Ginseng essence, a medicinal and edible herbal formulation, ameliorates carbon tetrachloride-induced oxidative stress and liver injury in rats

Kuan-Hung Lu1,*, Ching-Yi Weng1,*, Wei-Cheng Chen1, Lee-Yan Sheen1,2,3,*

1 Institute of Food Science and Technology, National Taiwan University, Taipei, Taiwan
2 Center for Food and Biomolecules, National Taiwan University, Taipei, Taiwan
3 National Center for Food Safety Education and Research, National Taiwan University, Taipei, Taiwan

Abstract

Background: Ginseng essence (GE) is a formulation comprising four medicinal and edible herbs including ginseng (Panax ginseng), American ginseng (Panax quinquefolius), lotus seed (Nelumbo nucifera), and lily bulb (Lilium longiflorum). This study was aimed at investigating the hepatoprotective effect of GE against carbon tetrachloride (CCl4)-induced liver injury in rats.

Methods: We treated Wistar rats daily with low, medium, and high [0.625 g/kg body weight (bw), 1.25 g/kg bw, and 3.125 g/kg bw, respectively] doses of GE for 9 wk. After the 1st wk of treatment, rats were administered 20% CCl4 (1.5 mL/kg bw) two times a week to induce liver damage until the treatment ended.

Results: Serum biochemical analysis indicated that GE ameliorated the elevation of aspartate aminotransferase and alanine aminotransferase and albumin decline in CCl4-treated rats. Moreover, CCl4-induced accumulation of hepatic total cholesterol and triglyceride was inhibited. The hepatoprotective effects of GE involved enhancing the hepatic antioxidant defense system including glutathione, glutathione peroxidase, glutathione reductase, glutathione S-transferase, superoxide dismutase, and catalase. In addition, histological analysis using hematoxylin and eosin and Masson’s trichrome staining showed that GE inhibited CCl4-induced hepatic inflammation and fibrosis. Furthermore, immunohistochemical staining of alpha-smooth muscle actin indicated that CCl4-triggered activation of hepatic stellate cells was reduced.

Conclusion: These findings demonstrate that GE improves CCl4-induced liver inflammation and fibrosis by attenuating oxidative stress. Therefore, GE could be a promising hepatoprotective herbal formulation for future development of phytotherapy.

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1. Introduction

Chronic liver disease and cirrhosis are the leading causes of death in Taiwan and have been responsible for an increasing number of fatalities in recent years [1]. Prevention of liver disease has become an important task for public health authorities in the absence of the discovery of an actual curative therapeutic agent. Numerous studies have demonstrated that oxidative stress is a mediator of acute and chronic liver injuries [2–4]. In addition, loss of balance between the antioxidant defense system and free radicals in the body can trigger inflammation and may lead to chronic diseases such as liver and cardiovascular diseases as well as diabetes and cancer [5,6]. Therefore, the use of antioxidants from herbal medicines or functional foods is a reasonable treatment strategy for inhibiting inflammation and oxidative damage to reduce the incidences of such diseases.
Medicine food homology (藥食同源 yào shí tóng yuán) means that food and traditional Chinese medicine originated at the same time in ancient China. Based on this concept, medicine food homology materials are considered a treasure house of functional factors for current functional foods [7]. Ginseng essence (GE) is an herbal formulation comprising four Chinese Materia Medica (中藥 zhōng yào) plants including ginseng (參 rén shen, Panax ginseng), American ginseng (西洋參 xiāng yáng shēn, Panax quinquefolius), lotus seed (蓮子 lián zi, Nelumbo nucifera), and the little bulb (百合 bái hé, Lilium longiflorum). They are allowed to be used not only as traditional Chinese medicine but also as food ingredients in Taiwan. Previous studies have indicated that ginseng and American ginseng as well as their main active compounds the ginsenosides have a number of biological benefits including hepatoprotective [8], anti-inflammatory [9], anti-diabetic [10], and tumor growth reduction [11]. Lotus seeds have been found to have hepatoprotective [12], blood sugar lowering [13], anti-inflammatory, and antioxidative activities, and the ability to prevent diabetes [14]. In addition, a few studies have shown that lily reduces inflammation [15], prevents cancer [16], inhibits fungal growth [17], and inhibits oxidative reactions [18]. Therefore, we hypothesized that the herbal formulation GE may have therapeutic potential for the prevention of liver injury via free radical scavenging as well as anti-inflammatory activities.

