HEPATOPROTECTIVE EFFECT OF GLYCYRRHIZA GLABRA L.

EXTRACTS AGAINST CARBON TETRACHLORIDE-INDUCED ACUTE LIVER DAMAGE IN RATS

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ABSTRACT

Hepatoprotective potential of aqueous extract of Glycyrrhiza glabra (QGG) and ethanol extract of G. glabra (EGG) and its possible mechanism were studied in rats intoxicated with carbon tetrachloride (CCl<sub>4</sub>) in the present study. For acute hepatopathy experimental animals were intraperitoneally injected with CCl<sub>4</sub> at a dose of 1.0 ml/kg as a 50% olive oil solution. The rats were orally given the QGG and EGG at doses of 250, 500 mg/kg beside reference drug Silymarin 20 mg/kg after 6 h of CCl<sub>4</sub> treatment. At 24 h after CCl<sub>4</sub> injection, samples of blood and liver were collected and then biochemical parameters and histological studies were carried out. Results showed that both extracts and Silymarin inhibited significantly the activities of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) and increased the activity of superoxide dismutase (SOD). Thus we can conclude that the QGG and EGG (250 mg/kg and 500 mg/kg) possesses dose dependent, significant protective activity against acute liver injuries induced by CCl<sub>4</sub> and this activity can be attributed to the antioxidant property of G. glabra.

KEYWORDS: Glycyrrhiza Glabra Carbon Tetrachloride Aspartate aminotransferase Alanine Aminotransferase Superoxide Dismutase

INTRODUCTION

Glycyrrhiza glabra L. (Fabaceae), commonly named as liquorice, considered one of the well-known medicinal plants. G. glabra is a hard herb or under shrub, growing to 1 m in height, with pinnate leaves. Flowers in axillary spikes, purple to pale whitish blue, produced in a loose inflorescence. The fruits is an oblong pod, containing several seeds (Anil and Jyotsna, 2012).

For centuries the roots of G. glabra used as sweetening and flavouring agent in food and drugs. In addition to traditional uses of plants for disorders like constipation, treatment Addison disease, cough and catarrhal affections (Al-Rawi and Chakravarty, 1964), also, it has showed Cytotoxic (Basar et al. 2015), hypocholesteremic (Lim et al., 2009), anti-ulcer (Wittschier et al., 2009), anti-inflammatory (Racková et al. 2007), antimicrobial (Soleimanpour et.al., 2015; Gupta et al., 2008), renoprotective (Bafna and Balaraman, 2005), hypoglycemic (Nakagawa et al., 2004), and antiatherogenic (Visavadiya et al., 2009) properties. Roots of G. glabra contain important active compounds like glycyrrhizin, liquiritin, isoliquiritin, kanzonol, estriol and glycyramarin (Asl and Hosseinzadeh, 2008).

Scientific reports showed that the changes associated with CCl<sub>4</sub>-induced liver damage are similar to that of acute viral hepatitis (Rubinstein, 1962), manydrug and chemicals (like Isoniazid, Rifampicin, Pyrazinamide,
Non-steroidal anti-inflammatory drugs (NSAIDs), etc. induced hepatopathy and oxidative stress (Recknagel et al., 1989; Pandit et al., 2012), so CCl₄-induced hepatotoxicity model is frequently used for the investigation of hepatoprotective activity of drugs and plant materials. *G. glabra* extract showed hepatoprotective and antioxidant activity on the carbon tetrachloride CCl₄-induced carp hepatocyte damage in vitro (Yin et al., 2011). *Silybum marianum* and *G. glabra* Combination have hepatoprotective effects against oxidative stress of rats with CCl₄-induced hepatic injury (Rasool et al., 2014). Licorice lead to protective effect against alcohol-induced liver injury in mice which mediated by inflammation and oxidative stress (Jung et al., 2016). The aim of this study was to evaluate the hepatoprotective activity of *G. glabra* extracts.

**MATERIALS AND METHODS**

**Chemicals and Reagents**

All the chemicals used in this experiment were obtained from Sigma Chemical Co. (St. Louis MO, USA). All other used reagents and solvents were of analytical grade.

**Plant Material and Extracts Preparation**

*G. glabra* root was purchased from a local herbal market. The plant were identified and authenticated by a pharmacognosist. Aqueous and ethanolic extract of *G. glabra* were prepared using the method described by Abdel-Barr et al. (2000). Briefly, dried plant materials were grinded by electric grinder to a fine powder. 50g of the powder were suspended in 500 ml of ethanol and distilled water and then stirred magnetically for 24 hours at 40 °C. Subsequently, each suspension was filtered (Whatman No.1) and concentrated under reduced pressure at 40°C. The water extract was freeze-dried, yield of aqueous and ethanolic extracts were 14.2 and 7.8% respectively. The preliminary phytochemical tests revealed the presence of saponins, flavonoids, polysaccharides, amino acids in the roots of *G. glabra* (Sakar and Tanker, 1991; Harbone 1998).

