PROTECTIVE EFFICACY OF GLYCYRRHIZA GLabra ON CCl₄ INDUCED LIVER INJURY IN RABBITS

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ABSTRACT

The present study was carried out to investigate the protective effects of Glycyrrhiza glabra L roots powder juice on some biochemical parameters in normal (non-CCl₄-induced hepatic injury) and CCl₄-induced hepatic injury rabbits. A dose of 2.0 g/kg body weight of aqueous extract of roots was orally administered daily to normal and CCl₄-induced hepatic injury rabbits for 7 days. The levels of AST, ALT, ALP, creatinine and urea were significantly (P<0.05) increased in the serum of CCl₄-induced hepatic injury rabbits as compared to the (non-CCl₄-induced hepatic injury rabbits. In contrast, glucose concentration was significantly decreased in CCl₄-induced hepatic injury rabbits, as compared to the non-CCl₄-induced hepatic injury rabbits. Significant reduction in the hepatic enzymes levels AST, ALT, ALP, serum creatinine, serum urea and improvement of serum glucose was found in animals treated with the extract before CCl₄-induced hepatic injury. The present study demonstrated that the G. glabra roots extract had a significant effect in liver functions in acute liver diseases when it was given in a single daily dose of 2.0 g/kg rabbit body weight for seven days. Therefore the aqueous extract of G. glabra roots can be used for prevention and treatment of liver disorders.

KEY WORDS: Glycyrrhiza glabra (Licorice); Carbon tetrachloride; Induced hepatic injury rabbits; Hepatic enzymes; Biochemical parameters.

INTRODUCTION

Carbon tetrachloride (CCl₄) is a very classic hepatic injury model, therefore, it is widely used for hepatoprotective drug screening [¹, ²]. The liver is especially sensitive to carbon tetrachloride because it enlarges, damages and destroys liver cells. Kidneys also are damaged, causing a buildup of wastes in the blood [³]. Several studies have demonstrated that
CCL₄ modulates toxic effects operate through its haloalkane metabolites. These reactive metabolites are produced during biotransformation of CCL₄ and may cause the oxidative damage of lipids, lipoproteins, and other cellular components, such as enzymes, DNA and proteins [⁴, ⁵].

The acute hepatotoxicity of CCL₄ lies in its biotransformation to trichloromethyl free radical (CCL₃) or trichloroperoxyl radical (CCL₃O₂⁻) produced by the mixed-function cytochrome P450 oxygenase system of the endoplasmic reticulum, which causes oxidative stress and membrane damage [⁶].

High exposure to carbon tetrachloride can cause liver, kidney, and central nervous system damage. These effects can occur after ingestion or breathing carbon tetrachloride, and possibly from exposure to the skin [³]. Glycyrrhiza glabra L, also known as (licorice), is a traditional medicinal herb grows in various parts of the world. In the traditional system of medicine, the roots and rhizomes of G. glabra (Family: Pappilionaceae/ Fabaceae) have been employed clinically for centuries for their anti-inflammatory, anti-ulcer, expectorant, antimicrobial and anxiolytic activities [⁷]. In modern medicine, Licorice extracts are often used as a flavoring agent to mask bitter taste in preparations and as an expectorant in cough and cold preparations [⁸].

There are many useful compound in licorice root such as, glycyrrhizin (saponin- Like glycoside -50 time sweeter than sugar) and its aglycone, glycyrrhetinic acid which are clinically used for hyperlipidemia [⁹]. many components isolated form licorice including water soluble, biologically active complex was composed of triterpene saponins, flavonoids, polysaccharides, pectins, simple sugars, amino acids, mineral salts and various other substances [¹⁰]. Glycyrrhiza glabra has been shown to have great antioxidant, freeradical scavenging [¹¹] and anticonvulsant activities [¹²].

This work was aimed to evaluate the hepatoprotective activity of G. glabra Juice against carbontetrachloride induced hepatotoxicity in rabbits.

**MATERIALS AND METHODS**

**Chemicals**

Carbon tetra chloride (CCL₄) was used to induce the hepatic injury of the experimental animal (rabbits).
All other chemicals used in the study were with known structure and functions.

