Original Article

Hepatoprotective effects of Gentianella turkestanerum extracts on acute liver injury induced by carbon tetrachloride in mice

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Abstract: Objective: To investigate the contents of secoiridoid compounds (i.e. sweroside, swertiamarin and gentiopicrin) from Gentianella turkestanerum extracts, and the potential effects of G. turkestanerum extracts against carbon tetrachloride (CCl4) induced liver injury in mice. Methods: The contents of swertiamarin, gentiopicroside and sweroside from different G. turkestanerum extracts were determined with high performance liquid chromatography (HPLC). CCl4 was used to induce acute liver injury in mice. The serum aspartate amino transferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), total protein (TP), total bilirubin (TB), superoxide dismutase (SOD), malondialdehyde (MDA), glutathione transferase (GSH) and catalase (CAT) were measured. HE staining was performed to investigate the pathological changes of liver. Results: Iridoid glycoside showed the highest content in the product extracted by butanol (designated as GBA), but lower in the products extracted by ethyl acetate and water designated as GEA and GW, respectively. All G. turkestanerum extracts showed protective effects against CCl4 induced acute liver injury in mice, among which GBA showed the maximal protective effects. G. turkestanerum extracts induced significant decrease in the serum ALT, AST, ALP and TB compared with those in the mice with acute lung injury (P<0.01). Obvious increase was noticed in serum TP (P<0.01). Moreover, such effects presented in a dose-dependent manner. Compared with the control group, the MDA was significantly elevated in the model group (P<0.01), while significant decrease was observed in the levels of SOD, GSH and CAT in model group compared with the control group (P<0.01). Whereas, such phenomenon was completely reversed by G. turkestanerum extracts in a dose-dependent manner. Conclusion: G. turkestanerum showed protective effects against CCl4 induced acute liver injury in mice.

Keywords: Gentianella turkestanerum, different polar parts, iridoid glycoside, HPLC, carbon tetrachloride, hepatoprotective effect

Introduction

Liver is the major organ involved in xenobiotic metabolism and its damage could be caused by virus infiltration, drugs, and toxic chemicals from infection or ingestion [1, 2]. The risk of liver dysfunction has significantly increased due to the exposure to environmental pesticides, toxins and chemotherapeutics [3]. Nowadays, the management of acute and chronic hepatic injury is still a challenge worldwide despite the advances of modern medical technologies [4]. This leads us to identify any potential agents that may alleviate the hepatic injury effectively. Plants of Gentiana genus, a productive source in traditional Chinese medicine, are often used to ease pain, dispel rheumatism, treat liver jaundice with damp-heat pathogen, chronic pharyngitis and headache [5]. Recently, several studies have indicated that plants of Gentianella genus may have hepatoprotective effects [6-8]. For example, G. cruciata and G. asclepiadea could significantly decrease the levels of serum aspartate amino transferase (AST) and alanine aminotransferase (ALT) in rats with hepatic injury, and contribute to the attenuation of the inflammation and necrosis in hepatic tissue [6, 7]. In addition, G. veitchiorum was reported to exert hepatoprotective effects against
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**Table 1. Quantitative determination of secoiridoid compounds in Gentianella turkestanerum extracts by HPLC analysis**

<table>
<thead>
<tr>
<th>Sample</th>
<th>Swertiamarin (mg/L)</th>
<th>Gentiopicroside (mg/L)</th>
<th>Sweroside (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GE</td>
<td>3.07</td>
<td>2.15</td>
<td>0.08</td>
</tr>
<tr>
<td>GW</td>
<td>2.30</td>
<td>0.96</td>
<td>0.16</td>
</tr>
<tr>
<td>GBA</td>
<td>6.12</td>
<td>5.69</td>
<td>0.75</td>
</tr>
<tr>
<td>GEA</td>
<td>0.27</td>
<td>1.54</td>
<td>0.11</td>
</tr>
<tr>
<td>GPE</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

Note: ND, Not detected.

