ABSTRACT

Objective: To evaluate the potential efficacy of *Glycyrrhiza glabra* Linn. (Fabaceae) in protecting tissues from peroxidative damage in CCl$_4$-intoxicated rats.

Material and Methods: Peroxidative hepatic damage in rats was studied by assessing parameters such as thiobarbituric acid reactive substances (TBARS), conjugated dienes (CD), superoxide dismutase (SOD), catalase (CAT), glutathione-S-transferase (GST), glutathione peroxidase (GSH-Px) and glutathione (GSH) in liver and kidneys. The effect of co-administration of *G. glabra* on the above parameters and histopathological findings of the liver in experimental animals was studied.

Results: The increased lipid peroxide formation in the tissues of CCl$_4$-treated rats was significantly inhibited by *G. glabra*. The observed decreased antioxidant enzyme activities of SOD, CAT, GSH-Px, GST, and antioxidant concentration of glutathione were nearly normalized by *G. glabra* treatment. Carbon tetrachloride-induced damage produces alteration in the antioxidant status of the tissues, which is manifested by abnormal histopathology. *G. glabra* restored all these changes.

Conclusion: *Glycyrrhiza glabra* is a potential antioxidant and attenuates the hepatotoxic effect of CCl$_4$.

KEY WORDS: Lipid peroxidation, hepatotoxicity, antioxidants
Material and Methods

Male albino rats of Sprague-Dawley strain, weighing between 120 g to 150 g were used for the experiment. They were housed in polypropylene cages under standard conditions (23±2°C, humidity 60-70%, 12 h light/dark cycles) and given standard pellet diet (M/s Hindustan Lever Ltd, Mumbai, India). Water was given ad libitum. The animals were divided into 3 groups of 6 rats each. Group I served as pair-fed control which received the normal feed. Groups II and III received a dose of 0.3 ml CCl₄ in liquid paraffin (3:1, v/v) per 100 g body weight subcutaneously twice a week for a period of two months. Group III rats, in addition to CCl₄ received a dose of 1000 mg/kg body weight/day of G. glabra root powder mixed with the feed for two months. The concentration of the powder in the feed was adjusted to the amount of food consumed. The dose of the medicinal plant was ascertained by a pilot study over a range of dosages varying from 500 mg/kg, body weight to 1500 mg/kg, body weight/day. Over the range of dosages studied, the plant did not show any toxicity. The dose of CCl₄ was adjusted to the amount of food consumed. Administration of G. glabra together with CCl₄ resulted in significant decrease of TBARS and CD in the liver and kidney compared with the corresponding CCl₄-intoxicated rats and normal (pair-fed) control rats, respectively.

Glycyrrhiza glabra roots were collected from the crude drug market, Pala, Kottayam district, Kerala. They were dried overnight at 45°C and powdered. This preparation was used for the experiment.

At the end of the experimental period, the animals were killed by decapitation. The liver and kidney were dissected out immediately and transferred into ice-cold physiological saline for various biochemical estimations. Sections of liver tissues were collected in 10% formal saline for proper fixation. Slices of fixed tissues were processed, embedded in paraffin, sectioned to a thickness of 5 mm, mounted on glass slides, and stained with hematoxylin and eosin for histopathological evaluation.

A 10% tissue (liver and kidney) homogenate was prepared using tris-HCl buffer (0.1M; pH 7.5) and used for the analysis. Lipid peroxidation was assessed in terms of thiobarbituric acid reactive substances (TBARS) and conjugated dienes (CD). Changes in the antioxidant status were determined by estimating the activities of catalase, superoxide dismutase (SOD), glutathione-S-transferase (GST), glutathione (GSH) and glutathione peroxidase (GSH-Px) in the liver and kidney. The protein content of the tissues was estimated by the method of Lowry et al. The results expressed as percent hepatoprotective activity (H) were calculated by the formula:

\[ H = \frac{|I - (HC-N)|}{(C-N)} \times 100 \]

where H, C and N are the hepatoprotective activity set at P<0.05.

