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Carbon Tetrachloride-induced Hepatotoxicity and Nephrotoxicity in Rats: Protective Role of Vitamin C

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ABSTRACT

Glutathione plays an important role in the detoxification of most toxic agents. This study was planned to investigate the protective effect of vitamin C on chronic and acute models of carbon tetrachloride (CCl_4)-induced oxidative stress and changes of the glutathione concentration (GSH) in the liver and kidney of Wistar rats. The administration of vitamin C (Vit. C) to the rats (100 mg/1 kg) with intraperitoneal injection of CCl_4 at dose level of 0.2 mL kg^{-1} (twice a week for 3 months) or 1 mL kg^{-1} (single dose) significantly reduced the elevated plasma levels of aminotransferases, alkaline phosphatase, blood hydroperoxide, malondialdehyde in liver and kidney and blood creatinine. Vit. C antagonized the decrease of GSH level caused by CCl_4. In CCl_4 (chronic dose) + Vit. C group, plasma cholesterol and triglycerides levels were significantly decreased, while high density lipoprotein and protein concentrations were significantly increased, when compared to CCl_4 group. The treatment of rats with CCl_4 as a single dose (1 mL kg^{-1}) has no significant influence on lipids. Plasma urea and uric acid levels of CCl_4+Vit. C group were significantly increased as compared to CCl_4 group. These results showed that vitamin C had a protective effect on hepatotoxicity and renal toxicity caused by CCl_4 induced oxidative stress via its antioxidative property, reducing the lipid peroxidation and normalizing the glutathione level with improved the alterations in the biochemical markers. Moreover, Vit. C showed hypolipidemic effect in CCl_4-treated rats.

Key words: Carbon tetrachloride, vitamin C, liver enzymes, glutathione, malondiadehyde, oxidative stress, kidney markers, lipids

INTRODUCTION

Carbon tetrachloride (CCl_4), a well-known model for hepatic injury, requires biotransformation by hepatic microsomal cytochrome to produce the hepatotoxic metabolite, trichlormethyl free radicals (Brattin *et al.,* 1985). Free radicals react with sulfhydryl groups such as glutathione and protein thiols which eventually lead to membrane lipid peroxidation and necrosis (Brent and Rumack, 1993; Brautbar and Williams, 2002). The reactive intermediates formed during the metabolism of toxicants are capable of binding covalently to tissue macromolecules, which in may in turn cause tissues damage (Eaton *et al.,* 1995). It was shown that CCl_4-induced cirrhosis in rats results in oxidative stress in the kidney as seen by increased lipid peroxidation and protein oxidation accompanied by altered antioxidant status. CCl_4 caused a marked rise in lipid

peroxidation level in liver and kidney tissues, whereas glutathione, catalase, superoxide dismutase levels were decreased (Manna *et al.,* 2006). The treatment of rats with CCl_4 increased the level of urine creatinine, protein and urobilinogen (Khan *et al.,* 2009). An association between hydrocarbon exposure and glomerulonephrities was reported (Ravnskov, 2000).

Intake of antioxidant vitamins which are widely distributed in fruits could be beneficial in protection against hepatotoxicity (Williams, 1995). Vitamin C (Vit. C) is a well known antioxidant, which can protect the body from damage caused by free radicals that can be generated during normal metabolism as well as through exposure to toxins and carcinoges (Banerjee *et al.,* 2009). Vit. C could be effective in the protection against stannous chloride (El-Demerdash *et al.,* 2005), arsenic (Banerjee *et al.,* 2009) and pesticides (El-Gendy *et al.,* 2010) induced oxidative stress and liver toxicity. Moreover, vitamin C supplementation ameliorates cisplatin-induced acute renal failure in mice (Ajith *et al.,* 2009). Vitamin C reduced the oxidative stress induced renal failure (Ferretti *et al.,* 2008). In the literature, we find few articles report protective effect of Vit. C against CCl_4 toxicity. In this study, our aim was to investigate the protective effects of Vit. C on hepatotoxicity, renal toxicity and oxidative stress caused by CCl_4 in male rats.

MATERIALS AND METHODS

Chemicals: 5,5 dithiobis-2 nitrobenzoic acid (DTNB) was purchased from Sigma,USA. Vitamin C was obtained from Fisher Scientific, UK. Trichloroacetic acid, carbon tetrachloride and thiobarbituric acid were purchased from Merk Company, Darmstadt.

