HEPATOProtective EFFECTS OF 18β-Glycyrrhetinic ACID ON CARBON TETRACHLORIDE-INDUCED LIVER INJURY: INHIBITION OF CYTOCHROME P450 2E1 EXPRESSION

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The protective effects of 18β-glycyrrhetinic acid (GA), the aglycone of glycyrrhizin (GL) derived from licorice, on carbon tetrachloride-induced hepatotoxicity and the possible mechanisms involved in this protection were investigated in mice. Pretreatment with GA prior to the administration of carbon tetrachloride significantly prevented an increase in serum alanine, aspartate aminotransferase activity and hepatic lipid peroxidation in a dose-dependent manner. In addition, pretreatment with GA also significantly prevented the depletion of glutathione (GSH) content in the livers of carbon tetrachloride-intoxicated mice. However, reduced hepatic GSH levels and glutathione-S-transferase activities were unaffected by treatment with GA alone. Carbon tetrachloride-induced hepatotoxicity was also prevented, as indicated by a liver histopathologic study. The effects of GA on the cytochrome P450 (P450) 2E1, the major isozyme involved in carbon tetrachloride bioactivation, were also investigated. Treatment of mice with GA resulted in a significant decrease of the P450 2E1-dependent hydroxylation of p-nitrophenol and aniline in a dose-dependent manner. Consistent with these observations, the P450 2E1 expressions were also decreased, as determined by immunoblot analysis. GA also showed antioxidant effects upon FeCl2-ascorbate-induced lipid peroxidation in mice liver homogenate and upon superoxide radical scavenging activity. These results show that protective effects of GA against the carbon tetrachloride-induced hepatotoxicity may be due to its ability to block the bioactivation of carbon tetrachloride, primarily by inhibiting the expression and activity of P450 2E1, and its free radical scavenging effects.

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INTRODUCTION

Many hepatotoxins including carbon tetrachloride, nitrosamines, and polycyclic aromatic hydrocarbons require metabolic activation, especially by liver cytochrome P450 (P450) enzymes, to form reactive, toxic metabolites, that in turn produce liver injury in experimental animals and humans [1]. Carbon tetrachloride, a well known model compound for the production of chemical hepatic injury, requires biotransformation by hepatic microsomal P450 to produce hepatotoxic metabolites, namely trichloromethyl free radicals (CCl3 and/or CCl 3 OO−) [2].

Trichloromethyl free radicals can react with sulphydryl groups, such as glutathione (GSH) and protein thiols, and the covalent binding of trichloromethyl free radicals to cell proteins is considered the initial step in a chain of events that eventually lead to membrane lipid peroxidation and finally to cell necrosis [2]. Although several isoforms of P450 may metabolize carbon tetrachloride, attention has been largely focused on the P450 2E1 isoform, which is ethanol inducible [3, 4]. Moreover, alterations in the activity of P450 2E1 are known to affect susceptibility to hepatic injury from carbon tetrachloride [5, 6]. Natural compounds that reduce chemical activating enzymes could be considered as good protective candidates against chemically induced toxicity, and P450 2E1 is well recognized for its role in the activation of many chemicals to toxic and carcinogenic agents [1, 3, 4].
Herbs have recently attracted attention as health-beneficial foods (physiologically functional foods) and as source materials for drug development. Herbal medicines derived from plant extracts are being increasingly utilized to treat a wide variety of clinical diseases, with relatively little knowledge regarding their modes of action. Licorice (Glycyrrhiza glabra L.) and its main water-soluble constituent glycyrrhizin (GL), a penta-cyclic triterpene derivative of β-amyrin type (oleanane), have been widely used as an antitussive, demulcent and as a folk medicine for generations in Asia and Europe. It is currently used as a sweetening and flavoring agent in food products. After oral administration or i.v. injection, GL has been shown to be hydrolyzed by the glucuronidase in intestinal bacteria to its active principle aglycone, 18β-glycyrrhetinic acid (GA), and the GA formed is absorbed into the blood [7]. Both GL and GA have been shown to possess several beneficial pharmacological activities, such as an antifibrotic effect [8], antinflammatory activity [9], direct and indirect antiviral activity [10], interferon inducibility [11], and an antihepatitis effect [12]. In addition, GL and GA have been shown to protect against a number of hepatotoxicants, such as carbon tetrachloride and d-galactosamine [12-14]. It has also been proposed that the antioxidantive action of GA plays an important role in its hepatoprotective effects against carbon tetrachloride-induced liver injury [12]. However, the mechanisms by which GA protects against carbon tetrachloride-induced hepatotoxicity are not fully understood.

