Effect of *Laggera alata* on hepatocyte damage induced by carbon tetrachloride *in vitro* and *in vivo*

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**A R T I C L E  I N F O**

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**A B S T R A C T**

Ethnopharmacological relevance: *Laggera alata*, as a traditional Chinese herbal medicine, has been widely used to ameliorate some ailments associated with inflammation including hepatitis in folk. **Aim of the study**: Based on anti-inflammatory activity of total phenolics from *Laggera alata* (TPLA), to further validate the remarkable curative effect *Laggera alata* in hepatitis, hepatoprotective effect of TPLA was examined. **Materials and methods**: TPLA was prepared and its principle components were quantificationally analyzed. The hepatoprotective effects of TPLA were studied using a CCl₄-induced injury model in primary cultured neonatal rat hepatocytes, and a CCl₄-induced acute and chronic damage model *in vivo*. **Results**: TPLA significantly reduced cellular leakage of hepatocyte aspartate aminotransferase (AST) and alanine aminotransferase (ALT) and improved cell viability *in vitro* and *in vivo*. **Conclusions**: This investigation verifies the hepatoprotective effect of TPLA *in vitro*/*in vivo* and clarifies its active components dicafeoylquinic acids responsible for hepatoprotective potential.

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1. Introduction

Traditional Chinese herbal medicines are known to possess a diversity of components or secondary metabolites which have various biological activities. However, to further validate the remarkable curative effect and elucidate its pharmacologic substance basis is a facing task. The genus *Laggera* (Asteraceae) is distributed mainly in tropical Africa and Southeast Asia. *Laggera alata* and *Laggera pterodonta* are the only two *Laggera* species found in China. Both are employed as folk medicines for over 300 years (Jiangsu New Medical College, 1977). *Laggera alata* was given wide attention in the last few years due to its remarkable curative effect, especially for the treatment of some ailments associated with inflammation including hepatitis. Most of studies concerned on *Laggera alata* focused on folk use and phytochemical analyses of this plant (Bohllmann et al., 1985; Raharivelomanana et al., 1998; Zheng et al., 2003a,b,c). In previous investigations, we examined the anti-inflammatory activities of total phenolics from *Laggera alata* (TPLA) and confirmed its potent inhibitory effects in models of acute and chronic inflammation (Wu et al., 2006a). But there have not yet been clarified on the pharmacologic action and mechanism of this plant in the treatment of hepatitis.

The correlative researches have indicated that hepatitis is induced by viruses, alcohol, lipid peroxidative products, and various drugs. However, as yet there is no suitable drug to treat patients with hepatitis. Koop advocated in *Science* that natural products should be considered important resources for future medicines (Koop, 2002). Based on our previous researches and folk use of this plant, it is postulated that *Laggera alata* is likely a good candidate for the development of new anti-hepatitis medicine. It is well known that hepatocyte injury is one of pathological changes of hepatitis. The amelioration of hepatocyte injury is the important aim of the treatment of hepatitis. CCl₄ is a widely used hepatotoxic agent that enhances the formation of free radicals, which cause lipid peroxidation of cellular and organelle membranes (Clawson, 1989). Oxidative stress has been noted to contribute to the pathogenesis of acute hepatitis. Free radicals are toxic to hepatocytes and initiate a reactive oxygen species-mediated cascade causing hepatocyte cell death and leading to acute hepatitis (Bedda et al., 2003; Okuyama et al., 2003). Silymarin from milk thistle is a hep-
The hepatoprotective effect of TPLA against CCl4-induced injury was examined in primary cultured neonatal rat hepatocytes, mice with acute hepatic damage and rats with chronic hepatic lesion. Silibinin was employed as the reference drug in vitro and in vivo tests. The results indicated TPLA possesses potent hepatoprotective effect on CCl4-induced hepatocyte injury in vitro and in vivo.