**Animals**

Adult male Wistar albino rats (7 weeks, weighing 200-250 g) were used in the present study. Animals were acclimatized and maintained under standard laboratory conditions (12 h light and 12h dark cycle, 22±2 °C) access to food and water ad libitum for 7 days prior to the experiments. The food was withdrawn on the day before the experiment, but free access of water was allowed. The rats were randomly assigned. All animal experiments were conducted in accordance with the "principle of laboratory animal care" (NIH Publication No. 85-23 revised 1985).guidelines and procedures (NIH, 1985).

**CCL4-Induced Acute Liver Damage Model in Rats and G. Glabra Reatment**

The animals were randomly divided into seven groups of six animals each. Group I: Normal; Group II: CCl₄ (1ml/kg ip); Group III: QGG (250 mg/kg); IV: rats QGG (500 mg/kg); Group V: EGG (250 mg/kg); VI: EGG (500 mg/kg); VII: Reference drug Silymarin (20 mg/kg). Treatments were administered orally by gavage. Rats were intraperitoneally (i.p.) injected with CCl₄ at a dose of 1.0 ml/kg as a 50% olive oil solution according to the reported methods (Feng et al., 2000) and control ones with the same dose of olive oil. Extracts were dissolved in distilled water and orally administered to rats injected with or without CCl₄ treatment at 6 hafter CCl₄ exposure. The control rats were orally given the same volume of distilled water. These animals were fasted with free accesssto water throughout the experiment.
Twenty-four hours after administration of CCl₄, blood samples were withdrawn by cardiac puncture. Animals had been anaesthetized by ketamine/xylazine mixture (ketamine 67 mg/kg, xylazine 6 mg/kg, i.p.). The animals were sacrificed by an overdose diethyl ether immediately after blood collection. Blood samples collected in heparinized tubes were centrifuged at 3000xg for 10 min to obtain serum. Serum were used to determine AST, ALT and SOD activities. On the other hand, the liver of each rat was promptly removed and used to determine the tissue level SOD.

**Serum AST and ALT Analysis**

The hepatic enzymes alkaline phosphates (AST or GOT) and alanine amino transferase (ALT or GPT) activities were measured by using Roche Liver Enzyme Kits based on the Randox company (Lewis et al., 2006). AST and ALT activities expressed in terms of units per liter (U/L) are the amount of enzyme oxidizing one µmol/L of NADH per minute.

**SOD Levels in Serum and Liver Tissues**

The sensitive SOD assay kit (SOD Assay Kit-WST) utilizes mitochondrial activity that produces a water-soluble formazan dye upon reduction with superoxide anion. The rate of the reduction with a superoxide anion is linearly related to the xanthine oxidase (XO) activity, and is inhibited by SOD. Therefore, the inhibition activity of SOD determined by a colorimetric method was used for the determination of the serum and liver SOD level in this study. Serum and liver homogenate collected from animals were used for measurement of SOD in blood and liver tissues. Procedure was carried out according to SOD Assay Kit-WST Technical Manual (Dojindo Laboratories, Kumamoto, Japan).

**Statistical Analysis**

The results analyzed using one-way analysis of variance (ANOVA) followed by post hoc Tukey's test using SPSS (version 13.0). The values are given as mean ± S.E.M. for six rats in each group. p-Values <0.05 and 0.01 were defined as statistically significant.

**RESULTS**

**Liver Protective Effects of G. Glabra on Acute Liver Damage in Rats**

When compared with the normal group, serum AST activities were significantly elevated (p < 0.01) by 6 h after the CCl₄ treatment. Post-administration of QGG and EGG significantly decreased the AST activities in serum in comparison with CCl₄-treated rats (p < 0.05, p < 0.01) (Table 1).

Compared with the normal group, the ALT activities in serum of the CCl₄ treated control group at an i.p. dose of 1.0 ml/kg showed significant elevation (p < 0.01) after 24 h. While treatment with QGG and EGG at 250 mg/kg decreased remarkably the levels of serum ALT in rats treated with CCl₄ though higher than the normal rats. The orally administered QGG and EGG at a higher dose of 500 mg/kg BW could significantly reduce the serum ALT level and restored them to normal levels when compared with rats treated with CCl₄ control (p < 0.01). This result showed that the oral treatment with 250 and 500 mg/kg BW of QGG, EGG and Silymarin could inhibit the elevated ALT activities in rats intoxicated with CCl₄ in a dose dependent manner (Table 1).