Carbon tetrachloride (CCl₄) is a well-known hepatotoxin, which is widely used to induce acute toxic liver injury in animals. Numerous studies have shown that CCl₄ is metabolized by the cytochrome P₄₅₀ enzyme system to yield reactive metabolic products including trichloromethyl free radicals, which can initiate the process of lipid peroxidation and ultimately result in the overproduction of reactive oxygen species (ROS) and hepatocyte injuries [19,20]. The rat model of CCl₄-induced liver injury is well established and is one of the methods for the evaluation of hepatoprotective agents recommended by the Ministry of Health and Welfare, Taiwan. In addition, silymarin (Silybum marianum) is an herbal product containing a mixture of flavonolignan isomers. Silymarin is used as a positive control in the animal model because numerous studies have shown that it can prevent CCl₄-induced lipid peroxidation and hepatotoxicity by decreasing the metabolic activation of CCl₄ and acting as a chain-breaking antioxidant [21–23]. Therefore, the aim of this study was to investigate whether GE can protect the rat liver against CCl₄-induced oxidative damage and inflammation. In this study, male Wistar rats were treated with GE [0.625 g/kg body weight (bw)/d, 1.25 g/kg bw/d, and 3.125 g/kg bw/d] or silymarin (positive control, 0.5 g/kg bw/d) for 9 wk. After the 10 wk of treatment, rats were gavaged with 20% CCl₄ at 1.5 mL/kg bw two times/wk to induce liver injury. After treating the animals, serum biochemical and antioxidant enzyme levels were determined, and histopathological observation of hepatic inflammation and fibrosis was performed to assess the hepatoprotective effect of GE against CCl₄-induced liver injury in rats.

2. Materials and methods

2.1. Preparation of GE

GE was obtained from Quaker Co., Ltd. (Taoyuan, Taiwan) and contained a mixture of P. quinquefolius, P. ginseng, N. nucifera, and L. longiflorum at a ratio of 1.66:1:1:1 (dry weight). The mixture was extracted with steam at 105°C for 30 min, cooled at 8°C for 12 h, and then filtered two times at 50°C. The filtrate was then freeze-dried to a powder, which was used to prepare different doses of GE in 0.5% carboxymethyl cellulose for the animal experiments.

2.2. Treatment of animals

Seventy-two male Wistar rats (weight: 240–260 g; age: 7 wk old) were obtained from BioLASCO Co., Ltd. (Yilan, Taiwan). All animals were handled in accordance with the guidelines of the National Taiwan University Animal Care Committee, which approved the study (Approval Number: NTU-99-EL-98). Standard experimental conditions were as follows: temperature, 22 ± 3°C; humidity, 50–70%; and a 12-h light/dark cycle. After 1 wk of acclimatization, the rats were randomly divided into six groups of 12 rats each including the control (normal control); CCl₄ (negative control); CCl₄ with silymarin (CCl₄ + silymarin); and CCl₄ with low-, medium-, and high-dose GE (CCl₄ + LGE, CCl₄ + MGE, and CCl₄ + HGE, respectively). The CCl₄ + silymarin, CCl₄ + LGE, CCl₄ + MGE, and CCl₄ + HGE groups were orally treated with silymarin (0.5 g/kg bw/d), LGE, MGE, and HGE (0.625 g/kg bw/d, 1.25 g/kg bw/d, and 3.125 g/kg bw/d, respectively), whereas the control and CCl₄ groups were orally treated with equal volumes of the vehicle (0.5% carboxymethyl cellulose). After 1 wk of treatment, rats in the CCl₄, CCl₄ + LGE, CCl₄ + MGE, and CCl₄ + HGE groups were further administered 20% CCl₄ (1.5 mL/kg bw, twice a week) for 8 wk to induce hepatic fibrosis, whereas rats in the control group were administered equal volumes of the vehicle (olive oil). Blood samples were then collected from the inferior vena cava of the rats, and each liver was isolated and stored at −80°C until further analysis. Schematic diagrams are shown in Fig. 1, which presents the design for the control, negative control (CCl₄), and treatment groups (CCl₄ + silymarin, CCl₄ + LGE, CCl₄ + MGE, and CCl₄ + HGE).

