellular carcinoma (Notas et al., 2009). Chronic injury leading to fibrosis in liver occurs in response to a variety of insults, including viral hepatitis, alcohol abuse, drugs, metabolic diseases due to over load of iron or copper, autoimmune attack of hepatocytes or bile duct epithelium, or congenital abnormalities (Michel et al., 2009).

Oxidative stress is defined in general as excess formation and/or insufficient removal of highly reactive molecules such as reactive oxygen species (ROS) and reactive nitrogen species (RNS) (Valko et al., 2007).

Carbon tetrachloride (CCl_4) is a classical hepatotoxicant that causes rapid liver damage progressing from steatosis to centrilobular necrosis. Long-term administration of CCl_4 causes chronic liver injury, and is a widely accepted model to produce hepatic fibrosis (Ha et al., 2005). In animal models, CCl_4 is administered by different routes and at various vehicle-to-CCl_4 ratios (Janakat & Al-Merie, 2002). Liver injuries induced by CCl_4 are the best-characterized system of the xenobiotic-induced hepatotoxicity which is the commonly used model for screening the anti-hepatotoxic or hepatoprotective activity of drugs (Lee et al., 2008).

An interest has increases in naturally-occurring antioxidants that can be used to protect human beings from oxidative stress damage (Mata et al., 2007). Plants used in traditional medicine for the treatment of liver disorders are of great interest, as they may serve as potential sources for new therapeutic agents that could be applied in the management and prevention of hepatic injury. Those rich in different photochemical derivatives such as triterpenes, flavonoids or polyphenols, have been reported to exhibit anti-hepatotoxic effect on experimental liver injury models induced by different types of hepatotoxicants (Liu et al., 1995) or those produced by pathogenic fungi and bacteria (Stickel et al., 2009). Herbal drugs have become increasingly popular and their use is widespread as a number of herbals show promising activity for antifibrotic and chronic hepatitis treatment (Adams et al., 2009).

Rosemary (*Rosmarinus officinalis*) is a small evergreen aromatic shrub with a Mediterranean origin and belonging to Lamiaceae (Labiatae) family. It has been widely used as preservative in food industry due to the antioxidant activity of some of its constituents. Oxidative stress models have been used as a tool to show that constituents of *Rosmarinus officinalis* are good scavengers of peroxyl radicals and are able to block the formation of the hydroxyl radical generated in non-lipid systems (Haraguchi et al., 1995). Therefore, the objective of the present study was to investigate the antioxidant and hepatoprotective effects of the aqueous extract of the Egyptian rosemary against CCl_4-induced hepatotoxicity.

Material and Methods

Chemicals

Carbon tetrachloride (CCl_4), perchloric acid (PCA), and trichloroacetic acid (TCA) (extra pure 99%) were obtained from SISCO Research Laboratories PVT LTD, Mumbai, India. Thiobarbituric acid (TBA) was obtained from MERCK, Darmstadt, Germany. Other solvents and chemicals used were either analar or of analytical grade unless otherwise specified.

Herb extraction

The Egyptian cultivated herb was purchased from a local supplier, Cairo, Egypt. The extraction was carried out according to the method of Dorman et al. (2003). Briefly, 50 g fine powdered herb were mixed with 500 ml distilled water in a quick fit flask round-bottom flask which connected to a hydrodistillation apparatus and the water was left to boil slowly for 120 minutes. The water from the flask was removed and another 300 ml of fresh distilled water were added and was boiled another 60 minutes. Water fractions were combined and filtered through qualitative No. 42 Whatman filter paper (Whatman International Ltd, Maidstone, England). The filtrate was then subjected to lypholyzation process through freeze drier (Snijders Scientific-tilburg, Holland) under pressure, 0.1 to 0.5 mbar and temperature -35 to -41°C conditions. The dry extract was stored at 4°C until used. Two doses (220 and 440 mg/kg b.w) were prepared daily.