Animals: Male Wister rats weighting 180-200 g obtained from animal house, Faculty of Pharmacy, King Saud University. The rats were maintained under standard laboratory conditions (12 h light, temperature 23±1°C). They fed dry ration *ad lib*. This study was conducted in Zoology Department, King Saud University, Saudi Arabia.

Experimental design: Fourty-eight male rats were divided into two main groups for studying the protective effect of Vit. C against chronic and acute effects of CCl_4. Each main group is divided into three subgroups of eight rats each as follows : control group, CCl_4 group and CCl_4 + Vit. C group. In the first main group, CCl_4 mixed with corn oil and was injected (ip) twice a week (0.2 mL kg^{-1}) while vitamin C was administered orally daily for three months at dose level of 100 mg kg^{-1} (Sheweita *et al.*, 2001). Corn oil was given to control group at the same volume as the vehicle. In the second main group, Vit. C was administered orally (100 mg kg^{-1}) daily for 10 days, followed by a single injection of CCl_4 (ip) at dose level of 1 mL kg^{-1}. After 4 days from CCl_4 injection, the samples were collected in acute model. At the end of the chronic or acute treatment, all animals were kept in individual metabolic cages to collect urine samples through 24 h, followed by withdrawn blood samples from retro-orbital plexus of each rat by fine glass capillary tubes. Plasma was separated and kept in deep freezer. The livers and kidney of the treatment groups were removed rapidly and cut into small portions for lipid peroxidation and glutathione estimation.

Biochemical assays

Estimation of lipid peroxides: Blood hydroperoxide level was evaluated using free radical analytical system (Iran, Parma, Italy). The test is a colorimetric test that takes advantage of the ability of hydroperoxides to generate free radicals after reacting with some transitional metals. When buffered chromogenic substances are added, a colored complex appears (Wolff, 1994). The complex can be measured by a spectrophotometer.

Lipid peroxidation in the liver and kidney was measured by the formation of the thiobarbituric acid reactive material, malondialdehyde (MDA) (Ohkawa *et al.,* 1979).

Assay of reduced glutathione (GSH) in the liver and kidney

Homogenates: GSH level was measured by the method of Ellman (1959). Trichloroacetic acid (5%) was added to the homogenate to precipitate the protein. After centrifugation, the supernatant was separated, DTNB solution was added. The absorbance was measured at 412 nm. A standard graph was drawn using different concentrations of GSH solution. With the help of the standard graph, GSH contents in the liver and kidney homogenates were calculated.

Determination of liver and kidney function tests: AST, ALT (Reichling and Kaplan,1988), ALP (Moss, 1987), cholesterol (Allain *et al.*,1974), triglycerides (Fossati and Lorenzo, 1982), HDL (Lopes-Virella *et al.*, 1977) and protein (Gornall *et al.*,1949) were measured in plasma samples obtained from all groups of rats. Urea (Patton and Crouch, 1977), uric acid (Artiss and Entwistle, 1981) and creatinine (Kroll *et al.*, 1987) were evaluated in plasma and urine. The measurements were determined colorimetrically by using BioMerieux kits (France) according to standardized assay methods. The intensity of the coloration was measured by using spectrophotometer, UV/visible-Model-80-2106-00, Pharmacia Biotech.cambridge, England.

Statistical analysis: Parametric data expressed as arithmetic Mean±Standard Error (SE) were analyzed by two way ANOVA, followed by Least Significant Difference (LSD) for comparison of various treatments using the SPSS 13.0

RESULTS

Lipid peroxidation markers: Table 1 reports the changes in the levels of blood hydroperoxide and liver and kidney malondialdehyde (MDA). Both hydroperoxide and MDA levels elevated significantly in all treatment groups as compared to control. On the other hand, their levels in CCl_4+Vit. C group were significantly ($p<0.01$) lower than those of CCl_4 groups. The administration of vitamin C to rats injected with chronic and acute doses of CCl_4 reduced the MDA level in liver by 73.83 and 19.73%, respectively as compared with CCl_4 group. Similarly, it reduced the MDA concentration in the kidney by 77.58 and 18.37%.