2.3. Phytochemical analysis

First, 1 g of freeze-dried GE powder was sonicated in 2 mL of 70% methanol for 1 h to obtain the extract, which was centrifuged at 6,000 rpm at 4°C for 30 min. The supernatant was then collected, filtered using a 0.22-µm syringe filter, and the filtrate was analyzed using high-performance liquid chromatography (HPLC). Qualitative analysis of the major active components (i.e., ginsenosides) in GE was further performed using HPLC (Jasco LC-Net II/ADC and Jasco PU-2089 Plus Quaternary gradient pump, Tokyo, Japan). The HPLC chromatographic conditions were maintained according to previous reports, but with slight

Fig. 1. Schematic diagrams showing the design for studying protective activity of ginseng essence on carbon tetrachloride (CCl₄)-induced liver injury in rats. Treatments of animals are detailed in the “Materials and Methods” section. Ac, acclimatization; CMC, carboxymethyl cellulose; HGE, high-dose ginseng essence; LGE, low-dose ginseng essence; MGE, medium-dose ginseng essence.
modifications [24,25]. The HPLC procedure was carried out on a Luna C18 column (5-μm pore size, 250 × 4.6 mm inner diameter; Scientific Hightek Co., Taipei, Taiwan) using a gradient solvent system consisting of phosphate buffer (Solvent A, pH 5.82) and acetonitrile (Solvent B). The two-solvent system was run as follows: 20% B (0.01 min), 20.3% B (25 min), 26.8% B (28 min), 26.8% B (38 min), 31% B (48 min), 31% B (58 min), 35.6% B (68 min), 50% B (78 min), 95% B (83 min), 95% (88 min), 20.3% B (90 min), and 20.3% B (95 min). The peaks were recorded using a UV/Visible detector (Jasco UV-2075 Plus) at 202 nm, and the solvent flow rate was maintained at 1.0 mL/min.

2.4. Serum biochemistry

To assess the liver damage in the rats, serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities, and albumin, total cholesterol (TC), and triglyceride (TG) levels were determined using SPOTCHEM EZ reagent strips (Arkray, Inc., Kyoto, Japan).

2.5. Histological analysis

For the histological examination, the anterior portions of the left lateral lobe of the rat livers were sectioned, fixed in 10% neutral-buffered formalin, embedded in paraffin, and sliced into 5-μm sections. The sections were then hematoxylin and eosin or Masson's trichrome stained. A blinded histological assessment of the liver sections was then performed by a veterinary pathologist at the Graduate Institute of Veterinary Pathobiology of the National Chung Hsing University, Taiwan. Histological changes were evaluated in nonconsecutive histological fields, randomly chosen at a magnification of 100×.

2.6. Hepatic antioxidant enzyme activities and total glutathione content

The frozen liver tissue was homogenized, centrifuged, and collected as previously described [26]. The resulting supernatant was then used to determine total glutathione content and antioxidant enzymatic activities including glutathione peroxidase (GPx), glutathione reductase (GRd), glutathione S-transferase (GST), superoxide dismutase (SOD), and catalase (CAT) using Cayman assay kits (Cayman, MI, USA).