2. Materials and methods

2.1. Preparation and quantification of TPLA

The whole herb of Laggera alata (D. Don) Sch.-Bip ex Olivier was collected from Tengchong county, Yunnan Province, China, in August 2003, and authenticated by Professor Liurong Chen, College of Pharmaceutical Sciences, Zhejiang University, China. A voucher specimen (No. ZY982003LA) was deposited in the herbarium of College of Pharmaceutical Sciences, Zhejiang University, China. Total phenolics from Laggera alata (TPLA) were prepared and its principle components were quantitatively analyzed according to the method we reported previously (Wu et al., 2006a). The results of quantification indicated that TPLA has a high content of phenolic compounds that made up half of the extract (52.6 g GAE/100 g extract). The HPLC analyses indicated that dicafeoylquinic acids (4.5-O-dicafeoylquinic acid, 3,5-O-dicafeoylquinic acid and 3,4-O-dicafeoylquinic acid) were the major components in TPLA whose content amounted to 51% (Wu et al., 2006a). TPLA was dissolved in 0.5% CMC-Na solution to be administered to the tested animals.

2.2. Animals

Male ICR mice weighing 20–25 g and male Sprague-Dawley rats weighing 180–220 g were kept in a room maintained at 22 ± 2 °C and at relative humidity between 40% and 70%. The animal experimental protocol was approved by the Animal Ethics Committee of Zhejiang University, in accordance with the Guiding Principles in the Use of Animals in Toxicology, adopted by the Society of Toxicology (USA) in July 1989 and revised in March 1999.

2.3. Chemicals

Carbon tetrachloride (CCl4), penicillin, streptomycin, dimethyl sulfoxide, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and silibinin were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Aspartate aminotransferase (AST), and alanine aminotransferase (ALT) diagnostic kits were also purchased from Sigma. Fetal bovine serum, 1640 medium and tris base were purchased from Gibco-BRL (Grand Island, NY, USA). Total protein, albumin, sialic acid, and hydroxyproline kits were purchased from Nanjing Jiancheng Bioengineering Institute (China). All other reagents were of the highest commercial grade available.

2.4. CCl4-induced hepatocyte injury in vitro

Neonatal rat hepatocytes were isolated from 3-day-old Sprague–Dawley rats according to the method of Anil Kumar et al. (2002). Cell viability was determined by trypan blue exclusion assay. The isolated hepatocytes were suspended in 1640 medium, and then transferred to 96-well culture plates at a density of approximately 5 × 103 cells/ml. After plating, the cells were incubated at 37 °C. After hepatocyte attachment to the culture plate, the medium was exchanged to remove unattached or dead cells. Twelve hours later, the hepatocytes were treated with hepatotoxic agents and tested in the following assays.

Cellular cytotoxicity induced by TPLA treatment was measured using the MTT assay as follows: hepatocytes were cultured in 1640 medium in the presence of 1–100 µg/ml TPLA for 48 h and then 10 µl of MTT (5 mg/ml) was added to cells in each well. After 4 h of culture, the medium was removed, and the blue formazan crystals that had formed were dissolved in dimethyl sulfoxide. The absorbency of formazan generated from MTT was measured at 570 nm using an ELX 800 universal microplate reader. Cell survival was defined as the amount of formazan production relative to that by cells not treated with hepatotoxic chemicals, and expressed as a percent.

After the hepatocytes had been incubated for 6 h with 8 mM CCl4 in 1640 medium at 37 °C (95% humidity, 5% CO2), the cells were then incubated for another 48 h in fresh culture medium containing 1–100 µg/ml TPLA. CCl4 was diluted with absolute ethanol before addition to the culture medium. Control experiments indicated that vehicles used in the study had no influence on the extent of celllar damage. Silibinin concentrations of 1–100 µg/ml were used as a reference drug for all studies. Hepatocyte injury was assessed by measuring the amount of AST and ALT leakage as well as cell viability. AST and ALT leakages into the medium were quantified using diagnostic kits for each enzyme. Viability was calculated as described above.

2.5. CCl4-induced acute hepatic damage in mice

Sixty mice were divided into six groups. Groups A and B, which served as vehicle and model control, respectively, received 0.5% CMC-Na solution at a dose of 10 ml/kg. Group C was served as drug control and received silybin at a dose of 100 mg/kg. Groups D–F received TPLA at doses of 50, 100, and 200 mg/kg, respectively. The vehicle and drugs were administered orally to the groups of mice, respectively, once per day for 7 days. One hour after the last administration, liver damage was induced in mice of Groups B–F by intraperitoneal injection of CCl4 (10 ml/kg, 1% in olive oil). The 10 mice of Group A were not given CCl4 but olive oil as a control. To enhance the hepatotoxicity, all animals were starved overnight after CCl4 treatment. Twenty-four hours after the last administration, mice were slightly anaesthetized with ether and blood samples were taken from the eyebut. The serum was separated for the measurement of AST and ALT.