**Plant material and preparation**

The dry roots of *Glycyrrhiza glabra* (Licorice, Sweet root) were obtained from local herbal market and was identified and authenticated by department of botany, faculty of science, university of Sirt, Sirt, Libya. The roots were cleaned and grounded into fine powder. A known weight of this powder, 40 grams, were suspended in 200 ml distilled water to prepare the juice for the experiment.

**Animals**

Fifteen rabbits weighing from 0.87-2.33 kg were used in the present study. The animals were grouped and housed in cages (70 x 44 x 103 cm) at the laboratories of the zoology department, Sirt University. The photoperiod was regulated at 12 hours light / 12 hours dark cycle and temperature was adjusted at 25±1°C. The rabbits were fed on commercial standard pellet and offered drink water *ad libitum*. The animals were acclimatized to laboratory conditions for one week before commencement of the experiment.

**Induction of hepatotoxicity by CCl₄**

Liver toxicity was induced by an oral single dose of CCl₄ 1.25ml /Kg rabbit body weight. diluted in a 1:1(v/v) olive oil.

**Experimental groups and protocol**

Rabbits were randomly distributed into three groups (5.0 rabbits/group).

**Group I:** control (G1)  normal control rabbits were given 2ml distilled water orally daily for 7 days , and in the 8th day were given 1.0ml olive oil.

**Group II:** Carbon tetra chloride treated group (G2) - rabbits were given 2.0ml distilled water orally daily for 7 days , and in the 8th day were given a single dose of CCl₄, 1.25 ml/Kg Body weight(b.wt). in a 1:1(V/V) olive oil.

**Group III:** Carbon tetrachloride + *G. glabra* treated group (G3) — rabbits were given 2.0 g/kg b.wt. *G. glabra*. in 10 ml distilled water orally daily for 7 days, and in the 8th day were given a single dose of 1.25 ml/Kg b.wt. CCl₄ in a 1:1 (v/v) in olive oil.
Collection of blood
At the end of the experimental period (8 days), overnight fasting rabbits were deprived of food but allowed for free access of drinking water. Animals were sacrificed by decapitation and the shed blood was collected in cleaned vials, without anticoagulant for serum separation. These vials were centrifuged at 3000 rpm for 20 minutes. The serum was analyzed to determine the Aspartate amino transferase (AST), Alanine amino transferase (ALT), and Alkaline Phosphatase (ALP). In addition creatinine, urea and glucose concentrations were also determined.

Statistical analysis
Data were expressed as mean ± SE, and were compared using One-way analysis of variance (ANOVA) and Student’s t test was used to detect the mean differences between groups. The significance levels were tested at (p < 0.05).

RESULTS AND DISCUSSION
Liver toxicity was assessed by the increased level of serum enzymes namely, aspartate transaminase (AST), alanine transaminase (ALT) and alkaline phosphatase (ALP). Table 1 showed that CCl₄ induced changes in the level of serum enzymes (AST, ALT and ALP) activities, where, they were significantly increased in the animals treated with CCl₄. The percentage elevations were 777.54%, 406.99% and 453.70% for the three enzymes respectively in comparison with the control animals. There are several studies suggesting that the treatment of animals with CCl₄ usually leads to elevation of the AST, ALT and ALP activities in serum.

Plasma AST increases in such cases could be due to their escapes to the plasma from the injured hepatic cells. In addition, plasma ALT level is also useful in indicating the existence of liver diseases, as this enzyme is present in large quantities in the liver. It increases in plasma when cellular degeneration or destruction occurs in this organ. CCl₄ is biotransformed by cytochrome p-450 in liver to produce highly reactive trichloromethyl free radical. This, in the presence of oxygen generated by metabolic leakage from mitochondria can causes’ membrane lipid peroxidation. This leads to loss of integrity of cell membranes and damage of hepatic tissue which is an evidence of the increased levels of serum marker enzymes, namely AST, ALT and ALP. Interestingly, this study show that pretreatment with Glycyrrhiza glabra before the oral treatment of CCl₄, significantly decreased the serum AST, ALT and ALP activity up to -77.55%, -56.52% and -88.96% respectively compared to
that of animals which administered with CCl₄. These results agree with the findings recorded [18].

