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Preventive Effect of Corosolic Acid on Lipid Profile Against Carbon Tetrachloride-Induced Hepatotoxic Rats

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Abstract: The present study was designed to investigate the hepatoprotective activity of corosolic acid in CCl_4-induced hepatotoxic male albino rats and included an assessment of the antihyperlipidemic properties. Liver necrosis was induced by intraperitoneal injection of CCl_4 (1.25 mL/kg b.wt.). Hyperlipidemic agents, such as lipid peroxidation, lipid profile and liver function markers were assessed. The activities of the hepatic marker enzymes such as aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) and gamma glutamyl transpeptidase (GGT) were increased significantly in CCl_4-treated animals. In the serum, increased levels of very low density lipoprotein cholesterol (VLDL-C) and low density lipoprotein cholesterol (LDL-C) and decreased high density lipoprotein cholesterol (HDL-C) were observed. Further, an increase in the levels of total cholesterol (TC), phospholipids (PL), triglycerides (TG) and free fatty acids (FFA) in serum and liver were observed in hepatotoxic rats, whereas these hepatic marker enzymes and lipid profiles were significantly protected in corosolic acid pretreated on CCl_4-treated animals. These results indicate that the corosolic acid can potentially ameliorate the lipids abnormalities and protect hepatic damage against CCl_4-induced hepatotoxicity rats.

Key words: CCL_4, liver, corosolic acid, silymarin, lipid profile

INTRODUCTION

Liver is a versatile organ of the body that regulates internal chemical environment. Liver injury induced by various hepatotoxins has been recognized as major toxicological problems arise in the modern world (Das et al., 2012). Because of Environmental Protection Agency (EPA) identifies the one of most serious hazardous environmental agents such as carbon tetrachloride (CCl_4) (EPA, 2005). In the past, carbon tetrachloride was widely used as a cleaning fluid, fire extinguishers and as a fumigant to kill insects in grain. Carbon tetrachloride has a sweet odor and most people can begin to smell it in air when the concentration reaches 10 parts carbon tetrachloride per million parts of air (ppm) (EPA, 2003). Due to this CCl_4 is released either from a large areas to enters the environment. The general population may be exposed to small amounts of carbon tetrachloride in the air, drinking water, food and soil, due to causes liver disease (ATSDR, 2005).

Liver injury induced by CCl_4 is the most intensively studied system for xenobiotic-induced oxidative hepatotoxicity (Ma et al., 2012). Liver damage caused by CCl_4 is characterized by inflammation in the early stage. In damaged hepatocytes, CCl_4 is reductively bioactivated by cytochrome P450 into a trichloromethyl radical, a highly reactive species that triggers lipid peroxidation and leads ultimately to hepatotoxicity (Deng et al., 2012). Simultaneously, the high level of blood cholesterol is a contributory factor of atherosclerosis and many lipid associated ailments like obesity, heart attacks and stroke and kidney failure. Previous study have shown that lipid associated disorders are not only attributed to the total serum cholesterol, but also to its distribution among different lipoproteins (Pushpavalli et al., 2010). The low density lipoproteins (LDLs) are the major carriers of cholesterol towards tissues having atherogenic potential, while the high density lipoproteins (HDLs) carry cholesterol from peripheral tissues to the liver (Agellon et al., 1991). HDLs thus give protection against many cardiac problems and obesity (Brinton et al., 1990). Although genetic factors recline behind these lipid disorders, in most of the cases it is allied with diets high in saturated fats or trans fats.

Developing therapeutically effective agents from natural products may reduce the risk of CCl_4 induced toxicity when the drug is used clinically (Shen et al., 2009). Modern medicines have little to offer for alleviation of hepatic diseases and it is chiefly the plant based preparations which are employed for the treatment of liver disorders (Somasundaram et al., 2010). There is a great demand for the development of an efficient hepatoprotective drug from the natural resource (Tandon et al., 2008). Pentacyclic triterpenes are a major group of ubiquitous secondary plant metabolites, organized in four classes according to their core structure (lupenyl, ursanyl, betulenyl or oleanyl) and further classified according to the presence, position and number of acidic, ketonic and hydroxyl moieties

(Caligiani *et al.*, 2013). They occur both in the form of free acids or aglycones in triterpenoid saponins, offering a wide range of health promoting activities both as pure substances or as blends. Among them, pentacyclic triterpenic acids are endowed with a plethora of health-promoting properties.