Liver samples were rapidly removed and fixed in 10% neutral-buffered formalin for histopathological analysis. Then, the fixed liver specimens were embedded in paraffin, sliced 5-µm thick, and stained with hematoxylin and eosin (HE). The pathological changes were assessed and photographed under an Olympus BX-51 microscope.

2.6. CCl4-induced chronic hepatic damage in rats

Sixty rats were divided into two groups with 50 and 10 animals. Liver damage was induced in 50 rats by subcutaneous injection of CCl4 (0.5 ml/kg, 10% in olive oil) twice a week for 13 weeks. To enhance the hepatotoxicity, all animals were starved overnight after CCl4 treatment every time. The other 10 rats (Group A) were not given CCl4 but olive oil as a control. After hepatic damage modes were induced for 13 weeks, the 50 rats with liver injury were randomly divided into five groups (Groups B–F) of 10 animals each. The blood sample of each animal from every group was collected and its serum was separated for assay of AST. The rat with the highest and the lowest AST levels in each group were eliminated, respectively. The rest 8 rats of every group were treated as follows.
Group A received 0.5% CMC-Na solution orally as the vehicle control. Group B, which served as the model control, received 0.5% CMC-Na solution orally. Group C received silybin at 100 mg/kg orally. Groups D–F received TPLA at 50, 100 and 200 mg/kg orally once a day for 2 months, respectively. The doses and treatment periods of the silybin and TPLA were determined by the results of the pretest. No apparent adverse effects were observed during the treatments. At 4 h after the last treatment of drug, blood samples were collected under light ether anesthesia by the eyepit puncture method, and the serum was separated by the eyepit puncture method, and the serum was separated. Liver samples were rapidly removed, rinsed in cold saline, and homogenized for hydroxyproline examination. The remaining liver was fixed in 10% neutral-buffered formalin for histopathological analysis.

For biochemical determinations, serum samples were processed as described in the instructions provided for the diagnostic kits. The serum levels of ALT, AST, total protein, albumin, and sialic acid were determined using the ALT, AST, total protein, albumin, and sialic acid detection kits, respectively. The degree of hepatic fibrosis was evaluated by quantifying the liver hydroxyproline and sialic acid contents. Liver samples were processed as described in the instructions provided for the test kits and measured using the hydroxyproline detection kits. For histopathological analysis, liver specimens fixed in 10% neutral-buffered formalin were embedded in paraffin, sliced 5-μm thick, and stained with hematoxylin and eosin (HE). The pathological changes were analyzed under an Olympus BX-51 microscope.

2.7. Statistical analysis

The data were expressed as mean ± standard deviations of the mean (S.D.) and subjected to a one-way analysis of variance (ANOVA) and Student’s t-test. P < 0.05 was chosen as the criterion of statistical significance. Statistical analyses were carried out using SPSS version 10.0 software.

3. Results

3.1. Protective effect of TPLA on CCl4-induced hepatocyte injury in vitro

Trypan blue exclusion assay indicated that cell viability of the isolated hepatocytes was over 90% in the study. The cytotoxicity of TPLA was tested in isolated rat hepatocytes. The result showed that TPLA concentrations of 1–100 μg/ml were almost nontoxic to the cells (Table 1). Cytotoxicity was induced in neonatal rat hepatocyte by exposure to 8 mM CCl4 and the cells were subsequently treated with TPLA. As shown in Table 2, TPLA at concentrations of 1–100 μg/ml significantly reduced cellular leakage of AST and ALT, and improved cell viability. Furthermore, TPLA afforded much stronger protection than the reference drug silybin.

3.2. Effects of TPLA on CCl4-induced acute hepatic damage in mice

The effects of the oral treatment of TPLA on the serum AST and ALT levels of hepatic-damaged mice are shown in Table 3. TPLA at doses of 100 and 200 mg/kg significantly reduced the serum AST and ALT levels of the mice with hepatic damage. At a dose of 100 mg/kg, silybin used as the standard drug indicated the similar effect.