Experimental animal design

Adult male Wistar albino rats weighting 120-150g (purchased from animal house colony, Giza, Egypt) were maintained on excess fresh tap water and standard pellets (proteins, 16.4; fats, 36.3; fibers, 41 g/kg and metabolizable energy 12.8 MJ); housed in artificially illuminated and thermally controlled suitable plastic cages and received humane care in compliance with the guidelines of the Animal Care and Use Committee of the National Research Centre, Cairo, Egypt. After an acclimatization period of one week, the animals were divided into eight groups (10 rats each) as following: group (I) subjected to intraperitonial injection of 10% saline-olive oil (1ml/kg twice a week) for four weeks and served as control; group (II) received oral dose of rosemary (440 mg/kg/day) for four weeks; group (III) received oral dose of rosemary (440 mg/kg/day) for eight weeks; group (IV) subjected to intraperitonial injection of CCl_4 in olive oil (10% V/V) at a dose of 1ml CCl_4 /kg twice a week for four weeks; group (V) received oral dose (220 mg/kg/day) of rosemary

for four weeks followed by another four weeks but in combination with CCl_4 injection; group (VI) received oral dose (220 mg/kg/day) of rosemary for four weeks, followed by CCl_4 injection for a similar period; group (VII) received oral dose (440 mg/kg/day) of rosemary for four weeks followed by another four weeks but in combination with CCl_4 injection and group (VIII) received oral dose (440 mg/kg/day) of rosemary for four weeks, followed by CCl_4 injection for a similar period. At the end the study, animals were fasted overnight, and following diethyl ether anesthesia, blood specimens were drawn from the retro-orbital venous plexus using sterile and heparinized capillary tubes into open vacutainer collecting tubes. Finally, all the animals were rapidly sacrificed and the liver's left lobe of each animal was dissected into two parts, one was placed immediately in 10% formalin-saline buffer for the histopathological examination and the other one was washed with saline, dried, weighted and homogenized in 50 mM ice cold- phosphate buffer solution (pH 7.4) to give 20% (w/v) homogenate (Lin et al., 1998). Both blood and liver homogenate specimens were centrifuged and its aliquots were stored at -70°C for biochemical measurements those included: serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities and bilirubin level were determined using reagent kit purchased from RANDOX Laboratories Ltd, Ardmore, Diamond Road, Croumlin Co. Antrone, United Kingdum; serum gamma glutamyltranspeptidase (GGT) activity was determined using reagent kit purchased from BioSystems S.A. Costa Brava 30, Barcelona, Spain; Serum alkaline phospatase (ALP) activity was determined using kit produced by Biodiagnostic Co., Dokki, Egypt; glutathione reductase activity of the liver homogenate was determined using a kit purchased from Oxis Research™ Co., USA; and malondialdehyde (MDA) (lipid peroxidation index) level of liver homogenate was determined chemically according to the method of Ruiz-Larrea et al. (1994).

Histopathological examination

After fixing in 10% formalin saline, liver specimens were washed in tap water overnight and subjected to dehydration in graded alcohol, clearing in xylene for 20 minutes and embedded in paraffin wax. Transverse serial sections were then cut at 5 µm thickness and mounted on albuminized slide. Sections were stained with hematoxylin and eosin (Hx&E) (Drury & Wallington, 1980) and then observed microscopically for the histopathological studies.

Statistical analysis

The obtained data were subjected to one way analysis of variance (ANOVA) using statistical analysis system (SAS) program software; copyright (c) 1998 by SAS Institute Inc., Cary, NC, USA. Tukey test was used to clarify the significance between the individual groups at probability level ≤ 0.05 (Steel & Torrie, 1960).

Results

The obtained data revealed that, intoxication with CCl_4 resulted in a significant increase in the activities of serum ALT, AST, GGT and ALP whereas, administration of 440 mg/kg/day rosemary for 4 or 8 weeks resulted in non significant decrease in their activities compared to the control group. Moreover, administration of rosemary (220 and 440 mg/kg/day) for 4 weeks before intoxication with CCl_4 alone, or CCl_4 in combination with rosemary, for a similar period resulted in a dose & duration-dependant decrease in the activities of those enzymes compared to CCl_4 intoxicated group (Table 1). In addition, administration of 440 mg/kg/day rosemary for 4 or 8 weeks resulted in non significant increase in hepatic glutathione reductase (GR) activity and non significant decrease of liver MDA and serum bilirubin levels, in contrast, it is clearly indicated that CCl_4 intoxication resulted in a significant increase in serum bilirubin (direct and indirect) and liver MDA levels accompanied with a significant reduction in the activity of hepatic GR compared to control group. Moreover, administration of 220 or 440 mg/kg/day rosemary for 4 weeks before intoxication with CCl_4 alone or CCl_4 in combination with rosemary another four weeks resulted in a dose and duration-dependant significant improvement in the tested parameters compared to CCl_4 intoxicated group (Table 2).