Statistical analysis

The results are presented as the mean ± SEM. One-way analysis of variance (ANOVA) followed by the Bonferroni test were applied for statistical analysis with the level of significance set at P<0.05.

Results

Feeding CCl₄ to rats for two months resulted in significant loss of body weight. Treatment with the medicinal plants along with CCl₄ prevented the loss in body weight.

There was a significant increase in the concentrations of TBARS and CD during CCl₄ treatment as compared with the pair-fed control. Administration of G. glabra together with CCl₄ resulted in significant decrease of TBARS and CD in the liver and kidney compared with the corresponding CCl₄-intoxicated group (Table 1).

Table 1

Effect of G. glabra on the level of TBARS, CD, GSH and the activities of SOD, CAT, and GSH-Px in the liver and kidney

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Tissue</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
<th>% Hepato protection</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>TBARS</td>
<td>Liver</td>
<td>1.32 ± 0.03</td>
<td>1.6 ± 0.66*</td>
<td>1.34 ± 0.06</td>
<td>92.86</td>
<td>6.13</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td></td>
<td>Kidney</td>
<td>1.27 ± 0.06</td>
<td>1.72 ± 0.06</td>
<td>1.32 ± 0.06</td>
<td>88.89</td>
<td>5.2</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>CD</td>
<td>Liver</td>
<td>63.28 ± 1.55</td>
<td>81.50 ± 2.82*</td>
<td>65.70 ± 2.96*</td>
<td>86.72</td>
<td>5.21</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td></td>
<td>Kidney</td>
<td>18.20 ± 0.45</td>
<td>22.02 ± 0.76</td>
<td>18.72 ± 0.84</td>
<td>8.63</td>
<td>4.1</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>SOD</td>
<td>Liver</td>
<td>5.62 ± 0.14</td>
<td>4.39 ± 0.15*</td>
<td>5.53 ± 0.25</td>
<td>92.68</td>
<td>5.2</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td></td>
<td>Kidney</td>
<td>5.48 ± 0.14</td>
<td>4.41 ± 0.15*</td>
<td>5.31 ± 0.24</td>
<td>84.11</td>
<td>5.9</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>CAT</td>
<td>Liver</td>
<td>212.52 ± 5.31</td>
<td>101.72 ± 3.53*</td>
<td>199.17 ± 8.96*</td>
<td>86.65</td>
<td>4.05</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td></td>
<td>Kidney</td>
<td>214.35 ± 5.33</td>
<td>181.23 ± 6.34*</td>
<td>210.06 ± 9.46*</td>
<td>87.05</td>
<td>5.2</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>GST</td>
<td>Liver</td>
<td>0.32 ± 0.01</td>
<td>0.24 ± 0.01*</td>
<td>0.31 ± 0.01</td>
<td>87.5</td>
<td>5.9</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td></td>
<td>Kidney</td>
<td>0.32 ± 0.01</td>
<td>0.19 ± 0.01*</td>
<td>0.32 ± 0.01</td>
<td>81.23</td>
<td>6.1</td>
<td>&lt;0.05</td>
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<tr>
<td>GSH-Px</td>
<td>Liver</td>
<td>9.71 ± 0.24</td>
<td>6.51 ± 0.23*</td>
<td>9.45 ± 0.41*</td>
<td>91.88</td>
<td>6.3</td>
<td>&lt;0.05</td>
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<tr>
<td></td>
<td>Kidney</td>
<td>7.81 ± 0.20</td>
<td>5.19 ± 0.18*</td>
<td>7.60 ± 0.34*</td>
<td>91.98</td>
<td>5.9</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>GSH</td>
<td>Liver</td>
<td>148.70 ± 3.81</td>
<td>110.93 ± 4.04*</td>
<td>143.31 ± 6.47*</td>
<td>85.73</td>
<td>5.73</td>
<td>&lt;0.05</td>
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<tr>
<td></td>
<td>Kidney</td>
<td>132.56 ± 3.31</td>
<td>109.76 ± 3.89*</td>
<td>129.15 ± 5.89*</td>
<td>85.09</td>
<td>4.2</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