Table 1: Blood hydroperoxide and tissue malondialdehyde levels of control, CCl_4 and CCl_4+ Vit. C groups

	Parameter								
				Malondialdehyde (nmol mg^{-1} protein)					
	Blood hydroperoxide (mg 100 mL^{-1})			Liver			Kidney		
Dose of CCl_4	Control	CCl_4	CCl_4+ Vit. C	Control	CCl_4	CCl_4+Vit. C	Control	CCl_4	CCl_4+Vit. C
0.2 mL kg^{-1} (i.p. injection twice a week for 3 months)	28.20±0.86	40.55±1.49**	31.55±0.90*b	0.30±0.02	1.49±0.06**	0.39±0.03*b	0.18±0.01	1.16±0.03**	0.26±0.03*b
1 mL kg^{-1} (Single injection i.p.)	24.85±0.52	63.76±2.95**	46.70±1.95**b	0.25±0.01	2.28±0.11**	1.83±0.07**b	0.21±0.01	1.96±0.08**	1.60±0.04**b

Each value is the Mean±SE, n = 8. Values marked with asterisks differ significantly from control value at *$p<0.05$, **$p<0.01$. Values marked with letter(b) differ significantly from CCl_4 group at $p<0.01$

Table 2: Effect of vitamin C on the GSH level (μg mg^{-1} protein) of rats terated with CCl_4 induced hepatic and renal damages

	Treatment					
	Liver			Kidney		
Dose of CCl_4	Control	CCl_4	CCl_4 + Vit. C	Control	CCl_4	CCl_4+Vit. C
0.2 mL kg^{-1} (i.p. injection twice a week for 3 months)	18.63±0.79	12.45±0.66**	17.48±1.04[b]	13.41±0.52	9.41±0.68**	13.00±0.82[b]
1 mL kg^{-1} (Single injection i.p.)	16.81±0.35	9.21±0.57**	12.75±0.42**[b]	14.50±0.61	7.38±0.34**	11.45±0.78**[b]

Each value is the Mean±SE, n = 8. Values marked with asterisks differ significantly from control value at **p<0.01. Values marked with letter(b) differ significantly from CCl_4 group at p<0.01

GSH level: The GSH level Table 2 in both liver and kidney homogenates decreased significantly (p<0.01) in the rats treated with chronic or acute dose of CCl_4. GSH level of CCl_4 (0.2 mL kg^{-1}) + Vit. C group did not change significantly, while it decreased significantly in CCl_4 (1 mL kg^{-1}) + Vit C group as compared to control. However, GSH level of CCl_4+Vit. C groups was significantly higher than those of CCl_4 groups.

Results of liver and kidney fuctions

Liver enzymes: Table 3 shows the levels of plasma AST, ALT and ALP of treatment groups. A significant high activities (p<0.01) of AST, ALT and ALP were found in rats treated with low or high dose of CCl_4. The activity of the enzymes in CCl_4 + Vit. C group was significantly lower than that of CCl_4 group.

Lipid profile: In the chronic model of CCl_4 a significant increase (p<0.01) in concentration of plasma cholesterol and triglycerides (Table 4) was recorded in all treatment groups. The values of CCl_4 + Vit. C group were significantly lower (p<0.01) than those of CCl_4 group. On the other hand, HDL-C of CCl_4 (Chronic dose) was significantly (p<0.05) lower than that of control, while it did not change significantly in CCl_4+Vit. C group. The treatment of rats with acute dose of CCl_4 or with CCl_4 + Vit. C has no significant influence on the previous parameters.

Protein level: The protein concentration Table 5 decreased significantly (p<0.01) in the rats treated with chronic and acute doses of CCl_4 by 21.17 and 26.23%, respectively as compared to control. Moreover, it decreased significantly in CCl_4 + Vit. C groups by 7.97 and 18.51%. However, the values of CCl_4 +Vit. C groups were significantly (p<0.01) higher than those of CCl_4 groups.

Markers of kidney function: The treatment of rats with CCl_4 (Chronic or acute dose) led to a significant decrease (p<0.01) in plasma level of urea and uric acid (p<0.01) and a significant increase (p<0.01) in creatinine level as compared to control (Table 6). These parameters were not change significantly in CCl_4 (0.2 mL kg^{-1}) + Vit. C group as compared to control, while they show significant difference in CCl_4 (1 mL kg^{-1}) +Vit. C group.

The results in Table 7 indicated a significant decrease (p<0.01) of urea and creatinine in urine of CCl_4 (chronic or acute)-treated rats and a significant increase (p<0.01) in uric acid level. The values of CCl_4 (0.2 mL kg^{-1}) + Vit. C group did not change significantly as compared to control, while they changed significantly in case of CCl_4 (1 mL kg^{-1}) + Vit. C group.