The administration of CCl4 caused liver damage (large area hepatocyte necrosis in the centrilobular zone, hepatocyte steatosis, etc.), which appeared in all animals of the model control group. No histological abnormalities were observed in vehicle control mice. Administration of different doses of TPLA resulted in significant recovery of hepatocytes in different sections of the liver. TPLA at a dose of 200 mg/kg showed near normalization of the tissues (Fig. 1). Silybin used as the reference drug showed the similar protection. The results suggested TPLA improved CCl4-induced acute hepatic damage.

3.3. Effects of TPLA on CCl4-induced chronic hepatic damage in rats

3.3.1. Effects of TPLA on serum parameters of chronic hepatic-damaged rats

The effects of the oral treatment of TPLA on the serum AST, ALT, total protein, and albumin levels of hepatic-damaged rats are shown in Table 4. The serum AST and ALT levels of the CCl4-only group were elevated, whereas the total protein and albumin contents and A/G values of this group were reduced, thus indicating that liver damage was markedly induced. Simultaneous treatment with TPLA significantly reduced the serum AST and ALT levels of the hepatic-damaged rats.

Table 1

<table>
<thead>
<tr>
<th>Group</th>
<th>Concentration (μg/ml)</th>
<th>Absorbency</th>
<th>Cell survival (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>–</td>
<td>0.882 ± 0.068</td>
<td>100</td>
</tr>
<tr>
<td>Silybin</td>
<td>100</td>
<td>0.826 ± 0.052</td>
<td>93.65</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>0.832 ± 0.035</td>
<td>94.33</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>0.843 ± 0.072</td>
<td>95.58</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>0.851 ± 0.040</td>
<td>96.48</td>
</tr>
<tr>
<td>TPLA</td>
<td>100</td>
<td>0.832 ± 0.077</td>
<td>94.33</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>0.836 ± 0.088</td>
<td>94.78</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>0.841 ± 0.092</td>
<td>95.35</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>0.858 ± 0.102</td>
<td>97.28</td>
</tr>
</tbody>
</table>

All determinations were performed in six replicates and values were expressed as mean ± S.D. No significant difference compared with the vehicle control.

Table 2

<table>
<thead>
<tr>
<th>Group</th>
<th>Concentration (μg/ml)</th>
<th>AS (IU/L)</th>
<th>ALT (IU/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>–</td>
<td>57.36 ± 3.02**</td>
<td>31.25 ± 1.67**</td>
</tr>
<tr>
<td>CCl4 control</td>
<td>–</td>
<td>108.36 ± 4.11</td>
<td>56.36 ± 3.27</td>
</tr>
<tr>
<td>Silybin</td>
<td>100</td>
<td>87.64 ± 3.24*</td>
<td>44.28 ± 1.54**</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>95.88 ± 6.85</td>
<td>50.25 ± 4.89</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>98.25 ± 5.76</td>
<td>53.14 ± 5.75</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>104.22 ± 5.03</td>
<td>56.02 ± 6.24</td>
</tr>
<tr>
<td>TPLA</td>
<td>100</td>
<td>65.36 ± 3.04**</td>
<td>36.48 ± 2.04**</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>66.22 ± 2.79**</td>
<td>38.46 ± 2.75**</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>73.12 ± 4.25</td>
<td>42.11 ± 3.39***</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>87.25 ± 3.05</td>
<td>44.28 ± 2.05***</td>
</tr>
</tbody>
</table>

All determinations were performed in six replicates and values were expressed as mean ± S.D.

* P < 0.05 compared with the CCl4 control.
** P < 0.01 compared with the CCl4 control.
*** P < 0.001 compared with the CCl4 control.
with TPLA significantly attenuated the CCl₄-induced elevation of the AST and ALT levels, the decrease of the total protein and albumin contents, and the reduction of A/G values. These data suggested that TPLA reduced the CCl₄-induced hepatic damage. Silibinin, used as the reference drug, indicated a similar effect.