Recently, we showed that oleanolic acid, a triterpenoid saponin found in Orienta herbs, is effective at inhibiting carbon tetrachloride-induced liver injury, and that this protective effect is associated with the inhibition of carbon tetrachloride biotransformation by the reduced expression of P450 2E1 [6]. Hence, we hypothesized that the hepatoprotective effects of GA against carbon tetrachloride might also be associated with the inhibition of carbon tetrachloride bioactivation. The present study was undertaken to evaluate the protective effects of GA on carbon tetrachloride-induced hepatotoxicity and to elucidate the mechanism underlying these protective effects in mice. Our results indicate that GA pretreatment significantly protected against carbon tetrachloride-induced hepatotoxicity.

**Materials and Methods**

**Chemicals**

GA, carbon tetrachloride, olive oil, diagnostic kits for serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST), thioctic acid, dehydroepiandrosterone, phenylmethoxysulfonyl fluoride, reduced GSH, and polyclonal antibody for β-actin were obtained from the Sigma Chemical Co. (St. Louis, MO, USA). All other chemicals and solvents were of the highest grade commercially available.

**Animals and Treatment**

Male ICR mice (25-30 g) were obtained from KFDA (Seoul, Korea). The animals were allowed free access to Purina Rodent Chow and tap water, and maintained in a controlled environment at 21 ± 2 °C and 50 ± 5% relative humidity under a 12-h dark/light cycle, and acclimated for at least 1 week before use. Mice received either 2% (v/v) Tween 80 in saline or GA (10-100 mg kg⁻¹, s.c.) once daily for 3 days consecutively. Three hours after the final treatment, mice were pretreated with carbon tetrachloride (20 mg kg⁻¹, i.p., dissolved in olive oil). Groups of control animals were given the corresponding vehicles. Twenty-four hours after the administration of carbon tetrachloride, mice were anesthetized with CO₂, blood was removed by cardiac puncture to determine the serum ALT and AST activities, and the animals were killed by cervical dislocation. After bleeding, the livers were weighed and frozen quickly in dry ice/methanol and stored at −70 °C for GSH content and lipid peroxidation analysis.

**Hepatotoxicity Studies and Hepatic GSH Determination**

Serum ALT and AST activities were measured using a spectrophotometric diagnostic kit obtained from the Sigma Chemical Co. (St. Louis, MO, USA). Hepatic lipid peroxidation was measured by the formation of the thiobarbituric acid-reactive material, malondialdehyde (MDA) [15]. Nonprotein liver GSH was estimated by a colorimetric method using Ellman’s reagent as described by Sedlak and Lindsay [16].

**Microsome and Cytosol Isolation**

Mice were treated with GA (10-100 mg kg⁻¹, s.c.), pyridine as P450 2E1 inducer (100 mg kg⁻¹, i.p.) or vehicle once daily for 3 days consecutively. Twenty-four hours after the last treatment, animals were sacrificed by cervical dislocation. Hepatic microsomal and cytosolic fractions were prepared by differential centrifugation, as described previously [17]. All preparations were stored at −70 °C until use. The microsomal fractions were used for P450 2E1-specific oxidative activities and immunoblot analysis of P450 2E1.