**Table 1: Effect of G. Glabra Extracts on CCl4 Induced Hepatotoxicity in Rats**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>AST (U/L)</th>
<th>ALT (U/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>63.4 ± 6.19</td>
<td>119.2 ± 7.01</td>
</tr>
<tr>
<td>CCl₄ (1ml/kg ip)</td>
<td>324.1 ± 4.52 †</td>
<td>333.9 ± 5.36 †</td>
</tr>
<tr>
<td>QGG (250 mg/kg)</td>
<td>166.9 ± 6.81 †,#</td>
<td>241.8 ± 5.41 †,#</td>
</tr>
</tbody>
</table>

* †, # p < 0.05, p < 0.01 compared to normal group.

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Effect of G. glabra on the Levels of Serum Sod and Liver Homogenate Sod Activities in Acute Liver Damage of Rats

The inhibition rate of serum SOD activities in control rats treated with CCl₄ alone was remarkably decreased after 24 h (p < 0.01) when compared with the normal groups, which showed the injured liver functions by CCl₄. While treated with QGG, EGG and Silymarin, the inhibition rates of SOD were significantly elevated (p < 0.01) compared with the control rats, especially the high dose at 500 mg/kg of both QGG and EGG which could restore the value to the normal level (Figure 1). Liver homogenate SOD activities were similar to serum expression.

Figure 1

![Figure 1: Effect of G. glabra on serum (A) and liver tissue (B) SOD activities in CCl₄-induced liver damage in rat. Results are mean±S.E.M. for seven rats in each group. *,**, p<0.05, 0.01 vs. control group. #,## p<0.05, 0.01 vs. CCl₄ group.](image-url)

**DISCUSSIONS**

Conventional drugs today either do not show complete cure or associated with many side effects. Therefore, there are continuous needs for developing new hepatoprotective agents. Many chemical or drugs substances are known to cause hepatic injuries, such as CCl₄, acetaminophen, d-galactosamine (GalN), dimethylnitrosamine (DMN) and aflatoxins, among which, liver injury induced by CCl₄ is one of the best-characterized systems of the xenobiotic-induced hepatotoxicity and used commonly for screening the drugs with hepatoprotective activity and/or anti-hepatotoxicity (Brattin et al., 1985). CCl₄-induced liver damage, can be expressed as free radical damage model.

lipid peroxidation resulted by oxidative stress could injure hepatocellular membrane this event will be followed by
a series of cascades of cellular events such as the massive release of inflammatory mediators or cytokines, which finally lead to liver injuries (Pessayre, 1995; Dizdaroglu et al., 2002; Higuchi and Gores, 2003). Superoxide dismutase (SOD) considered one of the most important antioxidative enzymes, CCl$_4$ injection lead to significantly decrease in SOD activities. Therefore SOD selected as parameter for the antioxidative effects of $G$. glabra. The present study showed that CCl$_4$ administration caused severe acute liver damage in rats, which manifested by significant increase in serum AST, ALT levels, decreased SOD activities (Table 1 and Figure1), and histopathological changes (data not shown), indicating that CCl$_4$-induced liver damage in rats such case can be useful in the evaluation the curative effect of $G$. glabra.

The data of this study consistently demonstrated that treatment with $G$. glabra both extracts especially at a dose of 500 mg/kg BW had a potent protective effect against oxidative stress and acute liver damage induced by CCl$_4$ in rats, as showed by significant increase in SOD activities in the liver and serum (Figure 2). Also, $G$. glabra could ameliorate acute liver damage dramatically, as demonstrated by the reduction of serum AST and ALT levels (Tables 1). It was previously reported that $G$. glabra effectively scavenged the free radicals (Franceschelli et al., 2011; Ojha et al., 2013), so the action mechanisms underlying hepatoprotection of $G$. glabra may be related to both its radical scavenging properties and indirect effects through regulating of antioxidative systems.

In this study, Aqueous and ethanolic extracts of $G$. glabra significantly lowered the elevated AST and ALT levels and improved SOD levels in serum and liver tissue when compared with CCl$_4$ control group. Preliminary phytochemical tests on $G$. glabra have shown the presence flavonoids as well as polysaccharides and this could be responsible for its hepatoprotective activity. Antioxidant activity can play the cornerstone in extracts activity hence antioxidants can show its activity by various mechanisms, among which are prevention of chain initiation, binding of transition metal ion catalysts, decomposition of peroxides, prevention of continued hydrogen abstraction, reductive capacity and radical scavenging (Mossa and Nawwar, 2011).

In conclusion, the results of this investigation show that Aqueous and ethanolic extracts of $G$. glabra displays hepatoprotective effect on acute liver injuries induced by CCl$_4$, which might be considered to be therapeutic effect in clinical situations.

**REFERENCES**


Licocalchone-C extracted from Glycyrrhiza glabra inhibits lipopolysaccharide-interferon-γ inflammation by improving antioxidant conditions and regulating inducible nitric oxide synthase expression. Molecules. 16(7):5720-5734.