Table 3: Plasma levels of AST, ALT and ALP of control and treated groups

	Liver enzymes								
	AST (μL^{-1})			ALT (μL^{-1})			ALP (μL^{-1})		
Dose of CCl_4	Control	CCl_4	CCl_4+ Vit. C	Control	CCl_4	CCl_4+Vit. C	Control	CCl_4	CCl_4+Vit. C
0.2 mL kg^{-1} (i.p. injection twice a weekfor 3 months)	47.81±1.25	148.35±4.44**	59.39±3.02**^{b}	27.07±1.45	99.62±1.70**	31.74±1.95^b	2.99.49±15.82	516.89±26.77**	394.12±16.97*^{b}
1 m kg^{-1} (Single injection i.p.)	49.97±1.54	336.70±31.28**	243.39±13.22**^{a}	22.31±1.06	141.34±7.41**	120.67±5.51**^{a}	294.62±6.41	692.22±31.71**	603.85±25.43**

Each value is the Mean± SE, n = 8. Values marked with asterisks differ significantly from control value at *p<0.05, **p<0.01. Values marked with letter(b) differ significantly from CCl_4 group at ^{a}p<0.05, ^{b} p<0.01

Table 4: Plasma levels of cholesterol, triglycerides and HDL of control and treated groups

	Parameters								
	Cholesterol			Triglycerides			HDL-C		
Dose of CCL_4	Control	CCl_4	CCl_4+ Vit. C	Control	CCl_4	CCl_4+Vit. C	Control	CCl_4	CCl_4+Vit. C
0.2 mL kg^{-1} (i.p. injection twice a week for 3 months)	79.26±3.34	180.71±4.81**	110.52±2.35**^{b}	70.14±1.19	138.27±2.00**	85.17±5.63**^{b}	35.78±2.10	30.15±1.20*	36.43±2.87
1 mL kg^{-1} (Single injection i.p.)	79.60±1.38	78.00±1.78	76.11±2.50	76.59±1.50	83.18±3.46	80.75±1.67	36.44±2.00	33.89±1.59	37.14±1.91

Each value is the Mean±SE, n = 8. Values marked with asterisks differ significantly from control value at *p<0.05, **p<0.01. Values marked with letter(b) differ significantly from CCl_4 group at p<0.01

Table 5: Concentration of protein (g 100 mL^{-1}) in plasma of control and treated groups

	Treatment		
Dose of CCl_4	Control	CCl_4	CCl_4 + Vit. C
0.2 mL kg^{-1} (i.p. injection twice a week for 3 months)	8.41±0.24	6.63±0.27**	7.74±0.15*^{b}
1 mL kg^{-1} (Single injection i.p.)	7.51±0.13	5.54±0.12**	6.12±0.11**^{b}

Each value is the Mean±SE, n = 8. Values marked with asterisks differ significantly from control value at *p<0.05, **p<0.01. Values marked with letter(b) differ significantly from CCl_4 group at p<0.01

Table 6: Plasma levels of urea uric acid and creatinine of control and treated groups

	Parameters								
	Urea (mg 100 mL^{-1})			Uric acid (mg 100 mL^{-1})			Creatinine (mg 100 mL^{-1})		
Dose of CCl_4	Control	CCl_4	CCl_4+ Vit. C	Control	CCl_4	CCl_4+Vit. C	Control	CCl_4	CCl_4+Vit. C
0.2 mL kg^{-1} (i.p. injection twice a week for 3 months)	45.00±1.18	35.00±1.80**	44.69±1.26^b	2.00±0.22	0.53±0.07**	1.99±0.16^b	0.43±0.01	0.52±0.03**	0.41±0.02^b
1 m kg^{-1} (Single injection i.p.)	44.98±2.36	34.18±1.00**	39.23±0.84*^{b}	2.16±0.11	0.28±0.04**	1.04±0.10**^{b}	0.46±0.01	0.72±0.03**	0.67±0.01**

Each value is the Mean±SE, n = 8. Values marked with asterisks differ significantly from control value at *p<0.05, **p<0.01. Values marked with letter(b) differ significantly from CCl_4 group at p<0.0

Table 7: Concentration of urea, uric acid and creatinine in urine of control and treated groups

