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ORIGINAL ARTICLE

Mangiferin, a natural polyphenol protects the hepatic damage in mice caused by CCl_4 intoxication

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Abstract Mangiferin is a natural polyphenolic (C-glucoxyl xanthone) antioxidant present in the bark, fruits, roots and leaves of *Mangifera indica* Linn. In the present study, we investigated the protective effect of mangiferin against carbon tetrachloride (CCl_4)-induced hepatotoxicity in mice and compared it with silymarin, a standard hepatoprotective drug. The pretreatment of mangiferin (30 mg/kg body weight, i.p.) has shown it to possess a significant protective effect by lowering the serum aspartate and alanine aminotransferases, alkaline phosphatase, bilirubin and inflammatory mediator TNF-α. This hepatoprotective action was confirmed by histological observation. In addition, pretreatment of mangiferin prevented the elevation of hepatic malondialdehyde formation and the depletion of antioxidant status (superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase, glutathione-*S*-transferase, and total reduced glutathione) activity in the liver of CCl_4-injected mice. The results suggest that mangiferin exhibits potent hepatoprotective effects on CCl_4-induced liver damages in mice.

Keywords Mangiferin · CCl4-induced hepatotoxicity · Lipid peroxidation · Antioxidant enzymes · Tumor necrosis factor alpha

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Introduction

Carbon tetrachloride (CCl_4), a well-known industrial solvent, is most extensively used to produce hepatic injury (Weber et al. 2003). In the liver, CCl_4 is accumulated in hepatic parenchyma cells and metabolized to trichloromethyl free radicals (CCl_3 and/or CCl_3OO) by liver cytochrome P450-dependent monooxygenase (Recknagel 1983). These trichloromethyl radical (CCl_3 and/or CCl_3OO) generated can bind with PUFA, forming alkoxy ($R_•$) and peroxy radicals ($ROO_•$) that generate lipid peroxide which may cause cell membrane damage, alteration in enzyme activity and finally induction of hepatic injury/necrosis (Pandit et al. 2004). The covalent binding of trichloromethyl free radicals to cellular proteins is considered the initial step in a chain of events that eventually lead to membrane lipid peroxidation and finally to cell necrosis (Brautbar and Williams 2002).

The use of natural remedies like Ayurveda, Chinese, European, and other system of medicines for the treatment of liver diseases is long known (Thyagarajan et al. 1998). The mango plant has been the focus of attention of many researchers searching for the next source of potent antioxidants (Garrido et al. 2004). Mangiferin is a natural polyphenolic (C-glucoxyl xanthone) antioxidant derivative present in the bark, fruits, roots and leaves of *Mangifera indica* Linn. (Anacardiaceae) (Ghosal et al. 1996). It is recommended in the Indian systems of medicine for the treatment of immuno-deficiency diseases such as arthritis, diabetes, hepatitis, cancer, autoimmune disorders, arteriosclerosis, and coronary heart diseases (Leiro et al. 2003). Mangiferin has been reported to exhibit antioxidant (Sanchez et al. 2000), antitumor (Guha et al. 1996), antiviral (Zheng and Lu 1990), anti-inflammatory, and antibacterial activity (Leiro et al. 2003). However, there is

no experimental evidence presently available in the literature with regard to its effect on CCl_4-induced hepatotoxicity. Taking this point into consideration, the present study was carried out in an attempt to investigate the possible hepatoprotective effect of mangiferin on CCl_4-induced hepatotoxicity in mice.

Methods

Animals

Male Swiss albino mice weighing about 25–30 g, of either sex were purchased from the Karigiri Hospital and Research Centre, Vellore, India. The animals were housed in large spacious cages. They were acclimatized for a week in a light and temperature–controlled room with a 12-h dark–light cycle and fed with commercial pelleted feed from Hindustan Lever Ltd. (Mumbai, India) and water ad libitum. The animals used in this study were treated and cared for well, in accordance with the guidelines recommended by the Committee for the Purpose of Control and Supervision of Experiments on Animals, Ministry of Culture, Government of India, Chennai.

Drugs and chemicals

The commercially available mangiferin powder was obtained from the Natural remedies private limited, Bangalore, India. Silymarin, a standard hepatoprotective drug was obtained from SRS Pharmaceuticals, Mumbai, India. All other reagents and chemicals used were of analytical grade.