The high level of flavonoids like luteolin, rutin, and apigenin in G. glabra possess antioxidant properties [21]. The flavonoid compound, rutin, is particularly having free radical scavenging property so inhibits the lipid peroxidation [22] the G. glabra contains flavonoides, glycosides, glycyrrhizin (the main active component, found in the root) and glycyrrhizic acid [10]. The glycyrrhizic acid has a lowering effect of elevated liver transaminases levels [23].

The author also, found that glycyrrhetin acid in the G. glabra may blocks the bioactivity of CCl₄ by inhibiting the activity of P4502E1 (the enzyme responsible for CCl₄ metabolism), thereby preventing the hepatoperoxidation [24]. Certain licorice constituents possess significant antioxidant and hepatoprotective properties. Glycyrrhizin and glabridin inhibit the generation of reactive oxygen species (ROS) by neutrophils at the site of inflammation [25, 26]. In vitro studies have demonstrated licorice isoflavones, hispaglabridin A and B, inhibit Fe³⁺-induced mitochondrial lipid per-oxidation in rat liver cells [27]. Other research indicates glycyrrhizin lowers lipid peroxide values in animal models of liver injury caused by ischemia reperfusion [28]. Licorice constituents also exhibit hepato-protective activity by lowering serum liver enzyme levels and improving tissue pathology in hepatitis patients [29, 30].

The carbon tetrachloride (CCl₄) is one of the environmental pollutants that have been studied and developed the conclusion of acute and chronic renal damage [31]. In the current study, Table 2 show, a significant increase in the level of creatinine and urea for rabbits treated with single dose of carbon tetrachloride these results agreed with other studies [32-34], indicated that the increase in the level of urea will lower the glomerular filtration rate as a result of carbon tetrachloride toxicity, and because the ratio of urea depends on the filtration rate.

The increase in the level of creatinine in the blood considered an indicator to the kidney damage [35].

The carbon tetrachloride has a role in the incidence of renal toxicity and poisoning of the urethra and the result of the presence of Cytochrome P 450 in the renal cortex which have a great sensitivity toward the compound carbon tetrachloride [31]. The effects of carbon tetrachloride on the structure and function of the kidney depends on the status and function of the liver, where, it can damage and lead to a breach in the dynamics of the liver (Hemodynamic) and therefore a defect in kidney function [36].
Table 2 show serum glucose concentrations. Serum glucose level was significantly decreased in animals treated with CCl₄, the percentage reduction of glucose was- 54.48% in comparison with the control animals. The results of this study agree with the findings recorded [37, 38]. The reduction in the level of glucose after treatment with carbon tetrachloride can be attributed to the decrease of liver content of glycogen content, which leads to a decrease in the process of gluconeogenesis (Gluconeogenesis) in the liver [37].

The results of the present study further added that the pretreatment of rabbits with licorice root at dose (2.0 g/kg body weight in 10 ml distelled water) before a dose of carbon tetrachloride had given a protective effect against the toxicity created by carbon tetrachloride through the significant improvement in renal function, which Valley to a decrease in the level of both urea and creatinine in the serum of rabbits compared to animals treated with only CCl₄. The roots of licorice contain high levels of flavonoid (Flavonoids) such as Tulane (Luteolin) and routine (Rutin) and Abganin (Apigenin), which has antioxidant properties [21]. Flavonoid and rutin in particular are scavengers of free radicals and thus inhibit the process of lipid peroxidation [22].

Table 1: Values of AST, ALT, and ALP (Means ± SE) for control and treated groups of rabbits.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>(G1) Means ± SE</th>
<th>(G2) Means ± SE</th>
<th>(G3) Means ± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>AST(U/L)</td>
<td>58.63 ± 4.34</td>
<td>514.50 ± 35.20</td>
<td></td>
</tr>
<tr>
<td>% of Change from G1</td>
<td></td>
<td>777.54</td>
<td></td>
</tr>
<tr>
<td>% of Change from G2</td>
<td></td>
<td>115.50 ± 9.19</td>
<td></td>
</tr>
<tr>
<td>ALT(U/L)</td>
<td>50.05 ± 6.13</td>
<td>253.75 ± 13.13</td>
<td></td>
</tr>
<tr>
<td>% of Change from G1</td>
<td></td>
<td>406.99</td>
<td></td>
</tr>
<tr>
<td>% of Change from G2</td>
<td></td>
<td>87.50 ± 12.14</td>
<td></td>
</tr>
<tr>
<td>ALP(U/L)</td>
<td>148.50 ± 2.61</td>
<td>822.25 ± 38.26</td>
<td></td>
</tr>
<tr>
<td>% of Change from G1</td>
<td></td>
<td>453.70</td>
<td></td>
</tr>
<tr>
<td>% of Change from G2</td>
<td></td>
<td>90.75 ± 9.61</td>
<td></td>
</tr>
</tbody>
</table>