**p-Nitrophenol and Aniline Hydroxylase Assay**

The hydroxylation of p-nitrophenol to 4-nitrocatechol was determined spectrophotometrically as described previously [18]. Aniline hydroxylase activity was determined by measuring p-aminophenol formation [18]. Microsomal protein was determined by the method of Bradford [19], using bovine serum albumin as a standard. For the inhibition study, GA was dissolved in saline and added to the incubation mixture.

**Immunoblot Analysis**

Immunochromatological detection of P450 2E1 was performed according to a method described previously [17]. Briefly, electrophoretic separation of microsomal
proteins was performed using 10% sodium dodecyl sulfate–polyacrylamide gels (SDS–PAGE) and then electrotransferred to a nitrocellulose membrane that was immuno-blotted with anti-rat P450 2E1 or anti-β-actin antibodies. Alkaline phosphatase-labeled rabbit anti-rat IgG was used as the secondary antibody and color developed using a mixture of 5-bromo-4-chloro-indolylphosphate and nitroblue tetrazolium.

FeCl₂–ascorbic acid stimulated lipid peroxidation in liver homogenate

The young male ICR mice weighing 20–25 g were killed by decapitation and their liver tissues were quickly removed. A 2 g portion of liver tissue was sliced and then homogenized with 10 ml of 150 mM KCl Tris–HCl buffer (pH 7.2). The reaction mixture was composed of 0.25 ml of liver homogenate, 0.1 ml of Tris–HCl buffer (pH 7.2), 0.05 ml of 0.1 mM ascorbic acid, 0.05 ml of 4 mM FeCl₂ and 0.05 ml of various concentrations of GA. The products of lipid peroxidation were quantified by the formation of the thiobarbituric acid-reactive material, MDA [15].

RESULTS

Effects of GA on carbon tetrachloride-induced hepatotoxicity

The effects of pretreatment with GA on the carbon tetrachloride-induced elevation of serum ALT and AST activities are shown in Table I. Pretreatment with GA (100 mg kg⁻¹, s.c., 3 days) caused no change in serum ALT and AST activities, compared to the control. A single dose of carbon tetrachloride (20 mg kg⁻¹, i.p.) caused hepatotoxicity in mice, as indicated by increased ALT and AST serum levels after carbon tetrachloride administration. GA pretreatment prevented the carbon tetrachloride-induced elevation of ALT and AST serum levels in a dose-dependent manner (Table I).

Effects of GA on hepatic lipid peroxidation and GSH levels

In order to evaluate the effect of GA pretreatment on carbon tetrachloride-induced liver lipid peroxidation, we monitored the levels of MDA, an indicator of oxidative damage, and one of the principal products of lipid peroxidation. As shown in Table I, the production of MDA in the carbon tetrachloride-treated group increased when compared with the control. Consistent with the serum levels of ALT and AST, pretreatment with GA significantly decreased carbon tetrachloride-induced hepatic lipid peroxidation in a dose-dependent manner (Table I). Carbon tetrachloride was given to two groups of mice, with or without GA pretreatment, and hepatic GSH levels were determined 24 h after the carbon tetrachloride administration. Whereas, the administration of carbon tetrachloride alone significantly depleted GSH levels, mice pretreated with GA were significantly protected from the GSH depletion produced by carbon tetrachloride (Fig. 1), and this protective effect occurred dose-dependently. However, no significant differences in the hepatic GSH levels and the activity of cytosolic glutathione-S-transferase in the controls or mice treated with GA alone were observed (data not shown).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Serum ALT (U l⁻¹)</th>
<th>Serum AST (U l⁻¹)</th>
<th>Liver lipid peroxidation (MDA, nmol g⁻¹ weight)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>62 ± 7†</td>
<td>32 ± 4†</td>
<td>11.5 ± 1.3†</td>
</tr>
<tr>
<td>GA 100 mg kg⁻¹</td>
<td>90 ± 8†</td>
<td>35 ± 4†</td>
<td>10.8 ± 1.2†</td>
</tr>
<tr>
<td>CCl₄</td>
<td>2636 ± 3465</td>
<td>1984 ± 2135</td>
<td>38 ± 4.7†</td>
</tr>
<tr>
<td>GA 100 mg kg⁻¹ + CCl₄</td>
<td>2512 ± 2627*</td>
<td>1595 ± 2185</td>
<td>33.2 ± 4.1†</td>
</tr>
<tr>
<td>GA 100 mg kg⁻¹ + CCl₄</td>
<td>1625 ± 2037**</td>
<td>1232 ± 1622</td>
<td>28 ± 3.2†</td>
</tr>
<tr>
<td>GA 100 mg kg⁻¹ + CCl₄</td>
<td>852 ± 964***</td>
<td>763 ± 977</td>
<td>16.2 ± 2.3†</td>
</tr>
</tbody>
</table>