2.7. Immunohistochemistry

Immunohistochemical analysis of the rat livers was performed as previously described with slight modifications [27]. In brief, the rats were killed, and their livers were trimmed into a strip, soaked in 10% formalin, embedded in paraffin, and then sectioned. The liver sections were dewaxed, hydrated, subjected to heat-induced antigen staining, and then incubated overnight at 4°C with an anti-alpha-smooth muscle actin (α-SMA) antibody (1:100; Dako, Denmark, Europe). The sections were then washed and further incubated with Super Enhancer and a poly-horseradish-conjugated reagent. The color was developed by incubating the sections with the 3,3′-diaminobenzidine and substrate reaction mixtures (1:38) as well as with hematoxylin. After washing the sections with water, the specific staining was visualized using light microscopy.

2.8. Statistical analysis

All the experimental data were represented as the mean ± standard deviation. The statistically significant differences in the data were analyzed using a one-way analysis of variance followed by Duncan multiple comparison test using statistical analysis software (SAS) version 9.2 (Cary, NC, USA). Differences were considered significant when p values are less than 0.05.

3. Results and discussion

3.1. Ginsenoside content of GE

Previous studies have indicated that ginsenosides Rb1 [28], Rg1 [8], and Rg3 [29] are the active components of ginseng with hepatoprotective effect. Therefore, in this study, we performed HPLC analysis to determine the composition of ginsenosides in the GE by comparing its retention time peaks with those of the reference ginsenoside standards (Fig. 2). The quantitative results of the chromatographic analysis revealed that GE contained ginsenosides Rg1, Re, Rb1, Rc, Rd, and Rg3 at levels of 31.74 ppm, 15.57 ppm, 52.79 ppm, 11.24 ppm, 11.69 ppm, and 3.24 ppm, respectively. Therefore, we reasonably presumed that these ginsenosides are the main active ingredients in the GEs that exhibit liver protection.

3.2. Changes in rat body and organ weights

A decrease in body weight is usually regarded as direct evidence of toxic injury in rodents. Administration of CCl4 induces physiological changes in the body of organisms that may slow body weight gain and even cause a decrease [30]. Table 1 shows that the final body weight of the CCl4 group was significantly lower than that of the control group. By contrast, the final body weight of the CCl4 + silymarin, CCl4 + MGE, and CCl4 + HGE groups was significantly higher than that of the CCl4 group (p < 0.05). The result indicates that changes in body weight were induced in rats administered CCl4. However, supplementation with MGE and HGE inhibited the decrease in body weight, and the result was similar to that of the silymarin group. Therefore, GE might inhibit the weight loss caused by CCl4 in rats. The results of the organ weight determinations shown in Table 1 revealed that the absolute liver weight of all CCl4-treated groups including the CCl4, CCl4 + silymarin, CCl4 + LGE, CCl4 + MGE, and CCl4 + HGE groups was significantly higher than that of the control group (p < 0.05). By contrast, the relative liver weight of the CCl4 + silymarin and CCl4 + HGE groups was significantly lower than that of the CCl4 group (p < 0.05). There was no significant difference in absolute spleen weight between all six groups, whereas the relative spleen weight of the CCl4 group was significantly higher than that of the other groups (p < 0.05). The absolute kidney weight of all the CCl4-treated groups was significantly higher than that of the control group (p < 0.05). However, the relative kidney weight of the CCl4 + silymarin, CCl4 + LGE, CCl4 + MGE, and CCl4 + HGE groups was significantly lower than that of the CCl4 group (p < 0.05). Evidence of change during the assessment of organ weight is often considered an important indicator of organ damage, and therefore can be used to evaluate responses to toxicants [31]. Damage to the liver could cause liver cell microvilli and sinusoidal endothelial cell fenestrae to disappear, alter the structure of the smooth endoplasmic reticulum, inactivate hepatic stellate cells (HSCs) and Kupffer cells, increase collagen content, result in accumulation of fibers in the liver, and then cause changes in liver weight [32]. Liver fibrosis can result in the blockage of blood flow to the liver and increase in portal pressure, which could lead to the retention of blood by the spleen, and thereby cause splenomegaly [33].
Our study results show that after chronic administration of CCl₄, the absolute and relative weights of the liver, spleen, and kidney were significantly heavier than those of the normal control animals. However, after 8-wk supplementation with GE, the relative liver weight of the high-dose group (CCl₄ + HGE) was significantly lower than that of the negative control group (CCl₄) and was close to that of the normal control. Furthermore, the relative spleen and kidney weights in all three GE-treated groups (CCl₄ + LGE, CCl₄ + MGE, and CCl₄ + HGE) were significantly lower than those of the negative control group. These results imply that supplementation with GE may significantly improve the swelling and inflammation induced by CCl₄ in the liver, spleen, and kidney of rats.