Carbon tetrachloride (CCl₄) induced hepatotoxicity in mice [8]. Gentianella turkestanerum, mainly distributed in Xinjiang and Inner Mongolia, has been frequently used for the management of diseases in these regions [9]. To our best knowledge, most of the studies on G. turkestanerum focused on the identification and quality control, but rare studies have been carried out to investigate the hepatoprotective effects of G. turkestanerum.

In this study, the effects and possible mechanisms of different G. turkestanerum extracts against CCl₄ induced hepatic injury were investigated. Our results showed these extracts presented protective effects on the hepatic injury in a dose dependent manner. Also, the safety of these extracts were approved with no toxicity in a dose of up to 2000 mg/Kg.

**Materials and methods**

**Animals**

ICR male mice (SPF grade, weighing 20±2 g) were obtained from the Experimental Animal Center of Xinjiang Medical University (Approve No.: SCXK 2011-0004). The animals were subject to adaptive feeding for 1 week on 12 h light/12 h dark cycle, at room temperature (24±2°C), with a relative humidity of 50±15%. All animals were access to standard diet and water. The study protocols were approved by the Animal Ethics Committees of the First Affiliated Hospital, Xinjiang Medical University.

**Chemicals and equipments**

Aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), total protein (TP), total bilirubin (TB) were purchased from the Mindray Bio-Medical Electronics Co., Ltd. (Shenzhen, China). Standard substances of swertiamarin (batch No. 2012-0129), gentiopicroside (batch No. 20120114) and sweroside (batch No. PN1125SA13) were purchased from the Yuanye Biotechnology Co. Ltd. (Shanghai, China). Silymarin was purchased from Sigma Aldrich (CA, USA). CCl₄, paraform (4%), ultrapure water, methanol were of chromatographic purity, and the other chemicals or reagents were of analytical grade.

**Plant material and preparation of the extracts**

G. turkestanerum was collected from the Gongnaisi and Bayinbuluke Forest Farm (Xinjiang, China). The collected plant material was authenticated by Professor Jiang XY in the Institute for Food and Drug Inspection (Xinjiang, China).

After grind, G. turkestanerum samples (0.5 kg) were extracted using 95% ethanol for 2 hrs. The extracts were concentrated under reduced pressure in a rotary evaporator to obtain ethanol extract (designated as GE). The GE was resuspended with sterile water, and extracted with petroleum benzene, ethyl acetate and butanol. Subsequently, the products extracted by petroleum benzene (GPE), ethyl acetate (GEA), butanol (GBA) and water (GW) were obtained, respectively.

**HPLC analysis**

The content of secoiridoides was analyzed using HPLC analysis according to the previous description [10]. Briefly, Cosmosol Packed Column 5C₁₈-PAQ (250 mm ×4.6 mm, 5 µm) was used for secoiridoid analysis. The temperature of the column was set at 25°C. The mobile phase was a mixture of methanol and 0.04% phosphoric acid (19:81, v/v). The flow rate was set to 1.0 mL/min, and a wavelength of 242 nm was used for the determination.

The standard substances swertiamarin, gentiopicroside and sweroside were dissolved in methanol and then diluted to the appropriate concentration ranges to establish the calibration curves. Linear regression analysis revealed an excellent linearity with correlation coefficient for swertiamarin (4.04-129.54 mg/L, r=0.9994), gentiopicroside (3.96-126.73 mg/L, r=0.9993) and sweroside (0.93-29.70 mg/L, r=0.9995), respectively.
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Acute toxicity testing

Acute oral toxicity study was performed according to the OECD guidelines [11]. Mice (n=3) were randomly selected for the acute toxicity study using each agent. The animals were fasted overnight prior to the experiment and maintained under standard laboratory conditions. The extracts (i.e. GE, GW, GBA, GEA) were given via lavage in each mice (18-22 g) with a dose of 200 mg/mL body weight. Then the hair color, activity, respiration, urination and defecation, as well as toxicity or even death were observed.