Experimental protocol

In this experiment, mice were divided into five groups, consisting of six animals each. The control group received corn oil (1 ml/kg body weight) only throughout the experimental period. The CCl_4-induced test group was treated with a single dose of CCl_4 (20 mg/kg body weight, i.p. dissolved in corn oil). The drug-treated group, were given mangiferin suspended in saline (30 mg/kg body weight, i.p.) once daily, for three consecutive days. Three hours after the final treatment, the mice were treated with CCl_4 (20 mg/kg body weight, i.p. dissolved in corn oil) (Hwang et al. 2009). The dose of mangiferin adopted in this study is based on our preliminary studies in our research group. The positive control group, was given silymarin suspended (25 mg/kg body weight, i.p.) once daily, for three consecutive days, and 3 h after the final treatment, the mice were treated with CCl_4 (20 mg/kg body weight, i.p. dissolved in corn oil). The placebo control group was administered with mangiferin alone suspended in saline (30 mg/kg body weight, i.p.). At the end of the experimental period (18 h after the administration of CCl_4), the mice were decapitated. The trunk blood was collected; the serum was separated, which was analyzed for various biochemical parameters. Tissue samples from the liver were obtained for biochemical and histological analysis.

Assessment of hepatoprotective activity through biochemical parameters

The activities of serum glutamyl oxaloacetate transaminase, serum glutamyl pyruvate transaminase, and alkaline phosphatase (ALP) and total bilirubin were estimated by using diagnostic kits (Span Diagnostics, India).

In the hepatic tissue samples, lipid peroxidation was determined by the procedure of Ohkawa et al. (1997). Malondialdehyde (MDA), formed as an end product of the peroxidation of lipids, served as an index of oxidative stress. Superoxide dismutase was assayed according to the method of Marklund and Marklund (1974). The unit of enzyme activity is defined as the enzyme required to give 50% inhibition of pyrogallol auto-oxidation. Catalase was assayed by the method of Sinha (1972). In this method, dichromate in acetic acid was reduced to chromic acetate when heated in the presence of hydrogen peroxide (H_2O_2), with the formation of perchloric acid as an unstable intermediate. Chromic acetate thus produced was measured colorimetrically at 610 nm. Glutathione peroxidase (GPx) was assayed by the method of Rotruk et al. (1973) based on the reaction between glutathione remaining after the action of GPx and 5,5′-dithiobis-(2-nitrobenzoic acid) to form a complex that absorbs maximally at 412 nm. Glutathione reductase was assayed by the method of Bellomo et al. (1987). Glutathione-*S*-transferase was assayed by the method of Habig et al. (1974). Total reduced glutathione (GSH) was determined by the method of Moron et al. (1979). The protein content was determined by the method of Lowry et al. (1951) using bovine serum albumin as a standard.

Effect of mangiferin and silymarin on TNF-α production

Tumor necrosis factor alpha (TNF-α) level in serum of control and experimental mice were determined by enzyme-linked immunosorbent assay (Cayman Chemicals, USA), according to the manufacturer's instructions.

Histopathological analysis of liver

Immediately after sacrifice, a portion of the liver was fixed in 10% formalin, then washed and dehydrated in

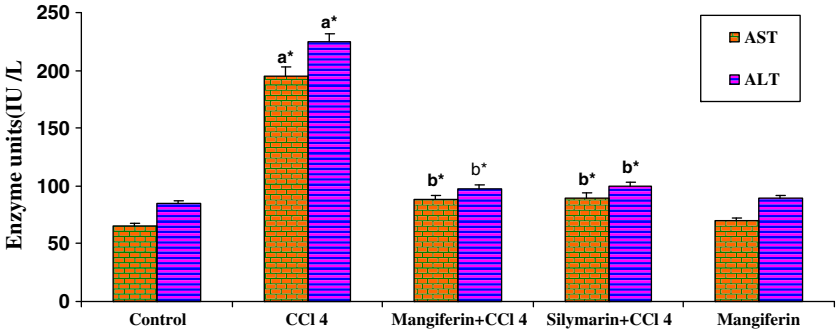

Fig. 1 Effect of mangiferin on liver functional markers (*AST* and *ALT*) in carbon tetrachloride (*CCl_4*)-intoxicated mice serum. Treatment of groups is as follows: group I, control (saline); group II, CCl_4 (20 mg/kg body weight, i.p.) single dose; group III, mangiferin+CCl_4 (30 mg/kg body weight, i.p.) once daily for three consecutive days (3 h after the final treatment, the mice were treated with CCl_4 (20 mg/kg body weight, i.p.)); group IV, silymarin+CCl_4 (25 mg/kg body weight, i.p.; 3 h after the final treatment, the mice were treated with CCl_4 (20 mg/kg body weight, i.p.)); group V, the placebo control group received mangiferin alone (30 mg/kg body weight, i.p.). Each value represents the mean±SD of six mice. Comparisons were made as follows: *a* control vs. CCl_4; *b* CCl_4 vs. mangiferin+CCl_4 and CCl_4 vs. silymarin+CCl_4. The symbols represent statistical significance at $*p<0.05$. Statistical analysis was calculated by one-way ANOVA followed by Student–Newman–Keul's test

descending grades of isopropanol and finally with xylene. The tissue was then embedded in molten paraffin wax. Sections were cut at 5 μm thickness, stained with hematoxylin and eosin for photomicroscopic observations of the liver histological architecture of the control and treated mice at ×400 magnification.