In this present scenario, we focus on the triterpenoid, corosolic acid (2a, 3b-dihydroxy-urs-12-en-28-oic acid), which is isolated from *A. valvata Dunn* and also has been discovered in many Chinese medicinal herbs, such as the *Lagerstroemia speciosa* L. and *banaba* leaves (Xu *et al.*, 2009). Corosolic acids possesses chemopreventive, antiproliferative and antioxidant activities, but are also endowed with specific antidiabetic properties (Stohs *et al.*, 2011). Corosolic acid in particular is gaining commercial and research interest for its ability to inhibit α-glucosidase and α-amylase and to decrease post challenge plasma glucose levels in humans (Sivakumar *et al.*, 2009; Vijaykumar *et al.*, 2006). Therefore, the present study was to evaluate the effect of corosolic acid on serum and tissue lipid profile in CCl₄-induced hepatotoxic rats.

MATERIALS AND METHODS

Chemicals: Carbon tetrachloride was purchased from Sigma-Aldrich Co. St. Louis, Missouri, USA. Corosolic acid was purchased from Mansite Pharmaceutical Co., Ltd (Chendu, China). All other chemicals used were of analytical grade obtained from E. Merck or HIMEDIA, Mumbai, India.

Experimental animals: Male albino wistar rats (180-200 g) were housed in clean cages at a 20-24°C temperature, 12 h light/12 h dark cycle and relative humidity 52% in the animal house at the College of Medicine, King Saud University. Ethics approval was obtained from the ethics committee of the college of medicine research center at King Saud University, Riyadh, Saudi Arabia (11/3215/IRB).

Experimental design: The animals were divided into four groups of six animals in each group. The corosolic acid was suspended in 0.1% dimethyl sulfoxide (DMSO) and fed to rats via an oral route at 20 mg/kg body weight for 7 days. Then a single oral dose of CCl₄ (1:1 in liquid paraffin) at 1.25 mL/kg BW (Saba et al., 2010) was given at an interval of 6 h after the administration of last dose of corosolic acid. Group I served as control rats received 0.1% dimethyl sulfoxide (DMSO) only, Group II served as control rats treated with corosolic acid 20 mg/kg BW. Group III was administered with CCl₄ (negative control). Groups IV was administered with corosolic acid at 20 mg/kg BW and also administered CCl₄ at an interval of 6 h after the administration of last dose of corosolic acid on the 7th day. Animals were sacrificed after 24 h of CCl₄ administration. Blood sample was collected in tubes containing a mixture of ethylene diamine tetra acetic acid (EDTA) for the estimation of plasma lipid peroxidation and antioxidants. Tissue was sliced into pieces and homogenized in appropriate buffer in cold condition (pH 7.0) to give 20% homogenate. The homogenate were centrifuged at 1000 rpm for 10 min at 0°C in cold centrifuge. The supernatant was separated and used for various biochemical estimations.

Biochemical estimations: AST and ALT were estimated by the method of Reitman and Frankel (1957). ALP and GGT were estimated by the method of Kind and King (1957) and Rosalki and Rau (1972), respectively. TC, TG, FFA and PL were estimated by the methods of Allain *et al.* (1974), McGowan *et al.* (1983), Falholt *et al.* (1973) and Zilversmit and Davis (1950), respectively. Serum HDL-C was estimated by the method of Izzo *et al.* (1981). LDL-C and VLDL-C were calculated by Friedewald's *et al.* (1972) formula.

Statistical analysis: Statistical evaluation was done using one-way analysis of Variance (ANOVA) followed by Duncan's Multiple Range Test (DMRT) using statistical package of social science (SPSS) 10.0 for Windows. Significance level was set at $p < 0.05$.

RESULTS

Figure 1 shows the activities of serum hepatic marker enzymes such as AST, ALT, ALP and GGT in control and CCl₄ hepatotoxic rats. Increased activities of AST, ALT, ALP and GGT levels were observed in CCl₄ induced rats. Administration of corosolic acid improved these parameters towards normalcy.

Table 1 and 2 show the levels of lipid profile in serum and liver of normal and CCl₄-hepatotoxic rats, respectively. The elevation of these lipid profiles such as free fatty acids (FFA), total cholesterol (TC), triglycerides (TG) and phospholipids (PL), in CCl₄ hepatotoxic rats in serum and liver. Pretreatment with corosolic acid these above lipid profiles were significantly protected to near normal levels.

Table 3 shows the levels of lipoproteins in serum of normal and CCl₄-induced hepatotoxic rats, respectively. Significant elevation of these lipoproteins such as LDL-C and VLDL-C and reduction of HDL-C were observed in CCl₄-induced hepatotoxic rats. Oral administration of corosolic acid the levels of lipoproteins significantly decreased LDL-C and VLDL-C and increased HDL-C levels.