Mice were pretreated with GA (10, 50 or 100 mg kg⁻¹, s.c.) once daily for 3 days consecutively. Control mice were given saline. Three hours after the final treatment, mice were treated with carbon tetrachloride (CCl₄, 20 mg kg⁻¹, i.p.). Hepatotoxicity was determined 24 h later by quantifying the serum activities of ALT and AST and the level of hepatic lipid peroxidation. Each value represents the mean ± SD of five mice. * Significantly different from CCl₄. † Significantly different from control. ** Significantly different from CCl₄ + GA.
Fig. 1. Protective effect of GA on the carbon tetrachloride-induced depletion of hepatic cellular GSH. Mice were pretreated with GA (10, 50 or 100 mg kg\(^{-1}\), s.c.) once daily for 3 days consecutively. Control mice were given saline. Three hours after the final treatment, mice were treated with carbon tetrachloride (CCl\(_4\); 20 mg kg\(^{-1}\), i.p.). Mice were killed 24 h after the administration of carbon tetrachloride. Hepatic cellular GSH contents were measured as described in MATERIALS AND METHODS. Each bar represents the mean ± sd for five mice. (\*) Significantly different from CCl\(_4\) at \(P<0.01\).

Effects of GA on carbon tetrachloride bioactivation-related P450 2E1 activity and expression

In mice, GA pretreatment showed a dose-dependent protective effect on carbon tetrachloride-induced hepatotoxicity, and it is known that carbon tetrachloride requires P450 2E1-associated bioactivation to produce liver injury. Therefore, the effects of GA on hepatic microsomal P450 2E1-specific microsomal monooxygenase activities were examined. As shown in Table II, hepatic microsomal fractions from mice treated with GA significantly decreased the hydroxylation of the two P450 2E1-specific substrates, \(p\)-nitrophenol and aniline, in a dose-dependent manner. The inhibitory activities of GA on hepatic microsomal P450 2E1-specific microsomal monooxygenase activities were confirmed in pyridine-induced hepatic microsomal incubations. In incubations with hepatic microsomes, GA showed a dose-dependent inhibition of P450 2E1-specific \(p\)-nitrophenol hydroxylase and aniline hydroxylase with IC\(_{50}\) values of 0.28 and 0.24 mg ml\(^{-1}\), respectively (Table II).

Immunoblot analysis was performed to examine the effect of GA on P450 2E1 protein expression. The hepatic microsomes from GA-treated mice were resolved by SDS–PAGE and immunoblotted with anti-P450 2E1. The immunoblot of P450 2E1 protein is shown in Figure 2. Pyridine, which is well known as P450 2E1 inducer, treatment markedly increased levels of P450 2E1 protein. P450 2E1 expressions were suppressed by GA treatment in a dose-dependent manner. This result is consistent with reduced microsomal \(p\)-nitrophenol and aniline hydroxylation activities (Table II).