3.3.2. Effects of TPLA on liver hydroxyproline and serum sialic acid contents of chronic hepatic-damaged rats

The effects of TPLA on the degree of hepatic fibrosis in rats with CCl₄-induced damage were shown in Table 5. In the CCl₄-only treatment group, the liver hydroxyproline and serum sialic acid

<table>
<thead>
<tr>
<th>Groups</th>
<th>Dose (mg/kg)</th>
<th>Total protein (g/l)</th>
<th>Albumin (g/l)</th>
<th>AST (IU/l)</th>
<th>ALT (IU/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>–</td>
<td>75.36 ± 3.23</td>
<td>35.35 ± 3.24</td>
<td>55.68 ± 3.58</td>
<td>34.28 ± 4.12</td>
</tr>
<tr>
<td>Model</td>
<td>–</td>
<td>65.15 ± 4.58</td>
<td>25.52 ± 3.21</td>
<td>214.22 ± 23.12</td>
<td>122.35 ± 11.24</td>
</tr>
<tr>
<td>Silybin</td>
<td>100</td>
<td>70.27 ± 7.24</td>
<td>32.11 ± 4.46</td>
<td>85.55 ± 8.23</td>
<td>71.23 ± 8.21</td>
</tr>
<tr>
<td>TPLA</td>
<td>50</td>
<td>72.15 ± 6.17</td>
<td>30.25 ± 1.75</td>
<td>105.33 ± 9.05</td>
<td>85.62 ± 5.29</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>72.94 ± 5.26</td>
<td>31.24 ± 2.75</td>
<td>92.14 ± 7.25</td>
<td>65.20 ± 7.15</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>73.53 ± 7.61</td>
<td>33.64 ± 3.01</td>
<td>87.21 ± 9.16</td>
<td>62.33 ± 5.21</td>
</tr>
</tbody>
</table>

All determinations were performed in 8 rats and values were expressed as mean ± S.D.

* P < 0.05 with respect to the model control.
** P < 0.01 with respect to the model control.
*** P < 0.001 with respect to the model control.
Table 5
Effects of TPLA on liver hydroxyproline and serum sialic acid contents of chronic hepatic-damaged rats.

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose (mg/kg)</th>
<th>Sialic acid (mmol/l)</th>
<th>Hydroxyproline (µg/g prot)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>–</td>
<td>3.56 ± 0.34***</td>
<td>8.69 ± 0.78***</td>
</tr>
<tr>
<td>Model</td>
<td>–</td>
<td>7.62 ± 1.02</td>
<td>30.43 ± 3.22</td>
</tr>
<tr>
<td>Silybin</td>
<td>100</td>
<td>5.67 ± 0.52**</td>
<td>15.68 ± 1.25***</td>
</tr>
<tr>
<td>TPLA</td>
<td>50</td>
<td>6.11 ± 0.45</td>
<td>21.37 ± 2.01***</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>5.12 ± 0.87**</td>
<td>16.95 ± 1.24***</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>4.88 ± 0.89*</td>
<td>15.88 ± 2.38***</td>
</tr>
</tbody>
</table>

Values were expressed as mean ± S.D. of 8 rats.

* P<0.05 with respect to the model control.
** P<0.01 with respect to the model control.
*** P<0.001 with respect to the model control.

contents, two hepatic fibrosis parameters were increased, thereby indicating that liver fibrosis was induced. However, the oral treatment with TPLA improved markedly the CCl₄-induced elevation of liver hydroxyproline and serum SA contents. Silibinin showed a similar effect. These results indicated that TPLA improved the CCl₄-induced hepatic fibrosis.

3.3.3. Histopathological effects of TPLA on chronic hepatic-damaged rats

Histopathological effects of TPLA on chronic hepatic-damaged rats were shown in Fig. 2. No histological abnormalities were observed in control rats. The administration of only CCl₄ for 13 weeks caused serious liver damage (hepatocyte necrosis and swelling, formation of vacuoles in cells, hepatocyte fat deposition, and fibrosis), which appeared in all animals of this group. Administration of different doses of TPLA resulted in significant