**Experimental design**

The mice were randomly divided into 15 groups (n=12 in each group) as follows: (i) Group 1 served as the control, which was given normal saline (NS) daily for 2 weeks; (ii) Group 2: subject to hepatic injury using CCl$_4$ (10 mL/kg) by intraperitoneal (IP) injection; and (iii) After induction of hepatic injury, the animals were subject to silymarin (100 mg/kg) (Group 3, positive control), GE extract dissolved in normal saline (NS) at 100 mg/kg (Group 4), 200 mg/kg (Group 5) and 400 mg/kg (Group 6), GW extract dissolved in NS at 100 mg/kg (Group 7), 200 mg/kg (Group 8) and 400 mg/kg (Group 9), GBA extract dis-

Table 2. Effect of different extracts from *G. turkestanorum* on mice body weight

<table>
<thead>
<tr>
<th>Group</th>
<th>Sex</th>
<th>Base level (g)</th>
<th>After treatment (g)</th>
<th>Body weight increase (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>F</td>
<td>20.620±1.195</td>
<td>27.940±0.513</td>
<td>35.50</td>
</tr>
<tr>
<td></td>
<td>M</td>
<td>21.140±0.999</td>
<td>29.680±0.672</td>
<td>40.40</td>
</tr>
<tr>
<td>GE</td>
<td>F</td>
<td>20.600±0.639</td>
<td>27.700±0.982</td>
<td>34.47</td>
</tr>
<tr>
<td></td>
<td>M</td>
<td>21.200±0.678</td>
<td>27.720±0.847</td>
<td>30.75</td>
</tr>
<tr>
<td>GW</td>
<td>F</td>
<td>20.820±0.887</td>
<td>26.560±0.654</td>
<td>27.57</td>
</tr>
<tr>
<td></td>
<td>M</td>
<td>21.340±0.713</td>
<td>27.420±0.540</td>
<td>28.50</td>
</tr>
<tr>
<td>GBA</td>
<td>F</td>
<td>20.667±0.582</td>
<td>27.060±0.907</td>
<td>30.93</td>
</tr>
<tr>
<td></td>
<td>M</td>
<td>20.550±1.294</td>
<td>27.260±0.555</td>
<td>32.65</td>
</tr>
<tr>
<td>GEA</td>
<td>F</td>
<td>20.517±1.057</td>
<td>26.860±0.673</td>
<td>30.92</td>
</tr>
<tr>
<td></td>
<td>M</td>
<td>21.100±0.725</td>
<td>27.520±0.531</td>
<td>30.43</td>
</tr>
</tbody>
</table>

Figure 1. HPLC chromatogram of mixed reference substances (A) and the samples of different *G. turkestanorum* extracts including GE (B), GW (C), GBA (D), GEA (E) and GPE (F). The peaks of 1-3 stands for swertiamarin, gentiopicroside and sweroside, respectively.
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Solved in NS at 100 mg/kg (Group 10), 200 mg/kg (Group 11) and 400 mg/kg (Group 12), GEA extract dissolved in NS at 100 mg/kg (Group 13), 200 mg/kg (Group 14) and 400 mg/kg (Group 15), respectively. On the last day of the treatment, the animals in groups 2-15 were subject to a single dose of 0.1% CCl₄ dissolved in peanut oil at 10 mL/kg intraperitoneally. Eighteen hours after CCl₄ administration, all the animals were sacrificed and blood samples were collected from the eyeballs. The liver and spleen were collected for biochemical analysis and histopathological analysis.

**Biochemical assay**

Collected blood samples were placed at room temperature for 30 min, and centrifuged at 3,000 rpm for 15 min at 4°C. Liver injury was evaluated by estimation of serum ALT, AST, ALP, TP and TB using automatic biochemistry analyzer.

**Liver antioxidant markers analysis**

Hepatic tissues (0.5 g) were homogenated in 0.9% NS, followed by centrifugation at 2,500 rpm for 15 min. Commercial kits were used for

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*Figure 2.* The effects of different extracts from *G. turkestanorum* on mice liver (A) and spleen index (B). Values are mean ± S.E.M., *n=10* in each group. *P < 0.05*, compared to the control group; **P < 0.01**, compared to the control group; *P < 0.05*, compared to the model group; ***P < 0.01***, compared to the model group.