3.3. Serum biochemical analysis

Previous studies have demonstrated that once the liver is exposed to CCl₄, ALT and AST are released and flow into the bloodstream. The notable increase in serum ALT and AST levels is considered an indicator of liver injury [34]. Figs. 3A and 3B show that compared with the control, administration of CCl₄ strongly elevated both serum ALT and AST levels of rats (control and CCl₄, 41 ± 5 IU/L and 1,387 ± 216 IU/L, and 110 ± 15 IU/L and 1,511 ± 459 IU/L, respectively). However, the levels of serum ALT and AST of the silymarin- and GE-treated groups were significantly lower than those of the control group (ALT: CCl₄ + silymarin, CCl₄ + LGE, CCl₄ + MGE, and CCl₄ + HGE groups, 766 ± 295 IU/L, 857 ± 314 IU/L, 784 ± 201 IU/L, and 844 ± 379 IU/L, respectively; AST: CCl₄ + silymarin, CCl₄ + LGE, CCl₄ + MGE, and CCl₄ + HGE groups, 1,061 ± 421 IU/L, 961 ± 415 IU/L, 899 ± 139 IU/L, and 980 ± 531 IU/L, respectively, p < 0.05). Therefore, the results suggest that GE has hepatoprotective effects against CCl₄-induced liver injury in rats.

In addition, Fig. 3C shows that the serum albumin level of CCl₄-treated rats obviously decreased compared with that of the controls (23.5 ± 2.6 g/L and 33.2 ± 1.2 g/L, respectively). Furthermore, supplementation with GE induced serum albumin levels in the CCl₄ + MGE and CCl₄ + HGE groups that were close to those of the CCl₄ + silymarin group (26.6 ± 2.4 g/L and 26.7 ± 3.1 g/L vs. 27.1 ± 2.2 g/L, respectively) and significantly higher than those of the CCl₄ group (p < 0.05). Previous studies have found that CCl₄ decreased serum total protein and albumin...
Values with different superscripts are significantly different among groups according to a one-way analysis of variance coupled with Duncan multiple test (p < 0.05). HGE, high-dose ginseng essence; LGE, low-dose ginseng essence; MGE, medium-dose ginseng essence.