Fig. 2. Histopathological effects of TPLA on chronic hepatic-damaged rats induced by CCl₄ (H&E 40×). (A) A control untreated rat showing a normal central vein and hepatocytes, (B) a CCl₄-treated rat showing the necrosis and swelling of hepatic cells, formation of vacuoles, hepatocyte fat deposition and fibrosis, (C) a silibinin (100 mg/kg)–CCl₄-treated rat showing obvious improvement of liver damage, (D) a TPLA (50 mg/kg)–CCl₄-treated rat, (E) a TPLA (100 mg/kg)–CCl₄-treated rat, and (F) a TPLA (200 mg/kg)–CCl₄-treated rat. (D)–(F) show the improvement of chronic liver damage.
recovery of hepatocytes in different sections of the liver. At a dose of 200 mg/kg, TPLA showed almost complete normalization of the tissues. These observations further supported the antihepatotoxic activity of TPLA.

4. Discussion and conclusions

The therapeutic benefits of traditional Chinese medicines have been recognized for centuries. Although there is still lack of evidence for clarification of their typical mechanisms, unlike with Western medicine, it is still widely accepted by people from East Asia (for example Japan and Korea, etc.) and beginning to be accepted by the rest of the world (for example Europe, North America and Australia, etc.). *Laggera alata*, as a traditional Chinese medicine, has been widely used to ameliorate some inflammatory ailments as hepatitis in folk. To further validate the remarkable curative effect of *Laggera alata*, the hepatoprotective effects of TPLA were studied using a CCl4-induced injury model in primary cultured neonatal rat hepatocytes, and a CCl4-induced acute and chronic damage model in vivo.

Chemical injury was induced in the cells by the classical hepatotoxic agents CCl4 to evaluate the hepatoprotective effect of TPLA in vitro. Cellular leakage of AST and ALT and decreased cell viability were observed in the cultured hepatocytes in response to the chemical agent, which are conventional inducers of hepatocyte injury. The stabilization of AST, ALT, and cell viability provides a clear indication of the improved functional status of the cells. Therefore, these parameters were used to assess the effect of TPLA in preventing hepatocyte injury. In cells exposed to CCl4, subsequent treatment with TPLA significantly reduced cellular leakage of AST and ALT and improved cell viability. Moreover, TPLA afforded much stronger protection than the reference drug silybinin, thus demonstrating the protective effect of TPLA on chemically injured hepatocytes.

Based on in vitro results of TPLA against CCl4-induced hepatocyte injury, we further studied the protection afforded by TPLA against CCl4-induced acute/chronic hepatic damage in mice/rats. In the whole animal model, the increased levels of AST and ALT, the decreased level of total protein and albumin, and the decrease in A/G values are classical indicators of liver damage. In CCl4-induced acute liver damage model, Administration of different doses of TPLA significantly reduced the serum AST and ALT levels. The increase with hepatic damage and resulted in significant recovery of hepatocytes in different sections of the liver. The results suggested TPLA ameliorated markedly CCl4-induced acute hepatic damage. In CCl4-induced chronic hepatic damage model, the degree of hepatic fibrosis was evaluated by quantifying the liver collagen and serum sialic acid contents. The total collagen content in the liver was determined by estimation of hydroxyproline, a characteristic amino acid in collagen. Treatment with TPLA significantly attenuated the elevation of liver hydroxyproline and serum SA contents, increase of the AST and ALT levels, the decrease of the total protein and albumin contents, and the reduction of A/G values. The results of biochemical tests proved the significant corrective effect of TPLA on the biochemical parameters of liver damage. Simultaneously, according to histopathological examinations, severe hepatic lesions induced by CCl4 were remarkably reduced by the administration of TPLA, in good agreement with the results of biochemical tests and in vitro research.

Hepatotoxic compounds such as CCl4 are known to cause remarkable increase in serum transaminases and induce liver injury through lipid peroxidation by free radical derivatives of the compound. The toxic effect of CCl4 is an example of free radical disease, due to its conversion by P-450 enzyme system to the highly reactive toxic free radical CCl3, which attacks the membranes of smooth and rough endoplasmatic reticulum (Farber et al., 1971; Gravela et al., 1979; Wolf et al., 1980; Yagi, 1987; Azri et al., 1992; Brent and Rumack, 1993; Fehér and Prónai, 1993). These changes are similar to those observed during cellular oxidative stress, which is considered to play a prominent role in the pathogenesis of many diseases, including liver injury (Park et al., 2005). Oxidative stress is the state of imbalance between the level of antioxidant defence system and production of oxygen-derived species. Increased O2− concentration and production of oxygen-derived species such as superoxide radical (O2−), hydroxyl radical (OH•) and hydrogen peroxide cause oxidative stress (Zhu et al., 2004). TPLA improved CCl4-induced injury in neonatal rat hepatocytes and in mice/rats, thus also suggesting the ability of TPLA to ameliorate oxidative stress, in accordance with the results we previously reported (Wu et al., 2006a).