Statistical analysis

Results were expressed as mean±SD and statistical analysis was performed using ANOVA, to determine the significant differences between the groups, followed by Student–Newman–Keul's test. $P<0.05$ implied significance.

Results

Effect of mangiferin on liver functional marker enzymes (AST, ALT, and ALP) in CCl_4-intoxicated mice

The activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT), and ALP enzymes in serum were significantly increased in the CCl_4-treated group when compared with the normal control group. The elevated levels of these biochemical parameters clearly indicated the damage of the hepatic cells. However, the mangiferin pretreatment significantly prevented the elevation of these enzymes induced by CCl_4. Standard drug silymarin also

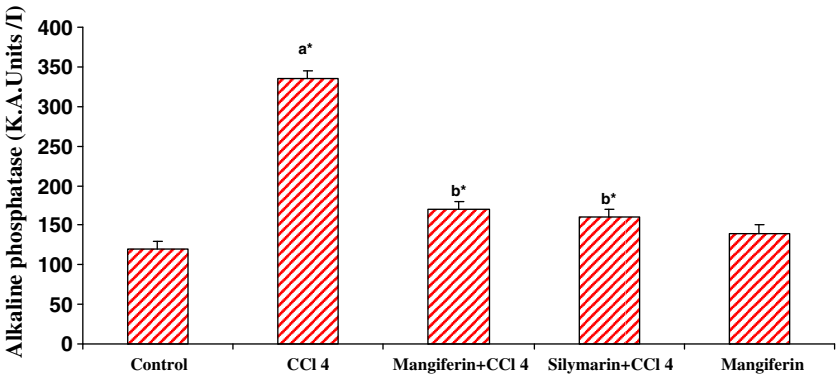

Fig. 2 Effect of mangiferin on alkaline phosphatase enzyme in carbon tetrachloride (*CCl_4*)-intoxicated mice serum. Treatment of groups is as follows: group I, control (saline); group II, CCl_4 (20 mg/kg body weight, i.p.) single dose; group III, mangiferin+CCl_4 (30 mg/kg body weight, i.p.) once daily for three consecutive days (3 h after the final treatment, the mice were treated with CCl_4 (20 mg/kg body weight, i.p.)); group IV, silymarin+CCl_4 (25 mg/kg body weight, i.p.; 3 h after the final treatment, the mice were treated with CCl_4 (20 mg/kg body weight, i.p.)); group V, the placebo control group received mangiferin alone (30 mg/kg body weight, i.p.). Each value represents the mean±SD of six mice. Comparisons were made as follows: *a* control vs. CCl_4; *b* CCl_4 vs. mangiferin+CCl_4 and CCl_4 vs. silymarin+CCl_4. The symbols represent statistical significance at $*p<0.05$. Statistical analysis was calculated by one-way ANOVA followed by Student–Newman–Keul's test

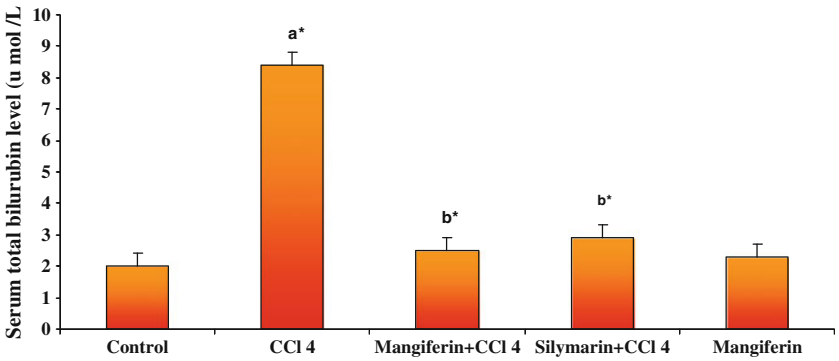

Fig. 3 Effect of mangiferin on serum total bilirubin in carbon tetrachloride (*CCl_4*)-intoxicated mice. Treatment of groups are as follows: group I, control (saline); group II, CCl_4 (20 mg/kg body weight, i.p.) single dose; group III, mangiferin+CCl_4 (30 mg/kg body weight, i.p.) once daily for three consecutive days (3 h after the final treatment, the mice were treated with CCl_4 (20 mg/kg body weight, i.p.)); group IV, silymarin+CCl_4 (25 mg/kg body weight, i.p.; 3 h after the final treatment, the mice were treated with CCl_4 (20 mg/kg body weight, i.p.)); group V, the placebo control group received mangiferin alone (30 mg/kg body weight, i.p.). Each value represents the mean±SD of six mice. Comparisons were made as follows: *a* control vs. CCl_4; *b* CCl_4 vs. mangiferin+CCl_4 and CCl_4 vs. silymarin+CCl_4. The symbols represent statistical significance at $*p<0.05$.Statistical analysis was calculated by one-way ANOVA followed by Student–Newman–Keul's test

showed remarkable protection towards CCl_4 intoxication (Figs. 1 and 2).