Values are given as mean ± SE for 5 rabbits in each group.

ª significant (P< 0.05) as compared with (G1).

ª significant (P< 0.05) as compared with the (G2).
Table 2: Values of Creatinine, Urea and Glucose (Means ± SE) for control and treated groups of male rabbits.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>(G1) Means ± SE</th>
<th>(G2) Means ± SE</th>
<th>(G3) Means ± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Glucose mg/dl</strong></td>
<td>73.73 + 6.22</td>
<td>33.56 + 1.46&lt;sup&gt;a&lt;/sup&gt;</td>
<td>73.90 + 8.55&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>% of Change from G1</td>
<td></td>
<td>- 54.48</td>
<td></td>
</tr>
<tr>
<td>% of Change from G2</td>
<td></td>
<td>_</td>
<td>120.20</td>
</tr>
<tr>
<td><strong>Creatinine mg/dl</strong></td>
<td>0.57 + 0.09</td>
<td>13.90 + 4.27&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.57 + 0.13&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>% of Change from G1</td>
<td></td>
<td>2338.60</td>
<td>0.00</td>
</tr>
<tr>
<td>% of Change from G2</td>
<td></td>
<td>_</td>
<td>- 2338.60</td>
</tr>
<tr>
<td><strong>Urea mg/dl</strong></td>
<td>53.43 + 0.49</td>
<td>56.69 + 1.07&lt;sup&gt;a&lt;/sup&gt;</td>
<td>53.43 + 1.19&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>% of Change from G1</td>
<td></td>
<td>6.10</td>
<td>0.00</td>
</tr>
<tr>
<td>% of Change from G2</td>
<td></td>
<td>_</td>
<td>- 6.10</td>
</tr>
</tbody>
</table>

Values are given as mean ± SE for 5 rabbits in each group.

<sup>a</sup> significant (P< 0.05) as compared with (G1).

<sup>b</sup> significant (P< 0.05) as compared with the (G2).

Figure 1: Values of AST activity (Means ± SE) for control and treated groups of rabbits.

<sup>a</sup> significant (P< 0.05) as compared with the (G1).

<sup>b</sup> significant (P< 0.05) as compared with the (G2).
Figure 2: Values of ALT activity (Means ± SE) for control and treated groups of rabbits.

- Significant (P < 0.05) as compared with the (G1).
- Significant (P < 0.05) as compared with the (G2).

Figure 3: Values of ALP activity (Means ± SE) for control and treated groups of rabbits.

- Significant (P < 0.05) as compared with the (G1).
- Significant (P < 0.05) as compared with the (G2).

Figure 4: Values of Glucose (Means ± SE) for control and treated groups of rabbits.

- Significant (P < 0.05) as compared with the (G1).
- Significant (P < 0.05) as compared with the (G2).
Figure 5: Values of creatinine (Means ± SE) for control and treated groups of rabbits.

- *significant (P < 0.05) as compared with the (G1).
- *significant (P < 0.05) as compared with the (G2).

Figure 6: Values of urea (Means ± SE) for control and treated groups of rabbits.

- *significant (P < 0.05) as compared with the (G1).
- *significant (P < 0.05) as compared with the (G2).

CONCLUSION

The present study demonstrated that the *G. glabra* roots extract had a significant effect in liver functions in acute liver diseases when it was given in a single daily dose of 2.0 gm/kg body weight for seven days. Therefore the aqueous extract of *G. glabra* roots can be used for prevention and treatment of liver disorders.

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