Quantification analysis of TPLA led to a conclusion that this extract fraction contains plenty of phenolic compounds, especially dicaffeoylquinic acids. Dicaffeoylquinic acids exhibit several pharmacological activities such as antioxidative, anti-inflammatory, hepatoprotective and antiviral, etc. (Kimura et al., 1987; Maruta et al., 1995; Peluso et al., 1995; Mcdougall et al., 1998; Gongora et al., 2003; Sun et al., 2004; Ooi Linda et al., 2006). The results obtained in this research clearly indicated that TPLA have pronounced protective potential against oxidative damage in accordance with the description of the pharmacological actions of phenolic compounds in the aforementioned references. In previous research, the potent anti-inflammatory effect of TPLA was proved and its action mechanisms are probably associated with the inhibition of prostaglandin formation, the influence on the antioxidant systems, and the suppression of lysozyme release (Wu et al., 2006a). Furthermore, the anti-inflammatory and hepatoprotective effects of the prepared pure dicaffeoylquinic acids have been confirmed. These data approved our prediction that TPLA possesses a good efficacy against liver injury for medicinal utilization. In addition, our previous study found that total flavonoids exact from the plant also possess hepatoprotective activity (Wu et al., 2006b). Taken together, dicaffeoylquinic acids such as 3,4-O-dicaffeoylquinic acid, 3,5-O-dicaffeoylquinic acid and 4,5-O-dicaffeoylquinic acid, may be the major active compounds responsible for the biological activity of *Laggera alata*. And flavonol compounds may also contribute to the curative effect of the plant in some inflammatory ailments including hepatitis.

Free radicals alter the structural and functional integrity of cells by a variety of mechanisms, including lipid peroxidation, sulfhydryl oxidation, proteolysis and shearing of the nuclear material. Healthy cells can scavenge free radicals effectively by their defensive system (antioxidant effects). In short, there is a dynamic relationship between reactive oxygen species and antioxidants in the human body. In some pathological conditions, such as cells suffering ischaemic insult, the sudden generation of reactive oxygen species can dramatically upset this balance with an increased demand on the antioxidant defence system. Natural antioxidants are depleted accompanied by accumulation of reactive oxygen species. In such a situation, natural products can play an important role in two aspects: enhance the activity of original natural antioxidants and neutralize reactive oxygen species by nonenzymatic mechanisms (Zhu et al., 2004). It is reported that oxidative stress is involved in many diseases like inflammation and liver damage (Wang et al., 2004; Park et al., 2005). CCl4-induced cell injury is closely related to the formation of oxidative stress. Hence, hepatoprotection of TPLA may be achieved by ameliorating oxidative stress from chemical-induced injury in hepatocytes.

In conclusion, this investigation verifies the hepatoprotective effect of *Laggera alata* in vitro/in vivo and clarifies its substance basis responsible for the hepatoprotective potential. The hepatoprotective action of TPLA is likely based on its active
components dicaffeoylquinic acids (such as 3,4-O-dicaffeoylquinic acid, 3,5-O-dicaffeoylquinic acid and 4,5-O-dicaffeoylquinic acid) and related to its potent antioxidative and anti-inflammatory activity. Neutralizing reactive oxygen species by nonenzymatic mechanisms and enhancing the activity of original natural hepatic-antioxidant enzymes may be the main mechanisms of action of TPLA against CCl₄-induced injury. Additional, flavonol compounds may also play important role in the biological activity of Laggera alata. These data provide a scientific explanation for the folkloric uses of Laggera alata in the treatment of hepatatitis. More detailed studies on dicaffeoylquinic acids are currently in progress.

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References