The effects of GE administration on serum and liver TG and TC levels in CCl4-treated rats are shown in Fig. 4. The result shows that the serum TG level of the CCl4 group (51 ± 5 mg/dL) was significantly lower than those of the CCl4+silymarin, GE-treated CCl4+LGE, CCl4+MGE, and CCl4+HGE groups (74 ± 25 mg/dL, 70 ± 19 mg/dL, 73 ± 22 mg/dL, and 71 ± 17 mg/dL, respectively, p < 0.05). Furthermore, the serum TC and liver TC and TG levels of the CCl4 group were significantly higher than those of the silymarin- and GE-treated groups (serum TC: CCl4, 59 ± 16 mg/dL; silymarin, CCl4, 59 ± 16 mg/dL; CCl4, 59 ± 15 mg/dL; GE, CCl4, 59 ± 15 mg/dL; liver TC: CCl4, 5,01 ± 20 mg/dL; silymarin, CCl4, 5,01 ± 20 mg/dL; CCl4, 5,01 ± 19 mg/dL; GE, CCl4, 5,01 ± 19 mg/dL; liver TG: CCl4, 38 ± 2 mg/dL; silymarin, CCl4, 38 ± 2 mg/dL; CCl4, 38 ± 2 mg/dL; GE, CCl4, 38 ± 2 mg/dL, respectively, p < 0.05). These results indicate that CCl4 inhibited the secretion of TG, and thereby enhanced fat accumulation. CCl4 induces an increase in hepatic TG levels, which causes its accumulation in the liver, leading to hepatomegaly. Previous studies have proposed various hypotheses to explain the mechanism of fatty liver induction by CCl4. Recknagel et al [40] demonstrated that CCl4 affected liver ribosomal division and protein synthesis, as well as inhibited the secretion of lipoproteins. In addition, CCl4-triggered lipid accumulation and inactivation of metabolism-related enzymes have been found to reduce liver cytochrome P450 content in vivo and in vitro [41,42]. Administration of CCl4 in rats for 6 wk increased liver TG but decreased it in the serum [43], and increased cholesterol in the liver and serum [44]. Supplementation with GE inhibits CCl4-induced lipid accumulation in the liver, which implies that GE might play an important role in lipoprotein synthesis and lipid transport by attenuating the inactivation of metabolizing enzymes.

### 3.4. In vivo antioxidant defense system

All aerobic organisms possess similar inherent and effective in vivo antioxidant defense systems, which include the antioxidant enzymes (SOD, GPx, and CAT) and nonenzymatic antioxidants such as glutathione. Antioxidant enzymes play an important role in the prevention of free radical-induced oxidative damage as well as reduction of antioxidant activities and capacity in vulnerable organisms [45]. Administration of CCl4 is known to lead to the generation of trichloromethyl free radicals and overproduction of ROS, leading to hepatic injuries in rats. The relationship between hepatoprotective effect and antioxidant scavenging activity is highly correlated. Table 2 shows the effects of GE on liver glutathione levels and antioxidant enzyme activities in CCl4-treated rats. Supplementation with GE resulted in significantly higher glutathione, GPx, CRd, GST, SOD, and CAT levels in the extract-treated groups than in the CCl4 group.
The enzyme levels of the GE-treated groups were close to those of the silymarin-treated group. In addition, glutathione is the major noncellular enzymatic antioxidant, which directly or indirectly scavenges free radicals effectively via enzymatic reactions. Previous studies have found that acute administration of CCl₄ depletes glutathione contents in mammals. The mechanism of CCl₄-induced liver injury showed that conjugation with glutathione plays a critical role in reducing the metabolism of toxins [46]. In this study, treatment with GE significantly inhibited the reduction of glutathione levels. These results indicate that the hepatoprotective effects of GE may be exerted by reducing ROS generation. Furthermore, the attenuation of oxidative stress by GE was similar to that shown by silymarin, which is consistent with previous studies [22,23].

3.5. Histopathological analysis

Hepatic inflammation and fibrosis are common outcomes of liver damage. Hematoxylin and eosin staining is performed to observe CCl₄-induced physiological changes in the rat liver,

\[ p < 0.05 \]. The enzyme levels of the GE-treated groups were close to those of the silymarin-treated group.

In addition, glutathione is the major noncellular enzymatic antioxidant, which directly or indirectly scavenges free radicals effectively via enzymatic reactions. Previous studies have found that acute administration of CCl₄ depletes glutathione contents in mammals. The mechanism of CCl₄-induced liver injury showed that conjugation with glutathione plays a critical role in reducing the metabolism of toxins [46]. In this study, treatment with GE significantly inhibited the reduction of glutathione levels. These results indicate that the hepatoprotective effects of GE may be exerted by reducing ROS generation. Furthermore, the attenuation of oxidative stress by GE was similar to that shown by silymarin, which is consistent with previous studies [22,23].

\[ \text{Table 2} \]