DISCUSSION

The present study demonstrates the hepatoprotective, prophylactic and antioxidant effects of corosolic acid against CCl₄-induced liver injury in rats. The liver mainly detoxifies toxic chemicals and drugs and becomes the

Table 1: Effect of corosolic acid on the levels of FFA, TG, TC and PL in serum on CCl_4 induced hepatotoxic rats

Groups	FFA (mg/dL)	Plasma TG (mg/dL)	Plasma TC (mg/dL)	Plasma PL (mg/dL)
Control	52.15±4.02[a]	61.13±4.24[a]	77.17±5.88[a]	101.25±7.72[a]
Control+corosolic acid (20 mg/kg b.wt.)	50.11±3.55[a]	59.25±4.11[a]	76.12±5.75[a]	103.34±8.08[a]
CCl₄-control (1.25 mL/kg b.wt.)	118.15±9.28[b]	145.20±10.48[b]	147.22±10.55[b]	167.12±10.26[b]
CCl₄+corosolic acid (20 mg/kg b.wt.)	59.47±4.18[a,c]	68.14±4.70[a,c]	85.25±6.29[a,c]	111.34±8.73[a]

Values are expressed as Means±SD for six rats in each group
Values not sharing a common superscript differ significantly at p<0.05 (DMRT)

Table 2: Effect of corosolic acid on the levels of FFA, TG, TC and PL in liver on CCl_4 induced hepatotoxic rats

Groups	FFA (mg/g wet tissue)	TG (mg/g wet tissue)	TC (mg/g wet tissue)	PL (mg/g wet tissue)
Control	6.86±0.45[a]	4.23±0.39[a]	4.66±0.36[a]	18.47±1.31[a]
Control+corosolic acid (20 mg/kg b.wt.)	6.98±0.52[a]	4.35±0.38[a]	4.61±0.38[a,c]	18.92±1.40[a]
CCl₄-control (1.25 mL/kg b.wt.)	15.54±1.15[b]	8.97±.50[b]	9.34±0.55[b]	46.66±2.92[b]
CCl₄+corosolic acid (20 mg/kg b.wt.)	7.89±0.44[a,c]	4.91±0.30[a,c]	5.18±0.36[a,c]	22.35±1.95[a,c]

Values are expressed as Means±SD for six rats in each group
Values not sharing a common superscript differ significantly at p<0.05 (DMRT)

Table 3: Effect of corosolic acid on the levels of serum lipoproteins and HDL/LDL cholesterol ratio in CCl_4 induced hepatotoxic rats

Groups	HDL-cholesterol (mg/dL)	VLDL-cholesterol (mg/dL)	LDL-cholesterol (mg/dL)
Control	52.14±3.36[a]	12.22±1.07[a]	12.81±1.22[a]
Control+corosolic acid (20 mg/kg b.wt.)	51.54±3.55[a]	11.85±1.01[a]	13.13±1.10[a]
CCl₄-control (1.25 mL/kg b.wt.)	20.45±1.60[b]	29.04±2.15[b]	97.73±6.12[b]
CCl₄+corosolic acid (20 mg/kg b.wt.)	47.47±3.22[a,c]	13.62±1.22[a,c]	24.16±2.02[c]

Values are expressed as Means±SD for six rats in each group
Values not sharing a common superscript differ significantly at p<0.05 (DMRT)
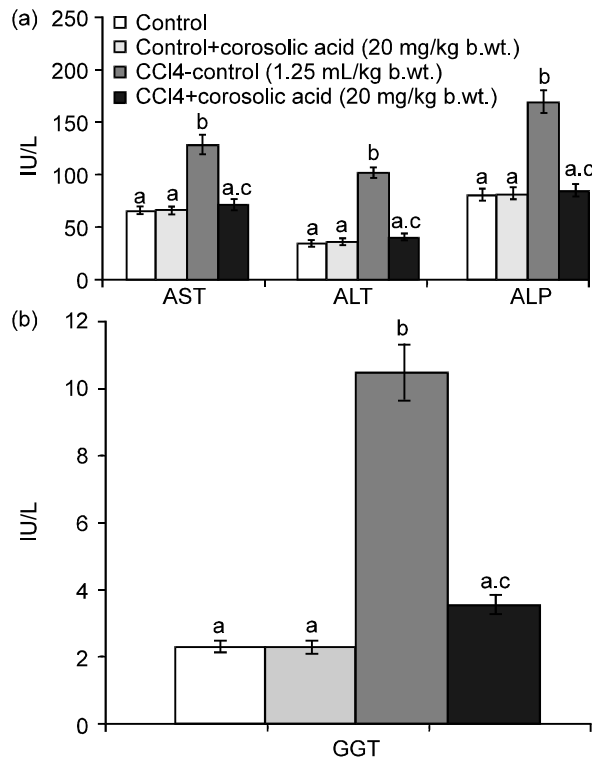