Effect of mangiferin on serum total bilirubin in CCl_4-intoxicated mice

The mice treated with CCl_4 alone showed a significant increase in serum bilirubin level when compared with the normal control group. Pretreatment with mangiferin exhibited a significant decrease in the bilirubin level. The results obtained were comparable with the standard drug silymarin (Fig. 3).

Effect of mangiferin on inflammatory mediator TNF-α in CCl_4-intoxicated mice

The levels of tumor necrosis factor alpha in the mice treated with CCl_4 alone were systemically overproduced in the serum. Mangiferin pretreatment significantly protected the tumor necrosis factor alpha elevation in the serum of CCl_4-

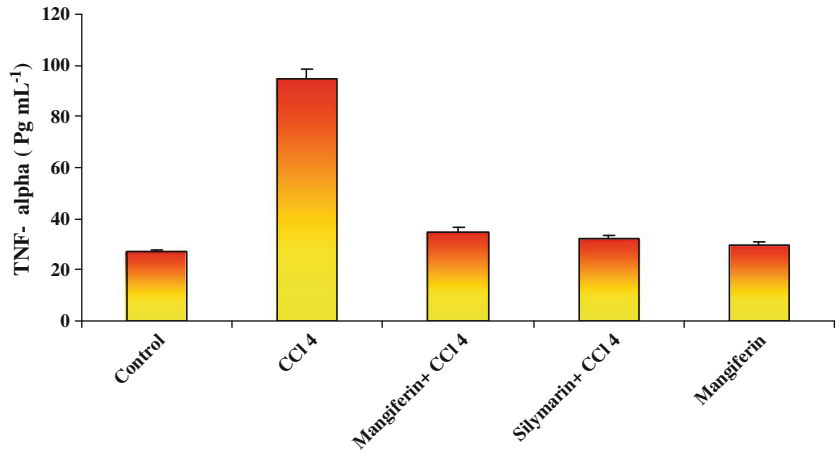

Fig. 4 Hepatoprotective effect of mangiferin on serum TNF-α levels in carbon tetrachloride (*CCl_4*)-intoxicated mice. Treatment of groups are as follows: group I, control (saline); group II, CCl_4 (20 mg/kg body weight, i.p.) single dose; group III, mangiferin+CCl_4 (30 mg/kg body weight, i.p.) once daily for three consecutive days (3 h after the final treatment, the mice were treated with CCl_4 (20 mg/kg body weight, i.p.)); group IV, silymarin+CCl_4 (25 mg/kg body weight, i.p.; 3 h after the final treatment, the mice were treated with CCl_4 (20 mg/kg body weight, i.p.); group V, the placebo control group received mangiferin alone (30 mg/kg body weight, i.p.). Each value represents the mean±SD of six mice. Comparisons were made as follows: *a* control vs. CCl_4; *b* CCl_4 vs. mangiferin+CCl_4 and CCl_4 vs. silymarin+CCl_4. The symbols represent statistical significance at $*p<0.05$. Statistical analysis was calculated by one-way ANOVA followed by Student–Newman–Keul's test

intoxicated mice (Fig. 4). The results obtained were found to be similar to standard drug silymarin.

Histopathological findings

The liver showed the following histopathological changes (Fig. 5). In the control and groups treated with mangiferin alone (Fig. 5a, e), the liver central vein was surrounded by hepatocytes with clear granular cytoplasm. Mice intoxicated with CCl_4 showed damage with enlarged cells, vaculated cytoplasm and large nuclei with condensed chromatin (Fig. 5b). However, CCl_4-intoxicated mice pretreated with mangiferin and silymarin showed central vein and hepatocytes with reactive and normal cells (Fig. 5c, d).