*Figure 3.* Effects of *G. turkestanerum* extracts and silymarin on the levels of liver index (A) and spleen index (B) after CCl₄ treatment in mice. Group 1-Control group; Group 2-CCl₄ (0.1% in peanut oil) 10 ml/kg i.p; Group 3-Silymarin (100 mg/kg) + CCl₄; Group 4-GE 100 mg/kg + CCl₄; Group 5-GE 200 mg/kg + CCl₄; Group 6-GE 400 mg/kg + CCl₄; Group 7-GW 100 mg/kg + CCl₄; Group 8-GW 200 mg/kg + CCl₄; Group 9-GW 400 mg/kg + CCl₄; Group 10-GBA 100 mg/kg + CCl₄; Group 11-GBA 200 mg/kg + CCl₄; Group 12-GBA 400 mg/kg + CCl₄; Group 13-GEA 100 mg/kg + CCl₄; Group 14-GEA 200 mg/kg + CCl₄; Group 15-GEA 400 mg/kg + CCl₄. Values are mean ± S.E.M., *n=12* animals in each group. **P < 0.01**, compared to the control group; *P < 0.05*, compared to the model group; ***P < 0.01***, compared to the model group.
the determination of TP, superoxide dismutase (SOD), malondialdehyde (MDA), glutathione (GSH) and catalase (CAT), according to the manufacturer’s instructions.

**Hematoxylin-eosin staining**

Liver slices were fixed in 4% paraform, and were embedded in paraffin after dehydration. The sections (4-5 µm) were stained with hematoxylin-eosin as routinely described. The images were observed under a light microscope.

**Statistical analysis**

The data were expressed as mean ± standard deviation. One-way analysis of variance (ANOVA) was used for the comparison among groups. Student’s t test was used for comparison between groups. Data analysis was carried out using SPSS 19.0 software. P < 0.05 was considered to be statistically significant.

**Results**

**HPLC analysis of secoiridoides**

Table 1 showed the content of secoiridoid compounds (i.e. sweroside, swertiamarin and gentiopicrin) in the GBA, GE, GW, GEA and GPE. Three secoiridoid compounds were identified in all the extracts except GPE. In addition, the content of these secoiridoid compound was much more higher in GBA than the others (Figure 1).

**Figures 1-3**

Acute toxicity testing

No mortality was noticed after administration of the extracts. The body weight was increased in each mice after administration of GBA, GE, GW, and GEA, but it showed no statistical difference compared with the control group (P > 0.05, Table 2). Besides, the liver index and spleen index in these groups showed no statistical difference compared with the control group (P > 0.05, Figure 2). This indicated GBA, GE, GE and GEA showed no effects on the growth of the mice. All the extracts of G. turkestanorum were found to be safe in a dose of up to 2,000 mg/kg. Hence 1/20, 1/10 and 1/5 of the maximum dose (i.e., 100, 200 and 400 mg/kg) were suitable for the study.