Microscopic examination

The transverse section in the liver of normal rats showed classical hepatic lobules; each is formed of cords of hepatocytes radiating from the central vein to the periphery of the lobule. The cell corda were separated by narrow blood sinusoids lined by endothelial cells and kupffer cells. The acidophilic cytoplasm around a pale stained nucleus could be seen. The portal tracts were found in the periphery of the lobules containing the portal vein (Figure 1-a). No pathological changes could be noticed in the liver of rats administrated with rosemary (440 mg/kg/day) either for four or eight weeks compared to control one (Figure 1-b).

Intoxication of rats with CCl_4 for four weeks induced macro and micro fatty changes (steatosis), and moderate fibrosis without formation of septa. Areas of hemorrhage, focal necrosis and mitotic figure were seen (Figure 1-c&d).

The microscopic examination of liver sections of the rats pretreated with rosemary (220 or 440 mg/kg/day) for four weeks followed by a similar period but in combination with CCl_4 or those of the rats administrated with rosemary (220 or 440 mg/kg/day) for four weeks only before they were intoxicated with CCl_4 for a similar period showed dose- and duration-dependant

Table 1. Protective effect of rosemary aqueous extract on serum markers of liver enzymes in CCl_4 intoxicated rats.

	Control	HD Ros. (4W)	HD Ros. (8W)	CCl_4 (4W)	LD Ros. (8W) CCl_4 (4W)	LD Ros.(4W) CCl_4 (4W)	HD Ros. (8W) CCl_4 (4W)	HD Ros. (4W) CCl_4 (4W)
	I	II	III	IV	V	VI	VII	VIII
ALT (IU/L)	22±0.65^{E}	21±0.65^{E}	20±0.74^{E}	118±2.66^{A}	80±1.49^{C}	88±2.08^{B}	59±1.14^{D}	78±2.14^{C}
AST (IU/L)	123±1.14^{E}	122±1.80^{E}	119±1.48^{E}	453±8.82^{A}	290±5.92^{C}	341±4.28^{B}	194±3.50^{D}	280±6.99^{C}
GGT (IU/L)	6.76±0.17^{E}	6.67±0.16^{E}	6.64± 0.86^{E}	13.44±0.39^{A}	10.16±0.20^{BC}	11.67± 0.3 4^{B}	8.15±0.2^{DE}	9.57±0.24^{CD}
ALP (IU/L)	115±2.47^{C}	114±2.27^{C}	114±1.24^{C}	158±3.21^{A}	127±1.22^{B}	135±3.38^{B}	116±1.17^{C}	117±0.91^{C}

Values are mean ± SE for 10 rats per group. Within each row, means with different letters are significantly different (P < 0.05) using one way (Tukey) ANOVA test

Table 2. Protective effect of rosemary aqueous extract on serum bilirubin and liver MDA levels and GR activity in CCl_4 intoxicated rats.

	Control	HD Ros. (4W)	HD Ros. (8W)	CCl_4 (4W)	LD Ros. (8W) CCl_4 (4W)	LD Ros.(4W) CCl_4 (4W)	HD Ros.(8W) CCl_4 (4W)	HD Ros.(4W) CCl_4 (4W)
	I	II	III	IV	V	VI	VII	VIII
T.Bilirubin (mg/dl)	0.295±0.007^{E}	0.291±0.008^{E}	0.289±0.009^{E}	0.782±0.020^{A}	0.471±0.009	0.503±0.008^{BC}	0.312±0.004^{D}	0.428±0.011^{C}
D.Bilirubin (mg/dl)	0.054±0.001^{E}	0.052±0.001^{E}	0.054±0.001^{E}	0.320±0.005^{A}	0.145±0.004	0.151±0.004^{BC}	0.082±0.002^{D}	0.132±0.003^{C}
Ind.Bilirubin (mg/dl)	0.241±0.008^{D}	0.240±0.008^{D}	234±0.009^{D}	0.462±0.022^{A}	0.326±0.006^{BC}	0.352±0.010^{B}	0.290±0.009^{C}	0.240±0.010^{D}
Liver GR (mU/g)	279±5.48^{A}	280±5.74^{A}	281±3.91^{A}	247±3.93^{B}	266±3.18^{AB}	265±5.25^{AB}	269±3.10^{A}	270±6.34^{AB}
Liver MDA (nmol/g)	27±0.05^{D}	26±0.57^{D}	25± 0.62^{D}	65±0.82^{A}	41±1.08^{B}	43±1.11^{B}	28±0.86^{D}	37± 0.73^{C}

Values are mean ± SE for 10 rats per group.
Within each row, means with different letters are significantly different (P < 0.05) using one way (Tukey) ANOVA test.