Effect of mangiferin on lipid peroxidation levels and antioxidant enzymes in CCl_4-intoxicated mice

In mice treated with carbon tetrachloride alone, the MDA level was increased significantly when compared with the control group, whereas a significant decrease in antioxidant enzymes (superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase, and glutathione-*S*-transferase) and total reduced glutathione was observed in CCl_4-treated mice. However, pretreatment of mangiferin significantly

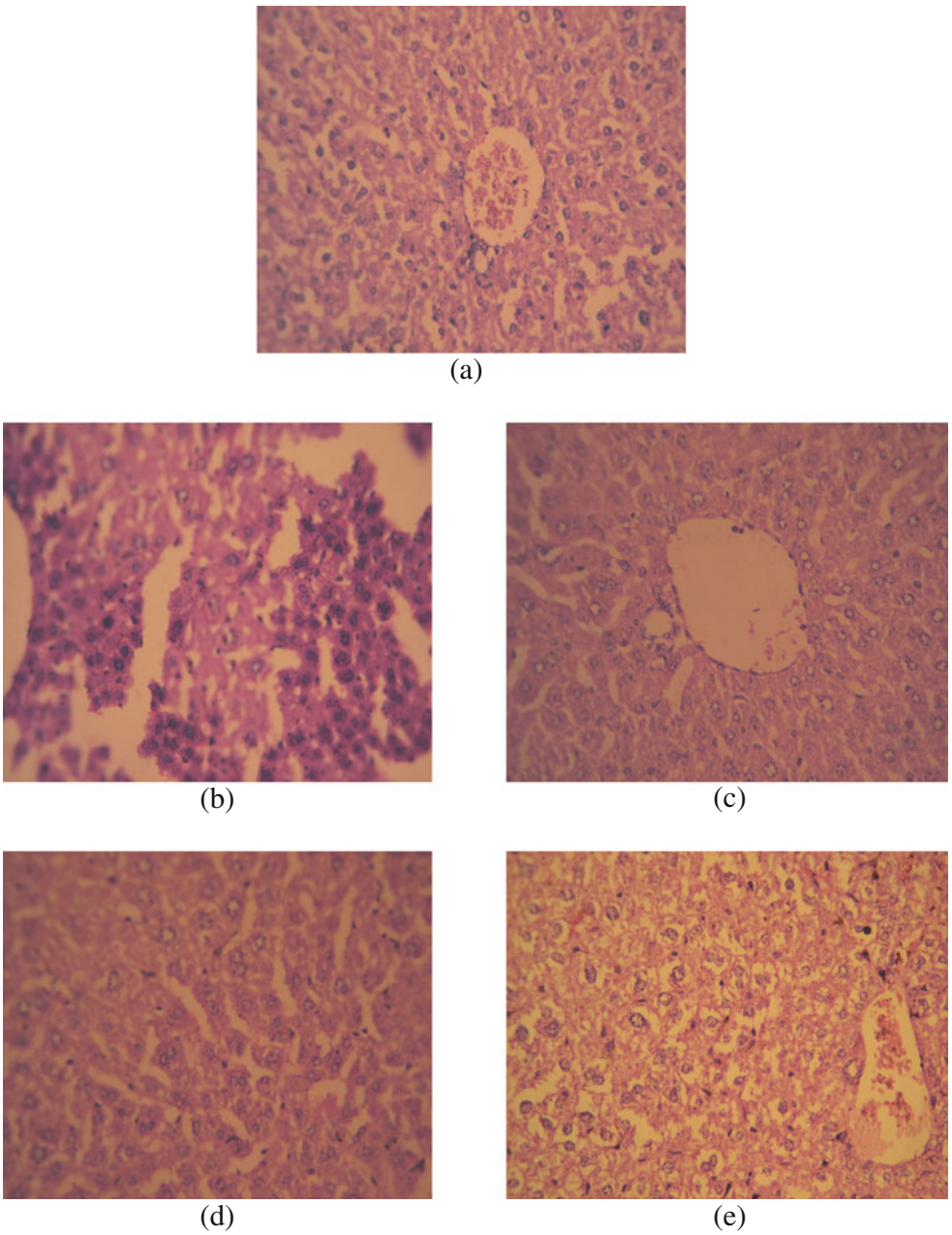

Fig. 5 Hematoxylin and eosin staining for photomicrographs of liver sections in mice at ×400 magnification. **a** Control group, showing central vein surrounded by hepatocytes with clear to granular cytoplasm. **b** CCl_4-treated group; showing damage with enlarged cells, vaculated cytoplasm and large nuclei with condensed chromatin. **c** Mangiferin+CCl_4-treated group; showing central vein and hepatocytes with reactive and normal cells. **d** Silymarin+CCl_4-treated group; showing reactive hepatocytes (binucleate) with normal and degenerative hepatocytes. **e** Mangiferin-treated group; showing central vein and hepatocytes with feathery cytoplasm were cells appear enlarged (ballooned)

prevented the MDA elevation and antioxidant enzymes and glutathione depletion induced by CCl_4 intoxication. Silymarin also protected the liver from elevating MDA levels and depleted antioxidant status and the results were comparable with normal values (Table 1).