**Table 3. Effects of G. turkestanorum extracts on ALI intoxicated mice**

<table>
<thead>
<tr>
<th>Group</th>
<th>ALT (U/L)</th>
<th>AST (U/L)</th>
<th>ALP (U/L)</th>
<th>TP (g/L)</th>
<th>TB (μM/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>50.44±7.26</td>
<td>129.91±19.35</td>
<td>48.82±6.38</td>
<td>48.98±4.50</td>
<td>2.66±0.47</td>
</tr>
<tr>
<td>2</td>
<td>283.80±26.91</td>
<td>304.40±21.61</td>
<td>68.76±5.74</td>
<td>39.18±2.91</td>
<td>5.37±0.63</td>
</tr>
<tr>
<td>3</td>
<td>304.40±21.61</td>
<td>45.15±7.06</td>
<td>43.33±4.66</td>
<td>43.33±1.91</td>
<td>4.72±0.41</td>
</tr>
<tr>
<td>4</td>
<td>304.40±21.61</td>
<td>261.89±18.34</td>
<td>45.94±4.66</td>
<td>45.94±4.66</td>
<td>4.72±0.41</td>
</tr>
<tr>
<td>5</td>
<td>304.40±21.61</td>
<td>261.89±18.34</td>
<td>45.94±4.66</td>
<td>45.94±4.66</td>
<td>4.72±0.41</td>
</tr>
<tr>
<td>6</td>
<td>304.40±21.61</td>
<td>261.89±18.34</td>
<td>45.94±4.66</td>
<td>45.94±4.66</td>
<td>4.72±0.41</td>
</tr>
<tr>
<td>7</td>
<td>304.40±21.61</td>
<td>261.89±18.34</td>
<td>45.94±4.66</td>
<td>45.94±4.66</td>
<td>4.72±0.41</td>
</tr>
<tr>
<td>8</td>
<td>304.40±21.61</td>
<td>261.89±18.34</td>
<td>45.94±4.66</td>
<td>45.94±4.66</td>
<td>4.72±0.41</td>
</tr>
<tr>
<td>9</td>
<td>304.40±21.61</td>
<td>261.89±18.34</td>
<td>45.94±4.66</td>
<td>45.94±4.66</td>
<td>4.72±0.41</td>
</tr>
<tr>
<td>10</td>
<td>304.40±21.61</td>
<td>261.89±18.34</td>
<td>45.94±4.66</td>
<td>45.94±4.66</td>
<td>4.72±0.41</td>
</tr>
<tr>
<td>11</td>
<td>304.40±21.61</td>
<td>261.89±18.34</td>
<td>45.94±4.66</td>
<td>45.94±4.66</td>
<td>4.72±0.41</td>
</tr>
<tr>
<td>12</td>
<td>304.40±21.61</td>
<td>261.89±18.34</td>
<td>45.94±4.66</td>
<td>45.94±4.66</td>
<td>4.72±0.41</td>
</tr>
<tr>
<td>13</td>
<td>304.40±21.61</td>
<td>261.89±18.34</td>
<td>45.94±4.66</td>
<td>45.94±4.66</td>
<td>4.72±0.41</td>
</tr>
<tr>
<td>14</td>
<td>304.40±21.61</td>
<td>261.89±18.34</td>
<td>45.94±4.66</td>
<td>45.94±4.66</td>
<td>4.72±0.41</td>
</tr>
<tr>
<td>15</td>
<td>304.40±21.61</td>
<td>261.89±18.34</td>
<td>45.94±4.66</td>
<td>45.94±4.66</td>
<td>4.72±0.41</td>
</tr>
</tbody>
</table>

Group 1-Control group; Group 2-CCl₄ (0.1% in peanut oil) 10 ml/kg i.p; Group 3-Silymarin (100 mg/kg) + CCl₄; Group 4-GE 100 mg/kg + CCl₄; Group 5-GE 200 mg/kg + CCl₄; Group 6-GE 400 mg/kg + CCl₄; Group 7-GW 100 mg/kg + CCl₄; Group 8-GW 200 mg/kg + CCl₄; Group 9-GW 400 mg/kg + CCl₄; Group 10-GBA 100 mg/kg + CCl₄; Group 11-GBA 200 mg/kg + CCl₄; Group 12-GBA 400 mg/kg + CCl₄; Group 13-GWA 100 mg/kg + CCl₄; Group 14-GWA 200 mg/kg + CCl₄; Group 15-GWA 400 mg/kg + CCl₄.

Values are mean ± S.E.M., n=12 animals in each group. *P < 0.01, compared to the control group; #P < 0.05, compared to the model group; **P < 0.01, compared to the model group.
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were significantly higher in the model group (*P* < 0.01). Silymarin and different *G. turkestanerum* extracts could significantly decrease the liver and spleen index induced by CCl₄ (*P* < 0.05).

**Effects of *G. turkestanerum* extracts on serum ALT, AST, ALP, TP and TB**

Significant increase was noticed in the serum ALT, AST, ALP and TB in the model group compared with the control group (*P* < 0.01). However, the level of TP was significantly lower in the model group than the control group (*P* < 0.01). In mice treated by silymarin, significant decrease was observed in the serum ALT, AST, ALP and TB compared with those in the mice treated by CCl₄ (*P* < 0.01). Whereas, silymarin induced obvious increase of serum TP (*P* < 0.01). Moreover, the effects presented in a dose-dependent manner, in which the GBA (400 mg/Kg) showed the maximal effects (*P* < 0.01, Table 3).