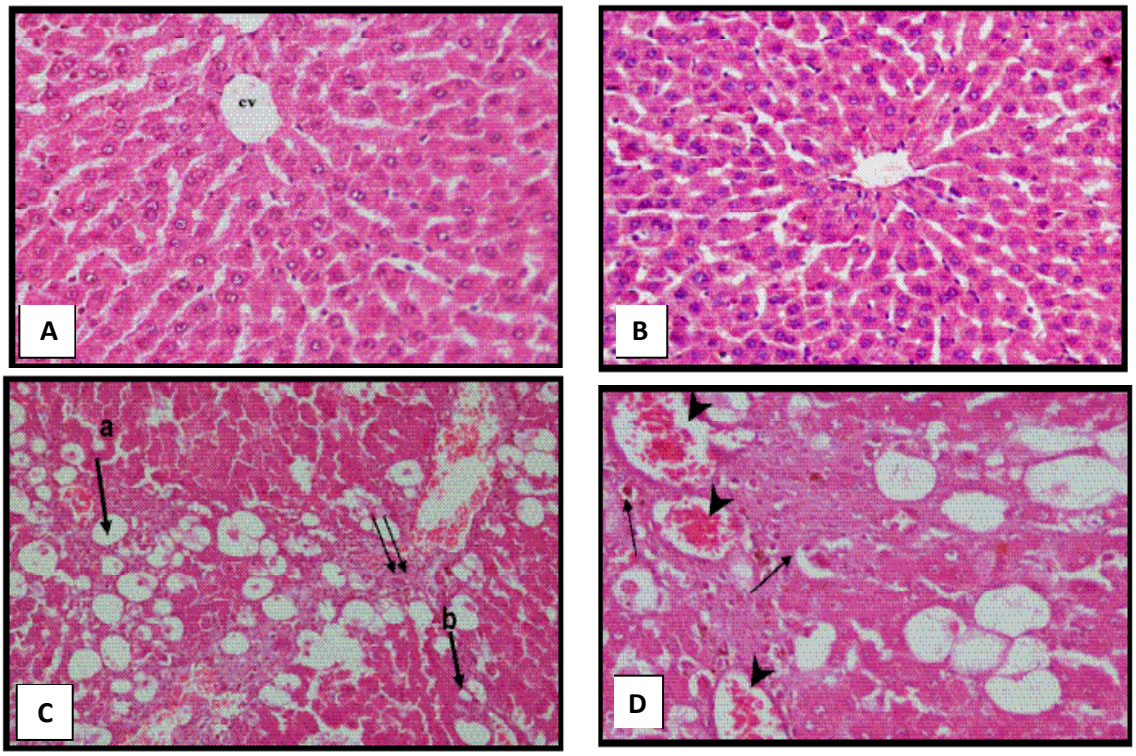

Figure 1. Sections of the livers of (A) control rat showing normal histological structure of hepatic lobules and central vein; (B) rat orally administrated with a high dose of rosemary extract for eight weeks showing normal histological structure; (C) rat intoxicated with CCl4 for four weeks showing macro (a) and micro (b) fatty changes (steatosis) (arrow), increase in area of fibrosis without formation of septa (double arrows); (D) another field of the liver of rat intoxicated with CCl4 for four weeks showing an area of hemorrhage (arrow head). (Hx & E x 200)

protective effects, such as diminution of fibrosis and fatty changes, while dilated blood sinusoids, vacuolar degeneration and hypertrophy of Kupffer cells were also seen, when compared to the liver sections of rats intoxicated with CCl_4 only or to each other (Figure 2- a & b) and (Figure 3-a, b & c).