	Parameters								
	Urea (mg 100 mL^{-1})			Uric acid (mg 100 mL^{-1})			Creatinine (mg 100 mL^{-1})		
Dose of CCl_4	Control	CCl_4	CCl_4+ Vit. C	Control	CCl_4	CCl_4+Vit. C	Control	CCl_4	CCl_4+Vit. C
0.2 mL kg^{-1} (i.p. injection twice a week for 3 months)	110.82±6.18	69.49±3.47**	108.74±3.03^b	190.63±13.37	330.00±12.34**	203.62±6.34^b	124.57±7.92	82.72±3.55**	119.11±4.26^b
1 m kg^{-1} (Single injection i.p.)	112.60±4.80	40.09±1.19**	79.41±2.43**^{b}	193.37±8.27	282.03±14.91**	235.38±4.95**	102.32±4.25	64.38±4.35**	79.55±2.32**

Each value is the Mean±SE, n = 8. Values marked with asterisks differ significantly from control value at *p<0.05, **p<0.01. Values marked with letter(b) differ significantly from CCl_4 group at ^{a}p<0.05, ^{b}p<0.01

DISCUSSION

A number of chemicals including various environmental toxicants can cause sever cellular damages in different organs of body through the metabolic activation to highly reactive substances such as free radicals. CCl_4 is one of such environmental toxicant. The reactive metabolite trichloromethyl radical (CCl_3) has been formed from the metabolic conversion of CCl_4 by cytochrome (Noguchi *et al.*, 1982). These free radicals initiate the peroxidation of membrane poly-unsaturated fatty acids which-results in the generation of Reactive Oxygen Species (ROS) and finally cell necrosis (Recknagel *et al.*, 1989). However, when oxidative stress reaches a certain limit, a defense mechanisms against ROS become insufficient (Halliwell and Gutteridge, 2000), led to a decrease in the intracellular concentration of GSH and antioxidant enzymes (Yamamoto and Yamashita, 1999). In the present study it has been observed that CCl_4 induced a significant elevation of blood hydroperoxide and malondialdehyde (lipid peroxidation products) in both liver and kidney. Moreover, this toxicant caused a significant decrease in glutathione content in renal and hepatic tissues. Evidence of lipid peroxidation by increased levels of malondialdehyde and hydroperoxide is one of the primary means by which researchers have associated oxidative processes with an overall of decrease of cellular function. Increase of MDA to indicate lipid peroxidation level is an index used to identify free radicals-induced injuries (Janero, 1990).

Administration of CCl_4 causes sever liver injuries in the present rats. This injury is recognized by an increase in serum levels of AST, ALT and ALP, which are indices of liver cell damage and leakage of enzymes from cells (Rajesh and Latha, 2004).

In the present study, it has been observed that CCl_4 (chronic or acute) induced a significant decrease in the levels of plasma urea, uric acid and protein and a significant increase in plasma creatinine concentration. Moreover, urea and creatinine levels in urine of CCl_4-treated rats decreased significantly, while uric acid level increased significantly. The controversy in the literature about the effect of CCl_4 on kidney function may be due to the time and route of CCl_4 exposure. Manna *et al.* (2006) reported that there was no change in either the urea nitrogen or creatinine in the serum of mice administered CCl_4 orally at dose level of 1 mL kg^{-1} for 2 days. They also concluded that the time of CCl_4 exposure to the animals was not enough for the renal damage although oxidative stress induced by that exposure. It was observed a decrease in serum urea and creatinine levels of rats injected with 0.5 mL kg^{-1} CCl_4 (SC) ever other day for one month (Ogeturk *et al.*, 2005). The observed decrease in urea and uric acid levels in plasma may be due to the decreased protein levels observed in the rats treated with CCl_4. The decrease in protein level is likely due to the impairement of protein synthetic activity during stress conditions (Rao *et al.*, 1995). Creatinine is a metabolite of protein and is excreted in the urine via glomerular filtration

an elevation of its level in the blood is thus an indication of impaired kidney function. It appears that the decreased level of glutathione in both liver and kidney with increased level of malondialdehyde involved in the development of CCl_4-induced injuries which may lead to changes in the present markers related to liver and kidney damages. Increased lipid peroxidation is generally believed to be an important underlying cause of the initiation of oxidation stress related various tissue injury and cell death (Akca *et al*., 2005).