Discussion

The most remarkable symptom of CCl_4-induced hepatotoxicity is the formation of reactive intermediate such as trichloromethyl and trichloro peroxymethyl free radicals, when CCl_4 is metabolized by cytochrome P450 system. These free radicals alkylate cellular proteins and other macromolecules with a simultaneous attack on polyunsaturated fatty acids, in the presence of oxygen produce lipid peroxides, leading to liver damage (Bishayee et al. 1995). Thus, free radical generation inhibition and suppression of cytochrome P450 system would result in a reduction in the level of reactive metabolites and is important in protection against CCl_4-induced liver lesions (Castro et al. 1974). Herbs have recently become an attractive source material for the development of drugs. Mangiferin from *M. indica* L. has been found to scavenge free radicals because of its antioxidant property. As reported in previous studies, mangiferin exhibited various pharmacological activities like antidiabetic, antioxidant, antiproliferative, anti-inflammatory (Leiro et al. 2003), and immunomodulatory properties (Andreu et al. 2005). Therefore in our study, the effect of mangiferin from *M. indica* L. on CCl_4-induced liver damage was investigated to assess its hepatoprotective effect based on its antioxidant activity.

Aspartate transaminase, alanine transaminase and alkaline phosphatase in plasma have been reported to be a sensitive indicator of liver injury. The disturbance in the transport function of the hepatocytes as a result of hepatic injury causes the leakage of enzymes from cells due to the altered permeability of membrane. This results in decreased levels of AST, ALT and ALP in the hepatic cells and a raised level in serum (Yadav and Dixit 2003). In accordance with those reports, a single dose of CCl_4 injection in our study developed hepatic damage in mice as evidenced by the substantial increment of hepatic marker enzymes (AST, ALT, and ALP), total bilirubin, and histophatological abnormalities. Pretreatment with mangiferin (30 mg/kg body weight, i.p.) for 3 days before the single dose of

Table 1 Effect of mangiferin on liver lipid peroxidation levels and antioxidant status in carbon tetrachloride (CCl_4)-intoxicated mice

Parameters	Group 1 control[a]	Group 2 (CCl_4)[a]	Group 3 (mangiferin+CCl_4)[b]	Group 4 (silymarin+CCl_4)[c]	Group 5 (mangiferin)[d]
LPO[e]	1.30±0.10	2.71±0.16[l, *]	1.46±0.13[m, *]	1.68±0.12[m, *]	1.25±0.13
SOD[f]	228.33±15.83	115.50±8.35[l, *]	206.67±12.53[m, *]	193.17±11.63[m, *]	221.67±16.83
CAT[g]	15.50±1.19	8.73±0.71[l, *]	14.15±1.07[m, *]	13.817±1.649[m, *]	15.16±1.75
GPx[h]	74.58±5.73	33.41±2.35[l, *]	71.08±4.43[m, *]	66.667±3.44[m, *]	73.08±4.74
GR[i]	125.13±10.91	74.58±6.20[l, *]	117.13±7.32[m, *]	112.13±5.89[m, *]	122.13±6.39
GST[j]	98.25±7.01	76.16±4.81[l, *]	93.08±6.58[m, *]	91.083±6.970[m, *]	97.41±6.86
Total reduced glutathione[k]	43.75±2.935	26.83±1.87[l, *]	41.75±2.68[m, *]	40.250±2.524[m, *]	42.41±2.86

Statistical analysis was calculated by one-way ANOVA followed by Student–Newman–Keul's test

LPO lipid peroxidation, *SOD* superoxide dismutase, *CAT* catalase, *GPx* glutathione peroxidase, *GR* glutathione reductase, *GST* glutathione-*S*-transferase

*$p<0.05$, statistically significance

[a] In 20 mg/kg body weight, i.p.

[b] In 30 mg/kg body weight, i.p.

[c] In 25 mg/kg body weight, i.p

[d] In 30 mg/kg body weight, i.p.

[e] In nanomoles of MDA formed per milligram of protein

[f] In micromoles of H_2O_2 consumed per minute per milligram of protein

[g] In units per milligram of protein (1 U=amount of enzyme that inhibits the auto-oxidation of pyrogallol by 50%)

[h] In micrograms of GSH utilized per minute per milligram of protein

[i] In nanomoles of NADPH oxidized per minute per milligram of protein

[j] In nanomoles of 1-chloro-2,4-dinitrobenzene-GSH conjugate formed per minute per milligram of protein

[k] In nanomoles per milligram per protein. Each value represents the mean±SD of six mice

[l] Comparison: control vs. CCl_4

[m] Comparisons: CCl_4 vs. mangiferin+CCl_4 and CCl_4 vs. silymarin+CCl_4

CCl_4 significantly lowered the levels of hepatic enzymes (AST, ALT, and ALP) and bilirubin. This fact was further supported by the histological observations as shown in Fig. 5.