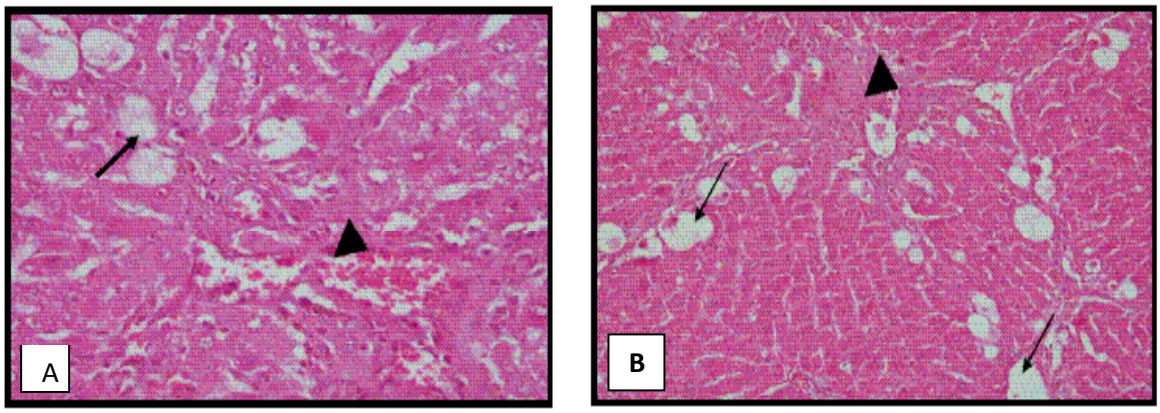

Figure 2. Sections of the livers of rats administered with a low dose of rosemary extract for four weeks before intoxicated with CCl_4 along with rosemary another four weeks. (A) Showing vacuolar degeneration (arrow) and large hemorrhagic area (arrow head) and (B) Another field showing mild fibrosis (arrow head) and fatty changes (arrows). (Hx & E x 200)