**Effects of *G. turkestanerum* extracts on SOD, MDA, GSH and CAT in liver**

As shown in Figure 4, compared with the control group, the level of MDA was significantly higher in the model group (*P* < 0.01), while the levels of SOD, GSH and CAT were significantly lower (*P* < 0.01). Silymarin could significantly inhibit the level of MDA, SOD, GSH and CAT compared with the model group (*P* < 0.01). Meanwhile, *G. turkestanerum* extracts showed similar effects, and presented in a dose-dependent manner. Among these extracts, GBA with a concentration of 400 mg/kg showed the strongest effects (*P* < 0.01).

**Histopathological examination**

The hepatic lobe was clearly displayed in the control group, together with no aberrant changes in the cellular structure (Figure 5A). The liver sections in model group revealed extensive liver injuries, characterized by loss of cellular...
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Figure 5. Photomicrographs of liver sections from: Group 1-Control group (A); Group 2-CCl<sub>4</sub> (0.1% in peanut oil) 10 ml/kg i.p (B); Group 3-Silymarin (100 mg/kg) + CCl<sub>4</sub> (C); Group 4-GE 100 mg/kg + CCl<sub>4</sub> (D); Group 5-GE 200 mg/kg + CCl<sub>4</sub> (E); Group 6-GE 400 mg/kg + CCl<sub>4</sub> (F); Group 7-GW 100 mg/kg + CCl<sub>4</sub> (G); Group 8-GW 200 mg/kg + CCl<sub>4</sub> (H); Group 9-GW 400 mg/kg + CCl<sub>4</sub> (I); Group 10-GBA 100 mg/kg + CCl<sub>4</sub> (J); Group 11-GBA 200 mg/kg + CCl<sub>4</sub> (K); Group 12-GBA 400 mg/kg + CCl<sub>4</sub> (L); Group 13-GEA 100 mg/kg + CCl<sub>4</sub> (M); Group 14-GEA 200 mg/kg + CCl<sub>4</sub> (N); Group 15-GEA 400 mg/kg + CCl<sub>4</sub> (O). H&E, original magnification 200×. Arrow: CV- central vein; BD- ballooning degeneration; C- congestion; LI- lymphocyte infiltration; FD- fatty degeneration; HE- hepatocytes edema.

boundaries, irregular shape, obvious hepatocyte edema, ballooning degeneration, fatty degeneration, necrosis, and the inflammatory cell infiltration around the central vein (Figure 5B).
The silymarin could significantly ameliorate the liver injury, and the hepatocyte structure was normal in the silymarin treated group (Figure 5C). Pretreatment with G. turkestanerum extracts could ameliorate the hepatocyte denaturation and necrosis induced by CCl₄ (Figure 5D-K). The GBA extracts (400 mg/kg) could significantly ameliorate the hepatocyte edema, ballooning degeneration and inflammatory cell infiltration (Figure 5L). Other extracts of a concentration of 400 mg/kg may induce necrosis and inflammatory cell infiltration (Figure 5M-O).

Discussion

In this study, we investigated the effects and the possible mechanism of different G. turkestanerum extracts against CCl₄ induced hepatic injury in mice. Our results indicated that G. turkestanerum extracts showed protective effects against CCl₄ induced acute lung injury in mice. Besides, secoiridoides may play crucial roles in the hepatoprotective effects.

CCl₄ has been widely used to induce liver injury in animal models [12, 13]. CCl₄ is metabolized to the trichloromethyl radical (CCl₃) by cytochrome P4502E1 (CYP2E1), which initiates lipid peroxidation and destroys polyunsaturated fatty acid [14-16]. High levels of ROS damage cells are involved in several human pathologies, including liver cirrhosis and fibrosis [17]. These processes affect the permeability of endoplasmic reticulum, mitochondrial, and plasma membranes, which finally lead to leakage of liver enzymes into the blood [18]. Therefore, estimation of enzyme activity in the serum is a useful tool to assess the hepatocellular damages [19].