Lipid peroxidation is one of the principal causes of CCl_4-induced liver and kidney injuries mediated by free radical derivatives of CCl_4. The antioxidative activity of some substances and inhibition of free radical generation are important in protecting the liver (Ozturk *et al*., 2009) and kidney (Ogeturk *et al*., 2005) from CCl_4 induced damage. Present results show that vitamin C is able to reduce the toxicity to the rat liver and kidney induced by CCl_4. This was demonstrated by MDA, hydroperoxide, marker enzymes of liver, creatinine, urea, uric acid and protein levels. Antioxidant. Property of vitamin C results in part from the fact that its oxidation product, semidehydroascorbate radical is unreactive and therefore, not damaging (Halliwell, 1990). Vitamin C might ameliorate oxidative damage by decreasing lipid peroxidation and altering antioxidant defense system (El-Gendy *et al*., 2010) or by donating electrons to free radicals and quench their reactivity (Bendich, 1990). Few researchers reported that vitamin C may prevent CCl_4-induced hepatotoxicity (Ademuyiwa *et al*., 1994; Sheweita *et al*., 2001). It was showed that ascorbate markedly attenuated CCl_4-induced alterations in hepatic glutathione and ascorbic acid contents (Nakagawa, 1993). In the present study, the administration of vitamin C with CCl_4 reduced the hepatotoxicity and renal toxicity. It is possible that the positive effect of vitamin C on CCl_4 toxicity, might be the result of its effect on reduction of lipid peroxidation increase in liver and kidney tissues and alleviation of glutathione depletion. Reduced glutathione acts as intracellular free radical scavengers and protect cells against radical mediated lipid peroxidation (Cuddihy *et al*., 2008). It was showed that ascorbate prevented hepatic glutathione depletion in chemical-induced hepatotoxicity in mice (Mitra *et al*., 1988). Moreover, Ozturk *et al*. (2009) showed that vitamin C treatment to CCl_4-intoxicated animals normalized the antioxidant enzymes, superoxide dismutase and catalase in the liver of rats. Furthermore, the observed protective effect of vitamin C may result from its effect on normalizing the uric acid level that decreased by CCl_4. Uric acid is a major antioxidant in human plasma and acts as radical scavenger (Becker *et al*., 1989).

Hypercholesterolemia and hypertriglyceridemia are risk factor for predicting coronary heart disease (Rosamond *et al*., 2007; Austin *et al*., 1998). The present study demonstrates an increase in the level of triglycerides and cholesterol with a decrease in HDL level of CCl_4-treated rats (twice a week for 3 months) while they did not change in the rats given, a single dose of CCl_4. HDL plays an essential role in the transport of cholesterol to the liver for excretion into bile (Dietschy, 1997). The observed increase in cholesterol level may result from the decline in HDL level or from increased liver fatty acid synthesis (Gans, 1973). Moreover, Kato and Nakazawa (1987) concluded that the triacylglycerol accumulation in the cultured rat hepatocytes caused by carbon tetrachloride might be mediated by the suppression of the secretion of lysosomal acid triacytglycerol lipase activity.

Ascorbic acid may affect the development of atherosclerosis and the onest of acute coronary events by several molecular mechanisms; it helps in maintaining arterial wall integrity, it can alter cholesterol metabolism by modulating the conversion at cholesterol to bile acids and it can affect plasma triglyceride levels via modulation of lipoprotein lipase activity (Villacorta *et al*., 2007). In the present study, vitamin C attenuated the increase of cholesterol and triglycerides in the rats

treated with CCl_4. Moreover, HDL-C did not change significantly in CCl_4+vitamin C group. Turley *et al.* (1976) showed that dietary vitamin C is involved in the regulation of cholesterol metabolism by lowering cholesterol absorption. It was stated that the hypertriglyceridemia was caused by a slow uptake and removal of very low density lipoprotein triglycerides from the plasma (Bobek *et al.*, 1983). Vitamin C is an antioxidant protect very low-density lipoprotein from oxidation and may therefore facilitate its uptake by the liver and hence promote its removal from the plasma (Hasegawa *et al.*, 2002).

In conclusion; significantly decreased activities of hepatic plasma markers (ALT, AST, ALP) and levels of lipid peroxidation markers (hydroperoxide and malondialdehyde) along with normalizing of endogenous GSH level and markers of kidney function either in plasma or in urine suggest that Vit. C as a strong antioxidant has a protective effect against CCl_4-induced hepatotoxicity and renal toxicity by reducing the oxidative stress. Moreover, Vit. C showed hypolipidemic effect in CCl_4-treated rats with the maintaining HDL-C level in normal range. Hence, Vit C may be considered as a protective agent against hyperlipidemia induced by CCl_4.

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