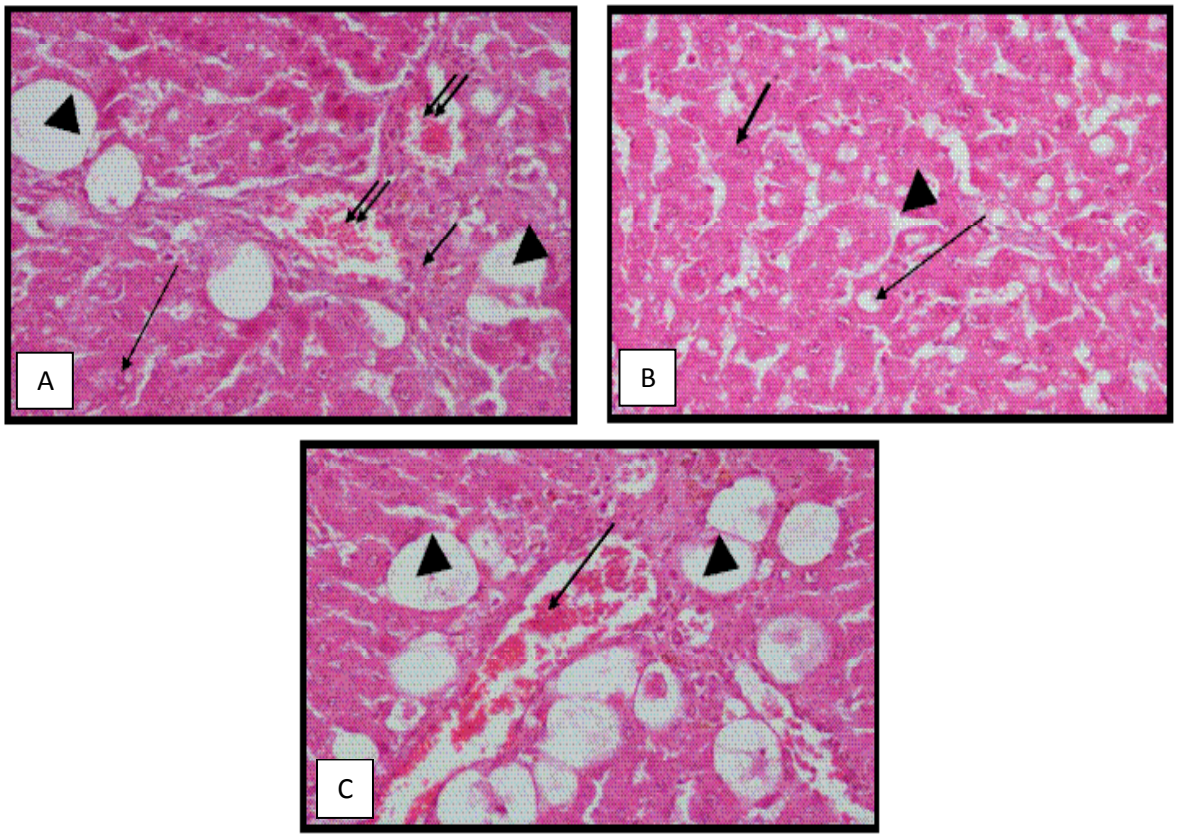

Figure 3. Sections of the livers of (A) rat administered with a low dose of rosemary extract for four weeks prior to CCl_4 intoxication for a similar period showing decrease in area of fibrosis (arrow), vacuolar degeneration (arrow head), hemorrhagic areas (double arrows) and pyknotic nuclei (long arrow) could be noticed; (B) rat administered with a high dose of rosemary extract for four weeks prior to intoxication with CCl_4 along with rosemary another four weeks showing no fibrosis or fatty changes, while dilated blood sinusoids (arrow head), vacuolar degeneration (arrow) and hypertrophy of kupffer cells (long arrow) still present and (C) rat administered with a high dose of rosemary extract for four weeks prior to CCl_4 intoxication for a similar period showing large hemorrhagic area (arrow) and vacuolar degeneration (arrow head). (Hx & E x 200)

Discussion

In the current study, the protective effects of rosemary aqueous extract (RE) against CCl_4-induced hepatotoxicity in adult male rats were investigated. In this respect, the effect of the extract on the liver functions and oxidative stress markers (hepatic glutathione reductase activity and malondialdehyde level) as well as histopathological changes of the liver were evaluated.

Administration of rosemary aqueous extract (440 mg/kg) for either four or eight weeks didn't adversely affect the activity of serum ALT, AST, GGT and ALP, and hepatic GR as well as the level of serum bilirubin and hepatic MDA compared to those of control rats. This reflecting the extract biological safety which confirmed here by the result of the toxicity test (data not shown) that didn't show any mortality or toxicity symptoms during the first 72 hours after administration.

The increase in the activity of serum ALT, AST, GGT and ALP as a result of CCl_4 intoxication is in coincidence with Wang et al. (2008). Carbon tetrachloride is a fat-soluble toxic chemical that enters the body and dissolves only in fatty structures not in water. This makes it difficult to be excreted from the body. Fat soluble chemicals have a high affinity for fat tissues and cell membranes, thus in adipose tissue, toxins may be stored for years, being released during times of exercise, stress or fasting (Seton et al., 2008). CCl_4 is metabolized by a drug-metabolizing enzyme system (Cytochrom P-450) in the hepatic cell into trichloromethyl free radical ($CCl_3^{\bullet}$) which either bind covalently with lipoproteins or reacts with oxygen to form a trichloromethylperoxy radical ($CCl_3OO^{\bullet}$) inducing peroxidation of polyunsaturated fatty acids in the hepatocytes membrane. Destructive lipid peroxidation products lead to breakdown of cellular membrane structure as well as function (Girotti, 1998) resulted in disturbance in the permeability and the transport function of the hepatocyte as a result of CCl_4 effect, therefore leakage of the cytosolic enzymes from hepatocyte to the serum (Zimmerman & Seeff, 1979). Moreover, these radicals can bind to the other cellular molecules such as nucleic acids and proteins, and adversely affect the permeability of mitochondria, and endoplasmic reticulum membranes, resulting in the loss of cellular calcium sequestration and homeostasis, which largely contributes to the subsequent hepatotoxicity damage (Kodai et al., 2007).

In addition, it was known that CCl_4 increases urinary taurine (taurine was postulated to be an important stabilizer of all cell membrane), therefore reduces its level in hepatic tissues, and consequently reduces the stability of hepatocytes membrane leading to release of these enzymes into the blood (Waterfield et al., 1991). It was suggested that many hydrolytic enzymes, appear to be stimulated by Ca^{++} elevation consequent to CCl_4 intoxication, including phoshpolipase A2 and C and non lysosomal proteases, those can initiate degradation of important metabolic and structural components in the liver (Cortan et al., 1994). Based on this mechanism, CCl_4 could reduce microsomal cytochrome P-45 content, cytochrome b-5, NADPH-cytochrome C reductase and glutathione (GSH) in the liver (Dhawan & Goel, 1996).