Oxidative stress is defined in general as excess formation and/or insufficient removal of reactive oxygen species and reactive nitrogen species. It is considered to play a prominent causative role in many diseases including liver damage (Valko et al. 2007). Thus, the antioxidant activity or the inhibition of the generation of free radicals is important in the protection against CCl_4-induced hepatotoxicity (Castro et al. 1974). Antioxidant enzymes such as superoxide dismutase, catalase, glutathione peroxidase, glutathione-*S*-transferase, glutathione reductase and blood glutathione represent one protection against oxidative tissue damage (Wang et al. 2004). SOD is an effective defense enzyme that converted the dismutation of superoxide anions into H_2O_2 (Reiter et al. 2000). Catalase is a hemeprotein in all aerobic cells that metabolize H_2O_2 to oxygen and water. Glutathione peroxidase plays an important role in the detoxification of xenobiotics in the liver and catalyses the reduction of H_2O_2 and hydroperoxides to nontoxic products. Glutathione reductase is a cytosolic hepatic enzyme involved in the detoxification of a range of xenobiotic compounds by their conjugation with GSH. These enzymes constitute a mutually supportive team of defense against reactive oxygen species (Venukumar and Latha 2002). In the present study, the hepatic MDA level was increased in CCl_4-intoxicated mice, whereas a significant decrease in antioxidant enzymes (superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase, and glutathione-*S*-transferase) and total reduced glutathione was observed. However, pretreatment of mangiferin significantly prevented the MDA elevation and antioxidant status depletion induced by CCl_4 intoxication. The antiperoxidative role of magniferin, a natural polyphenol observed in our study could have been attributed due to its maintenance on cellular oxidant–antioxidant balance by decreasing the localized O_2 concentration and generating mangiferin phenoxy radicals, thereby reducing free radical mediated lipid peroxidation (Ghosal et al. 1996). As per earlier reports, polyphenols reduce oxidative stress by preferentially neutralizing the reactive free radicals, as their reaction with several damaging free radicals produces radicals that are less reactive towards biomolecules (Priyadarsini et al. 2002). Moreover, free radical scavenging activity of mangiferin is well established in other previous studies (Leiro et al. 2003).

Tumor necrosis factor alpha (TNF-α) is an important cytokine in the development of various liver diseases. The proinflammatory cytokines induced by TNF-α were thought to be responsible for the pathogenesis of liver diseases (Youssef and McCullough 2002). In our present study, tumor necrosis factor-α level was systemically overproduced in the serum of CCl_4-treated mice. Mangiferin pretreatment significantly protected the tumor necrosis factor-α elevation in CCl_4-intoxicated mice (Fig. 4). It has been previously reported that mangiferin, a polyphenolic free-radical scavenger is capable of inhibiting TNF-α production as well as the expression of genes coding TNF-α (García et al. 2002).

In conclusion, the present study indicates that mangiferin treatment prevents CCl_4-induced liver damage in mice, possibly through its antioxidant action.

References

Andreu GP, Delgado R, Velho JA, Curti C, Vercesi AE (2005) Iron complexing activity of mangiferin, a naturally occurring glucosylxanthone, inhibits mitochondrial lipid peroxidation induced by Fe(2+)-citrate. Eur J Pharmacol 513:47–55

Bellomo G, Mirabelli F, Dimonte D, Richelmi P, Thor H, Orrenius C, Orrenius S (1987) Formation and reduction of glutathione-mixed disulfides during oxidative stress. Biochem Pharmacol 36:1313–1320

Bishayee A, Sarkar A, Chatterjee M (1995) The hepatoprotective activity of carrot (*Daucas carota* L.) against carbon tetrachloride intoxication in mouse liver. J Ethnopharmacol 47:69–74

Brautbar N, Williams J (2002) Industrial solvents and liver toxicity: risk assessment, risk factors and mechanisms. Int J Hyg Environ Health 205:479–491

Castro JA, Ferrya GC, Castro CR, Sasame S, Fenes OM, Gilette JR (1974) Prevention of carbon tetrachloride-induced necrosis by inhibitors of drug metabolism. Further studies on the mechanism of their action. Biochem Pharmacol 23:295–302

García D, Delgado R, Ubeira Leiro J (2002) Modulation of rat macrophage function by the *Mangifera indica* L. extracts and mangiferin. Int Immunopharmacol 2:797–806

Garrido G, Gonzalez D, Lemus Y, Garcia D, Lodeiro L, Quintero G (2004) In vivo and in vitro anti-inflammatory activity of *Mangifera indica* L. extract (VIMANG). Pharmacol Res 50:143–149