Elevation of serum ALT and AST levels contributed to liver damage as they were localized in the cytoplasm normally and released into the serum after the increase of membrane permeability [20, 21]. ALP and TB are indicators of pathological alteration in biliary flow [22], and serum ALP and TB were increased in the presence of liver damage [23, 24]. The TP level will be decreased under hepaticotoxic conditions because of defective protein biosynthesis in liver [25, 26]. In our study, compared with the control group, the liver and spleen index, the levels of ALT, AST, ALP and TB were significantly higher in the model group (P < 0.01), while the level of TP was significantly lower (P < 0.01).

Our results indicated that G. turkestanerum extracts could significantly decrease the liver and spleen index. Besides, these extracts induced significant decrease of ALT, AST, ALP and TB in a dose dependent manner. Moreover, it could significantly increase the level of TP (P < 0.05), especially in mice treated by GBA (400 mg/Kg, P < 0.01). Taken together, it is reasonable to conclude that G. turkestanerum shows protective effects against CCl₄ induced acute liver injury in mice.

Oxidative stress induced by reactive oxygen species (ROS) plays a key role in hepatotoxicity [27]. It could cause liver injury through initiating lipid peroxidation, changing biomembrane function, and destroying the enzyme activity [28]. MDA is the end product of lipid peroxidation, and the elevation of MDA in serum is an indicator of liver injury [29-32]. GSH is an important intracellular antioxidant that could protect cell from damage by peroxides, free radicals and toxins [33-36]. SOD and CAT were the major components of the antioxidant system, and the decreased levels represented the liver damage [2, 37]. In our study, G. turkestanerum extracts induced significant decrease of MDA and obvious increase of SOD, GSH and CAT in a dose depended manner, especially after treatment of GBA (400 mg/kg, P < 0.01). These findings suggested that G. turkestanerum may be useful as a hepatoprotective agent against CCl₄ induced liver injury. The mechanisms of protection may include the increase in antioxidant enzyme activity and the inhibition of lipid peroxidation process.

Liver pathological section could directly reflect the degree of liver damage. In our study, the liver sections after CCl₄ treatment revealed extensive liver injuries, characterized by obvious hepatocyte edema, ballooning degeneration, fatty degeneration, necrosis, and the inflammatory cell infiltration around the central vein. Pretreatment with G. turkestanerum extracts (400 mg/kg) could ameliorate the hepatocyte denaturation and necrosis induced by CCl₄. Also, the effects of G. turkestanerum extracts on liver pathological section showed a dose depended manner, especially in mice treated with GBA (400 mg/Kg, P < 0.01).

In our study, toxicity studies showed that no mortality was observed up to a dose level of 2000 mg/kg body weight. Besides, no signs of
changes were found in the skins, furs and muscle of all animals. Central nerve response, respiration, behavior patterns, and sleep were similar to the normal group. The food intake was normal and no salorrhea, lacrimation, rhinorrhea, dyspnea, diarrhea, constipation, and intestinal tympanites were noticed. Our results showed that all the extracts of *G. turkestanerum* were found to be safe in a dose of up to 2000 mg/kg.

Phytochemical studies revealed that secoiridoides was the mainly active constituent in gentian [5, 38, 39]. In our study, HPLC showed that the content sequence of the total secoiridoides in extracts was as follows: GBA (12%) > GE (5%) > GW (3.4%) > GEA (1.9%) > GPE (0). The sequence of the protective effects for extracts was as follows: GBA > GE > GW > GEA. Our results indicated that secoiridoides may be the main active constituent in *G. turkestanerum* playing hepatoprotective effects against lung injury. In future, further studies are still needed to investigate the exact protective effects of the *G. turkestanerum* extracts and the potential mechanisms. In conclusion, *G. turkestanerum* played protective effects against CCl₄ induced acute liver injury in mice. The mechanism may be related with the inhibition of lipid peroxidation, and the improved anti-oxidant levels. Moreover, the hepatoprotective effects were correlated with the content of secoiridoides. The protective effects of *G. turkestanerum* against CCl₄ induced acute liver injury were presented in a dose-dependent manner, especially the mice treated with GBA (400 mg/Kg). We conclude that secoiridoides may play an important role in the hepatoprotective effects mediated by *G. turkestanerum*.

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Disclosure of conflict of interest

None.

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