The obtained data indicated dose- and duration-dependant significant improving effect of RE on the altered activities of serum ALT, AST, GGT and ALP induced by CCl_4-intoxication. It was reported that intake of oxygen radical scavengers (antioxidants and phytochemicals) may be a good defense mechanism for hepatoprotection. Furthermore, improvement of phase II detoxifying and antioxidant enzymes and elevation of the antioxidant substance content is one of the mechanisms to improve the antioxidant status (Lee et al., 2007). Rosemary is one of the plants rich in different phytochemical derivatives such as triterpenes, flavonoids or polyphenols. Its extracts are able to donate electrons to reactive radicals, converting them to more stable and non reactive species, therefore preventing them from reaching biomolecules, such as lipoproteins, polyunsaturated fatty acids, DNA, amino acids, proteins and sugars, in susceptible biological systems. Also, it was concluded that rosemary extracts have a high scavenging capacity of different types of reactive oxygen and nitrogen species, mostly free radicals, is thought to be one of the main mechanisms of the antioxidant action exhibited by phenolic phytochemicals (Haraguchi et al., 1995). In addition, water soluble extracts of rosemary was reported to induce xenobiotec detoxification enzymes (XME) in rat liver, produce a significant increase in all enzyme activities of phase I [ethoxyresorufin O-deethylase (EROD), methoxyresorufin O-demethylase (MROD), pentoxyresorufin O-dealkylase (PROD), P-nitrophenol hydroxylase (PNPH) and nitric oxide (NO)] and phase II [quinone reductase (QR), GST and UDP-glucuronosyl transferase (UGT)) (Sotélo-Félix et al., 2002), enhance both cytochrome P (CYP) and detoxifying enzymes (Debersac et al., 2001) and attenuate the depletion in hepatic GSH and catalase (CAT) (Fahim et al., 1999).

In the current study, high dose (as well as continuous administration) of the RE induced high degree of protection and improvement. This could be attributed to the high percentage of antioxidants in the high dose, which resulted in strongest inhibition of oxidative radicals from reaching the biological molecules. By the same manner, the continuous administration of rosemary extract besides CCl_4 built a continuous resistant-barrier with more protection battery against oxidative radicals.

The current study recorded a significant increase in the levels of serum total, direct and indirect bilirubin due to CCl_4 intoxication. As

mentioned before CCl_4 is metabolized by drug metabolizing system into intermediate radical that may react with O_2 producing more active peroxy radicals, or react directly with the membrane lipids resulting in destructive lipid peroxidation products that impair the integrity, structure and function of the hepatocyte, therefore leading to defective secretion of the bile (bilirubin, cholesterol & bile acids) due to the damaged bile ducts, consequently elevation of serum bilirubin.
The alteration and loss of integrity of red blood cells (RBCs) membrane precede the onset of CCl_4-induced liver cirrhosis (Beutler, 1986), thus the direct exposure to molecular oxygen and circulating components in the blood, and the loss of the de novo synthesizing capacity of new enzyme molecules during RBCs maturation put the erythrocytes at a high risk of damage by reactive molecules.

One, besides to the normal functions of the liver, is to excrete the breakdown product of hemoglobin (namely bilirubin) into the bile (Plaa & Hewit, 1982). As CCl_4 affects the integrity of liver cells, by the same manner it affects on the structure and function of the erythrocyte membrane, and increase the erythrocyte fragility, this in turn lead to erythrocyte hemolysis and hemoglobin breakdown. Additional bilirubin resulted in rising of bilirubin level.

The current study found that administration of 220 and 440 mg/kg/day RE prior to CCl_4 intoxication or continues in combination with CCl_4 induced, dose- and duration-dependant, significant decrease in the bilirubin levels compared to the group intoxicated with CCl_4. The antioxidative, radical scavenging and electron donation properties of RE which can stabilize the reactive radicals and converting them into stable species before reaching the parenchyma of the liver; induction of the hepatic endogenous antioxidant enzymes; improvement of hepatic excretory function of bile or/and the amelioration of erythrocyte integrity against CCl_4 damaging effect could explain the improved bilirubin level.
Injection of rats with CCl_4 for four weeks induced an increase in the hepatic MDA level associated with a decrease in the activity of hepatic GR compared to control. These finding is in accordance with the previous study of Wang et al. (2008).