Ghosal S, Rao G, Saravanan Misra N, Rana D (1996) A pausible chemical mechanism of the bioactivities of mangiferin. Indian J Chem 35B:561–566

Guha S, Ghosal S, Chattopadhyay U (1996) Antitumor, immunomodulatory and anti-HIV effect of mangiferin, a naturally occurring glucosylxanthone. Chemotherapy 42:443–451

Habig WH, Pabst MJ, Jakoby WB (1974) Glutathione-*S*-transferases. The first enzymatic step in mercapturic acid formation. J Biol Chem 249:7130–7139

Hwang YP, Choi JH, Jeong HG (2009) Protective effect of the *Aralia continentalis* root extract against carbon tetrachloride-induced hepatotoxicity in mice. Food Chem Toxicol 47:75–81

Leiro JM, Alvarez E, Arranz JA, Siso IG, Orallo F (2003) In vitro effects of mangiferin on superoxide concentrations and expression of the inducible nitric oxide synthase, tumour necrosis factor-alpha and transforming growth factor-beta genes. Biochem Pharmacol 65(8):1361–1371

Lowry OH, Rosebrough NJ, Farr AI, Randall PRJ (1951) Protein measurement with the folin phenol reagent. J Biol Chem 193:265–275

Marklund SL, Marklund G (1974) Involvement of superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase. Eur J Biochem 47:469–474

Moron MS, Depierre JW, Mannervik B (1979) Levels of glutathione. glutathione reductase and glutathione-*S*-transferase activities in rat lung and liver. Biochem Biophys Acta 582:67–78

Ohkawa H, Ohish N, Yagi K (1997) Assay for lipid peroxides in animal tissues by thiiobarbituric acid. Anal Biochem 95:351–358

Pandit S, Sur TK, Jana U, Debnath PK, Sen S, Bhattacharyya D (2004) Prevention of carbon tetrachloride-induced hepatotoxicity in rats by *Adhatoda vasica* leaves. Indian J Pharmacol 36 (5):312–320

Priyadarsini KI, Khopde FM, Kumar SM, Mohan H (2002) Free radical studies of ellagic acid, a natural phenolic antioxidant. J Agric Food Chem 50:2200–2206

Recknagel RO (1983) A new direction in the study of carbon tetrachloride hepatotoxicity. Life Sci 33:401–408

Reiter RJ, Tan D, Osuna C, Gitto E (2000) Actions of melatonin in the reduction of oxidative stress. A review. J Biomed Sci 7:444–458

Rotruk JT, Pope AL, Ganther HE, Swanson AB, Hafeman DG, Hekstra WG (1973) Selenium, biochemical role as a component of glutathione peroxidase purification and assay. Science 179:588–590

Sanchez GM, Re L, Giuliani A, Nunez-Selles AJ, Davison GP, Leon-Fernandez OS (2000) Protective effects of *Mangifera indica* L. extract, mangiferin and selected antioxidants against TPA-induced biomolecules oxidation and peritoneal macrophage activation in mice. Pharmacol Res 42:565–573

Sinha AK (1972) Colorimetric assay of catalase. Anal Biochem 47:389–394

Thyagarajan SP, Jayaram S, Gopalakrishnan V, Hari R, Jeyakumar P, Visioli F, Bellomo G, Galli C (1998) Free radical-scavenging properties of olive oil polyphenols. Biochem Biophys Res Commun 247:60–64

Valko M, Leibfritz D, Moncol J, Cronin MTD, Mazur M, Telser J (2007) Free radicals and antioxidants in normal physiological functions and human disease. Int J Biochem Cell Biol 39:44–84

Venukumar MR, Latha MS (2002) Antioxidant activity of *Curculigo orchioides* in carbon tetrachloride-induced hepatopathy in rats. Indian J Clin Biochem 17:80–87

Wang BJ, Liu CT, Tseng CY, Wu CP, Yu ZR (2004) Hepatoprotective and antioxidant effects of Bupleurum kaoi Liu (Chao et Chuang) extract and its fractions fractionated using supercritical CO2 on CCl4-induced liver damage. Food Chem Toxicol 42:609–617

Weber LWD, Boll M, Stampfl A (2003) Hepatotoxicity and mechanism of action of haloalkanes: carbon tetrachloride as a toxicological model. Crit Rev Toxicol 33:105–136

Yadav NP, Dixit VK (2003) Hepatoprotective activity of leaves of *Kalanchoe pinnata* Pers. J Ethnopharmacol 86(23):197–202

Youssef W, McCullough AJ (2002) Diabetes mellitus, obesity, and hepatic steatosis. Semin Gastrointest Dis 13:17–30

Zheng MS, Lu ZY (1990) Antiviral effect of mangiferin and isomangiferin on herpes simplex virus. Chin Med J 103:160–165