### Table II

<table>
<thead>
<tr>
<th>Treatment</th>
<th>(p)-Nitrophenol Hydroxylation (nmol mg(^{-1}) protein min(^{-1}))</th>
<th>Aniline 4-Hydroxylation (nmol mg(^{-1}) protein min(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>In vivo(^a)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>2.47 ± 0.20</td>
<td>0.68 ± 0.08</td>
</tr>
<tr>
<td>GA 10 mg kg(^{-1})</td>
<td>2.06 ± 0.24</td>
<td>0.97 ± 0.06</td>
</tr>
<tr>
<td>GA 50 mg kg(^{-1})</td>
<td>1.62 ± 0.20(^*)</td>
<td>0.41 ± 0.05(^*)</td>
</tr>
<tr>
<td>GA 100 mg kg(^{-1})</td>
<td>1.06 ± 0.15(^*)</td>
<td>0.22 ± 0.04(^*)</td>
</tr>
<tr>
<td>In vitro(^b)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>6.29 ± 0.66</td>
<td>8.53 ± 0.91</td>
</tr>
<tr>
<td>GA 0.01 mg ml(^{-1})</td>
<td>5.59 ± 0.60</td>
<td>8.25 ± 0.64</td>
</tr>
<tr>
<td>GA 0.1 mg ml(^{-1})</td>
<td>4.09 ± 0.44(^*)</td>
<td>6.21 ± 0.67(^*)</td>
</tr>
<tr>
<td>GA 1 mg ml(^{-1})</td>
<td>1.28 ± 0.14(^*)</td>
<td>2.31 ± 0.25(^*)</td>
</tr>
</tbody>
</table>

\(^a\) Mice were pretreated with GA (10, 50 or 100 mg kg\(^{-1}\), s.c., 3 days) and killed 24 h after the final treatment. Each value represents means ± sd of five mice. \(^b\) Liver microsomes were obtained from mice treated with pyridine (100 mg kg\(^{-1}\), i.p., 3 days). Each value represents means ± sd of three independent experiments, performed in triplicate. \(^*\) Significantly different from control at \(P<0.01\).
Effects of GA on FeCl₃–ascorbic acid stimulated lipid peroxidation and superoxide scavenging activity

In order to determine the antioxidant effects of GA in terms of the mechanism of its hepatoprotective effect, antilipid peroxidation in liver homogenate and the superoxide scavenging activity of GA were investigated. Consistent with the results of carbon tetrachloride-induced hepatic lipid peroxidation, GA showed a dose-dependent inhibition of the FeCl₃–ascorbic acid stimulated lipid peroxidation with an IC₅₀ value of 0.65 mg ml⁻¹ in liver homogenate. GA also showed superoxide scavenging activity with an IC₅₀ value of 0.47 mg ml⁻¹ (Table III).

**DISCUSSION**

Liver injuries induced by carbon tetrachloride are best-characterized system of xenobiotic-induced hepatotoxicity and is a commonly used model for the screening of antihapatotoxic/hepatoprotective activity of drugs [2]. Increased serum levels of lactate dehydrogenase, AST and ALT have been attributed to the damaged structural integrity of the liver, because these are cytoplasmic in location and are released into circulation after cellular damage [2]. The results of the present study demonstrate that the pretreatment of mice with GA effectively protected mice against carbon tetrachloride-induced hepatotoxicity, as evidenced by decreased serum aminotransferase activity, and hepatic lipid peroxidation in a dose-dependent manner (Table I). This result is consistent with previous reports. The antihapatotoxicity of GA on carbon tetrachloride-induced liver injury has been previously reported [14].