Both $CCl_3^{\bullet}$ and subsequent CCl_3OO; radicals (CCl_4 metabolites) combine with the cellular lipids inducing a chain of lipidic peroxidation that usually measured through lipid catabolites such as MDA (Ilavarasan et al., 2003). CCl_4 intoxication significantly decreases the expression of antioxidant enzymes in the liver, including glutathione reductase and glutathione–S-transferase (Lee et al., 2007); lowered the hepatic GSH (Song & Yen, 2003) and elevated the hepatic oxidized glutathione (GSSG) (Takahashi et al., 1996).

The current results indicated that, administration of 220 and 440 mg/kg/day RE before CCl_4-intoxication induced a dose- and duration-dependant decrease in level of MDA associated with an elevation in GR activity. The decrease in the MDA level, by rosemary herein may be attributed to either the well-known scavenging action against $Cl_3COO^{\bullet}$ and $^{\bullet}OH$ or the antioxidant properties that inhibited lipid peroxidation, this in turn stabilize the reactive radicals, preserve the cellular integrity and restrain the severity of CCl_4. Another notion can be introduced here, rosemary extract showed ability to prevent CCl_4-induced decrement of GR level, suggesting that rosemary may be protect the GR –SH group from the reactive radicals that produced from CCl_4-intoxication.

It was postulated that the liver fibrosis is a consequence of chronic liver injury from different causes, including alcohol, toxin, chronic viral infection and metabolic disease. In the liver there is an increased deposition of extracellular matrix (ECM) in perisinusoidal and periportal spaces. The activated hepatic stellate cells have now been identified as the primary source of ECM in liver fibrogenesis. The accumulation of ECM proteins distorts the hepatic architecture by formation of fibrous scar and subsequent development of nodules regenerating hepatocytes defines cirrhosis (Xu et al., 2006).

Cirrhosis produces hepatocellular dysfunction and increased intrahepatic resistance to blood flow (Hung et al., 2006). The hepatic fibrosis developed due to increased accumulation of MDA, the stable end product of lipid peroxidation. MDA in this oxidative stress causes various diseases (Lee et al., 2004). On the other hand, Onell et al. (2000) found that histological examination of livers from CCl_4 intoxicated rats revealed that hepatocytes denaturation and necrosis were not obvious and pseudolobules were not found.

Regarding the histological study of the present work, intoxication of rats with CCl_4 for four weeks induced a moderate fibrosis without formation of septa and macro and micro fatty changes (Steatosis). Fatty changes those are induced by CCl_4 have been explained by Liu et al. (2007) who postulated that the liver steatosis states in the central perivenular area but it may involve the total surface of the lobule. The accumulation of fats is attributed to a defect in the synthesis, as well as secretion, of lipoprotein resulting in interference of the toxin with assembly of tubulin in microtubules. Fat metabolism is responsible for fatty disease which may be due to imbalance in energy consumption and combustion resulting in lipid storage or may be a consequence of peripheral resistance to insulin, where by the transport of fatty acids from adipose tissues to the liver is increased (Reddy & Rao, 2006).

Chronic liver damage is a wide spread pathology characterized by a progressive evolution from steatosis, fibrosis and cirrhosis. The production of reactive oxygen species (ROS) is considered to be a major factor in oxidative cell

injury. The antioxidant activity or the inhibition of free radicals generation is important in providing protection against such hepatic damage (Vitaglione et al., 2004).

It is realized that administration of rats with rosemary extract in this work alleviated the deleterious effect of CCl_4 on liver. In addition, rosemary may act as a co-factor in the synthesis of biological endogenous antioxidant material such as glutathione-s-transferase (GST) and quinine reductase (QR) (Singletary, 1996), therefore administration of rats with rosemary extract before or in combination with CCl_4 showed improvement in the pathological changes when compared with rats intoxicated with CCl_4.

Conclusions

In conclusion, rosemary inhibited and reduced the CCl_4-induced hepatotoxicity in rats possibly by scavenging or blocking the formation of free radicals generated during CCl_4 metabolism. These improving effects of rosemary could be attributed to the bioactive constituents that alleviated the deleterious effect of CCl_4 either by the well-known scavenging action or the antioxidant properties that inhibited lipid peroxidation, stabilized the reactive radicals, preserve the cellular integrity and restrain the severity of CCl_4.

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