*P<0.05 as compared to Group I.
*P<0.05 as compared to Group II.
Values are mean±SEM of 6 animals in each group. Values expressed as mM/100 g tissues for TBARS and CD, units/mg protein for SOD, CAT, GST, GSH-Px and mg/100 g tissue for GSH.
One unit of enzyme activity was taken as the enzyme reaction which gave 50% inhibition of NBT reduction in 1 min.
1mmol of H₂O₂ consumed per minute.
1mg of glutathione consumed per minute.
The activities of SOD, catalase, GST and GSH-Px in the tissues studied were significantly decreased in the CCl\textsubscript{4}-treated rats compared with pair-fed control. There was also a decrease in the content of GSH in the tissues of Group II rats. Administration of \textit{G. glabra} along with CCl\textsubscript{4} restored the activities of the above antioxidant enzymes and the level of glutathione to near normal compared to the corresponding CCl\textsubscript{4} administered rats.

Histopathological studies (compared to controls) demonstrated fatty change and ballooning degeneration of hepatocytes induced by CCl\textsubscript{4}-liquid paraffin. The liver also showed distorted architecture with nodule formation, distorted central vein and the portal triad showed fibrous portal expansion with moderate fibrosis and moderate inflammation (Figures 1, a-c). Administration of the root powder of \textit{G. glabra} at a dose of 1000 mg/kg, body weight exhibited significant improvement (Figure 1d).

As a part of the pilot study, we also evaluated the effect of \textit{G. glabra} on normal rats. Over the range of dosages studied, the plant did not show any alteration of the antioxidant defense and on liver function tests.

**Discussion**

The reactive metabolites such as trichloromethyl (CCl\textsubscript{3}·) and trichloromethyl peroxy (CCl\textsubscript{3}OO·) radicals emanated from CCl\textsubscript{4} initiate peroxidation of membrane unsaturated fatty acids. This lipid peroxidation of membrane seriously impairs its function and produces liver injury.

The antioxidant enzymes SOD, catalase and peroxidases constitute a mutually supportive team of defense against reactive oxygen species (ROS).\textsuperscript{15-16} The decrease in the activity of SOD in the liver and kidney of CCl\textsubscript{4}-treated rats may be due to the increased lipid peroxidation or inactivation of the enzyme by cross-linking with malondialdehyde. This will cause an increased accumulation of superoxide radicals, which could further stimulate lipid peroxidation. GST binds to lipophilic compounds and acts as an enzyme for GSH conjugation reactions.\textsuperscript{3} The decrease in the activity of GST during CCl\textsubscript{4} toxicity may be due to the decreased availability of GSH and suggests a total inhibition of drug metabolism during CCl\textsubscript{4}-intoxication.

Depletion of GSH results in enhanced lipid peroxidation, which in turn causes increased GSH consumption.\textsuperscript{3} The medicinal herb-treated rats restored the changes in the activity of the antioxidant enzymes and the level of glutathione.

Hepatotoxins develop hypoxic conditions which can damage the perivenular zone of the hepatic acinus. The highest expression of Cytochrome P450 2E1 (CYP2E1) in the perivenular region produces oxy-radicals that contribute to the injury. Moreover, hepatocytes in the perivenular area contain less antioxidant factors and antioxidant enzymes.\textsuperscript{17} Thus, while the lipid peroxidation mediated by oxy radicals is likely to be the highest in the perivenular area, the detoxifying capacity of the hepatocytes here is reduced, therefore, the production may exceed the detoxification in the perivenular area.

In short, CCl\textsubscript{4}-induced damage produces alteration in the antioxidant status of the tissues, which is manifested as an abnormal histopathology. \textit{G. glabra} restored all these changes. So, it can be concluded that the herb is a potential antioxidant and attenuates the hepatotoxic effect of CCl\textsubscript{4} by acting as an \textit{in vivo} antioxidant and thereby inhibiting the initiation and promotion of lipid peroxidation or by an accelerated scavenging of free radicals and their products by conjugation with GSH aided by GST.

**Acknowledgement**

The first author is extremely grateful to M.G. University, Kottayam, Kerala, India for providing financial assistance in the form of JRF.

**References**


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