It is now generally accepted that the hepatotoxicity of carbon tetrachloride is the result of reductive dehalogenation, which is catalyzed by P450, and which forms the highly reactive trichloromethyl free radical. This then readily interacts with molecular oxygen to form the trichloromethyl peroxyl radical. Both trichloromethyl and its peroxy radical are capable of binding to proteins or lipids, or of abstracting a hydrogen atom from an unsaturated lipid, initiating lipid peroxidation and liver damage and by doing so playing a significant role in pathogenesis of diseases [2]. Therefore, the suppression of P450 could result in reduced levels of reactive metabolites, and thus decreased tissue injury. The metabolic activation of carbon tetrachloride is thought to be mediated through P450 2E1 [3, 4]. Our results support this hypothesis, because a good correlation was found between decreased P450 2E1 enzyme activity in GA-treated hepatic microsomes in vivo, and decreased P450 2E1-induced hepatic microsomes in vitro and the level of protection against carbon tetrachloride-induced hepatotoxicity in mice (Tables I and II and Fig. 1). The hydroxylation of p-nitrophenol and aniline, as used in the present study, has been used extensively to probe the activity of P450 2E1 [3, 5, 6, 18]. In our immunoblot analysis, GA reduced the expression of P450 2E1 (Fig. 2). The decreased levels of P450 2E1 by GA were consistent with the results of these monooxygenase activities.

The observations above suggest that the inhibition of P450 2E1 by GA in mice plays an important role in GA-induced hepatoprotection against carbon tetrachloride. Several previous studies have demonstrated that carbon tetrachloride-induced hepatotoxicity can be modulated by substances that influence the activity of P450 2E1. In particular, compounds or drugs that induce P450 2E1 potentiate the hepatic toxicity of carbon tetrachloride [21, 22], conversely, compounds that inhibit P450 2E1 protect against carbon tetrachloride-induced toxicity [5, 6]. The induction or inhibition of carbon tetrachloride biotransformation may subsequently influence the metabolic activation or detoxification of carbon tetrachloride. P450 2E1 participates in the metabolism of small organic molecules, such as carbon tetrachloride, acetonitrobenzenes, and nitrosamines [1, 3, 4]. In addition, P450 2E1 plays a critical role in the metabolism of many carcinogens, including the nitrosamines, which require metabolic activation to exert their carcinogenic effects [1]. Thus, the inhibition of P450 2E1 by GA not only plays an important role by protecting against the hepatotoxicity of carbon tetrachloride, but also may play a role in reducing xenobiotic toxicity by decreasing metabolic activation.

In contrast to the toxic activation of carbon tetrachloride via the P450 2E1 pathway, the detoxification pathway involves GSH conjugation of the trichloromethyl radical, a P450 2E1-mediated carbon tetrachloride metabolite. Previous studies on the mechanism of carbon tetrachloride-induced hepatotoxicity have shown that GSH plays a key role in the detoxification of the reactive toxic metabolites of carbon tetrachloride and that liver necrosis begins when GSH stores are markedly depleted [2]. GSH is largely mediated through the activity of glutathione-S-transferase, and forms adducts with the toxic metabolites of carbon tetrachloride. Moreover, GSH contributes to the detoxification of carbon tetrachloride,

**Table III**

<table>
<thead>
<tr>
<th>Treatment (mg ml⁻¹)</th>
<th>Inhibition of lipid peroxidation (mg ml⁻¹)</th>
<th>Treatment (mg ml⁻¹)</th>
<th>Inhibition of scavenging activity (mg ml⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GA 0.01</td>
<td>8.7 ± 1.1</td>
<td>GA 0.01</td>
<td>3.2 ± 0.5</td>
</tr>
<tr>
<td>GA 0.1</td>
<td>22.4 ± 2.4</td>
<td>GA 0.1</td>
<td>16.2 ± 1.9</td>
</tr>
<tr>
<td>GA 0.5</td>
<td>42.5 ± 4.4</td>
<td>GA 0.5</td>
<td>53.4 ± 5.8</td>
</tr>
<tr>
<td>GA 1.0</td>
<td>71.2 ± 7.3</td>
<td>GA 1.0</td>
<td>86.2 ± 9.2</td>
</tr>
</tbody>
</table>

Values are presented as the mean of the percentage inhibition ± SD for three independent experiments, performed in triplicate. * Mouse liver homogenates were stimulated with FeCl₃–ascorbic acid in the presence or absence of GA and lipid peroxidation was measured as described in MATERIALS AND METHODS. ** Superoxide was generated by the oxidation of xanthine/xanthine oxidase in the presence or absence of GA and scavenging activity was measured as described in MATERIALS AND METHODS.