Fig. 1(a-b): Effect of corosolic acid on the activities of hepatic marker enzymes in the serum on CCl₄ induced hepatotoxic rats. Values are expressed as Means±SD for six rats in each group. Values not sharing a common superscript differ significantly at p<0.05 (DMRT)

main target organ for all possible toxic xenobiotics. Being a potent hepatotoxin, CCl₄ is the most extensively used chemical agent to investigate hepatoprotective activity on various experimental animal models. The experimental hepatic damage caused by CCl₄ histologically also resembles viral hepatitis (Ravikumar and Gnanadesigan, 2012; Shim *et al.*, 2010). AST, ALT, ALP and GGT are the serum hepatobillary enzymes present normally in the liver in high concentrations. Upon necrosis or hepatic damage these enzymes will be leaked into the circulation; raising serum concentration of these enzymes (Zeashan *et al.*, 2008). Present study, elevated serum AST, ALT, ALP and GGT levels in CCl₄ treated animals indicates to cause cellular breakage and loss of functional integrity of cell membranes in liver. Administration of corosolic acid significantly decreased the activities of these hepatic marker enzymes indicating hepatoprotective activity. Similar observations during CCl₄ induced hepatotoxicity were elsewhere recorded (Bilgin *et al.*, 2011). In addition, the antioxidative activity and/or the inhibition of free-radical generation are important in terms of protecting the liver from CCl₄-induced damage (Manibusan *et al.*, 2007).

The liver is the major site for the synthesis and metabolism of cholesterol, bile acids and phospholipids (Yang *et al.*, 2011). Distinct alterations in lipid metabolism have been reported in CCl₄-induced hepatotoxicity in rats (Singhal and Gupta, 2012). The present results were significantly elevated levels of serum and liver total cholesterol, triglycerides, free fatty acid and phospholipids in CCl₄ induction rats. The increase in cholesterol levels to increases the

membrane fluidity, regulates membrane permeability and alters internal viscosity and also the internal chemical composition (Sathish *et al.*, 2003). This increased LDL concentration in the serum of CCl₄-induced rats might be due to defect in LDL-C receptor either through failure in its production (or) function. HDL-C is protective by reversing cholesterol transport, inhibiting the oxidation of LDL-C and by neutralizing the atherogenic effects of oxidized low density lipoprotein-C. A greater increase of LDL-C and VLDL-C may also cause a greater decrease of HDL-C and decreased HDL-C may also be due to diminished lecithin cholesterol acyl transferase activity (Sabesin *et al.*, 1975). In our present study, the levels of TC, TG, FFA, PL, LDL-C, VLDL-C were found to be increased and the level of HDL-C decreased in CCl₄-hepatotoxicity in rats. Corosolic acid administrative rats resulted in significantly protected the abnormalities of lipid profiles against CCl₄-hepatotoxicity rats. These results indicate that corosolic acid could provide an antihyperlipidemic activity. Earlier study have documented the beneficial effects of red beet (beetroot) is associated with numerous of bioactive constituents. The major bioactive constituents such as tricarboxylic acids and phenolic compounds demonstrated by *in vitro* and pre-clinical studies include hypoglycemic, anti-inflammatory, antiproliferative, antitumor, antimicrobial, anti-acetylcholinesterase, antimutagenic and lipid-lowering benefits, protection from cardiovascular disease, induction of phase II enzymes activities (Chidambara Murthy and Shivapriya, 2012). All of these pharmacological properties are associated directly or indirectly with free radical-scavenging abilities of bioactive molecules, which are abundant in corosolic acid involved preventive scenario at CCl₄ altered lipid profiles.

Conclusion: In conclusion, it may be mentioned that the altered biochemical profiles due to CCl₄ exposure is regulated towards normalization by corosolic acid administrated rats. The contents of the corosolic acid not only protect the integrity of plasma membrane peroxidation but, at the same time maintained lipid profile in the regenerative and reparative capacity of the liver. These results suggest that the corosolic acid efficiently act as on the liver to keep it normally functioning and minimizing cell membrane disturbances.

Conflict of interest statement: There are no conflicts of interest.

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