/**References**


and it has been hypothesized that one of the principal causes of carbon tetrachloride-induced liver injury is lipid peroxidation caused by its free radical derivatives [2]. Our results show that pretreatment with GA significantly inhibits lipid peroxidation (Table I) and significantly reduces carbon tetrachloride-induced hepatic GSH depletion (Fig. 1). This is attributed to the decreased bioactivation of carbon tetrachloride caused by the GA pretreatment (Table II). Glutathione-S-transferase is a soluble protein located in the cytosol, and plays an important role in the detoxification and excretion of xenobiotics [23]. Moreover, glutathione-S-transferase functionally binds GSH and endogenous or exogenous substances. Since it increases the solubility of hydrophobic substances, it also plays an important role in the storage and excretion of xenobiotics. A compound that increases the activity of glutathione-S-transferase, which metabolizes toxic to nontoxic compounds, protects the liver. However, GA pretreatment, by itself, did not affect hepatic GSH levels (Fig. 1). In addition, GA did not significantly change cytotoxic glutathione-S-transferase activity (data not shown). These results show that the protection afforded by GA against carbon tetrachloride-induced hepatotoxicity may not be related to increased cellular GSH content or increased glutathione-S-transferase activity.

Lipid peroxidation, is accepted to be one of the principal causes of carbon tetrachloride-induced liver injury, and is mediated by the production of free radical derivatives of carbon tetrachloride. Thus, antioxidant activity and/or the inhibition of free radicals generation is important in terms of protecting the liver from carbon tetrachloride-induced damage [24]. In vitro lipid peroxidation in a liver homogenate can proceed in a nonenzymatic way. The process is induced by ascorbate in the presence of Fe²⁺/Fe³⁺, and it has been reported that Fe³⁺ and ascorbic acid stimulated lipid peroxidation in rat liver microsomes and mitochondria. In order to clarify the mode of action of GA, in vitro lipid peroxidation experiments were carried out. According to the results obtained, GA inhibited FeCl₂-ascorbic acid-stimulated lipid peroxidation in liver homogenate (Table III). Moreover, our experimental results demonstrate that GA exerts free radical scavenging activity upon the superoxide radical generated using the xanthine–xanthine oxidase system (Table III), and may therefore, act by scavenging free radicals and reactive oxygen species formed during carbon tetrachloride metabolism. The hepatoprotective effects of GA on carbon tetrachloride-induced liver injury in vitro have been reported to be due to its anti-oxidative activity [12]. In addition, Ju et al. [25], showed that GA is not only an oxygen free radical scavenger but also an inhibitor of oxygen free radical generation in phorbol myristate acetate-stimulated polymorphonuclear leukocytes. Active oxygen species and free radicals are known to be involved in a variety of diseases, such as arteriosclerosis, liver disease, diabetes, inflammation, cancer, and the aging process [26]. Any compound, natural or synthetic, with antioxidant properties that might contribute towards the partial or total alleviation of this damage, may have a significant role in maintaining health when used as a medicine or as a food. Another study reported that the hepatoprotective effects of GA on carbon tetrachloride-induced liver injury might be caused by β-glucuronidase inhibition [27]. They also reported that silymarin inhibited rat liver microsomal β-glucuronidase and had the hepatoprotective effect [28]. There have been demonstrated that liver damage caused an increase of β-glucuronidase in blood [29].

In conclusion, the results of this study demonstrate that GA has a potent hepatoprotective action upon carbon tetrachloride-induced hepatic damage in mice. Our results show that the hepatoprotective effects of GA may be due to its ability to block the bioactivation of carbon tetrachloride by inhibiting P450 2E1 activity and its expression. This results in the decreased formation of trichloromethyl radicals, and GA’s antioxidant activity, in combination with GA’s ability to scavenge free radicals and inhibit lipid peroxidation, all of which are capable of hepatocellular injury.

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