Effects of ginseng essence on liver glutathione and antioxidant enzyme activities of rats with carbon tetrachloride-induced liver injury

| Group \(^{1}\) | Glutathione (nmol/mg protein) | GPx (nmol/min/mg protein) | GRd (nmol/min/mg protein) | GST (nmol/min/mg protein) | SOD (U/mg protein) | CAT (U/mg protein) |
|--------------|-----------------------------|---------------------------|--------------------------|--------------------------|-------------------|-------------------|
| Control      | 71 ± 8^b                    | 54 ± 8^b                  | 40 ± 8^bc                | 493 ± 29^a               | 1,446 ± 323^a     | 129 ± 16^a        |
| CCl₄         | 43 ± 9^c                    | 35 ± 11^b                 | 28 ± 6^c                 | 354 ± 11^b               | 535 ± 87^b        | 53 ± 13^b         |
| CCl₄ + silymarin | 97 ± 8^a                  | 61 ± 13^c                | 54 ± 12^b                | 524 ± 18^b               | 1,292 ± 307^b     | 129 ± 24^b        |
| CCl₄ + LGE   | 98 ± 5^a                    | 51 ± 18^a                 | 50 ± 20^b                | 460 ± 58^a               | 1,368 ± 252^b     | 136 ± 39^a        |
| CCl₄ + MGE   | 102 ± 14^a                  | 52 ± 8^a                  | 64 ± 27^a                | 528 ± 58^a               | 1,401 ± 167^a     | 152 ± 30^a        |
| CCl₄ + HGE   | 99 ± 6^a                    | 51 ± 6^a                  | 52 ± 14^b                | 570 ± 33^a               | 1,247 ± 244^a     | 131 ± 31^a        |

Data are represented as the mean ± standard deviation (n = 12). Values with different superscripts within the same column are significantly different among groups according to a one-way analysis of variance coupled with Duncan multiple test (p < 0.05).

\(^{1}\) Control, vehicle (0.5% CMC + olive oil); CCl₄, 20% CCl₄; CCl₄ + silymarin, 20% CCl₄ + silymarin 0.5 g/kg bw/d; CCl₄ + LGE, 20% CCl₄ + GE 0.625 g/kg bw/d; CCl₄ + MGE, 20% CCl₄ + GE 1.25 g/kg bw/d; CCl₄ + HGE, 20% CCl₄ + GE 3.125 g/kg bw/d; bw, body weight; CAT, catalase; CCl₄, carbon tetrachloride; CMC, carboxymethyl cellulose; GPx, glutathione peroxidase; GRd, glutathione reductase; GST, glutathione S-transferase; HGE, high-dose ginseng essence; LGE, low-dose ginseng essence; MGE, medium-dose ginseng essence; SOD, superoxide dismutase.
whereas Masson’s trichrome staining is a commonly used collagen staining method for liver fibrosis detection [47]. Figs. 5 and 6 show the histological analyses of liver inflammation and fibrosis, respectively. The liver portal peripheral inflammation (vacuoles) and fibrosis were evaluated and scored by a blinded veterinary pathologist. As shown in Table 3, scores of the liver portal peripheral inflammation in the CCl₄ + LGE and CCl₄ + HGE groups (2.60 ± 1.07 and 2.40 ± 0.97, respectively) were slightly lower than those of the CCl₄-treated group. In addition, the CCl₄ + MGE group showed significantly lower scores than the CCl₄ group (2.10 ± 1.29 and 3.20 ± 0.79, respectively, p < 0.05). The liver fibrosis scores of the GE-treated CCl₄ + LGE, CCl₄ + MGE, and CCl₄ + HGE groups (1.70 ± 0.95, 1.10 ± 0.74, and 1.60 ± 0.84, respectively) were close to those of silymarin + CCl₄ and significantly lower than those of the control (1.00 ± 0.82 and 2.40 ± 0.70, respectively, p < 0.05). Therefore, GE obviously inhibited CCl₄-induced hepatic inflammation and fibrosis in rats.

3.6. Immunohistochemical staining of α-SMA

α-SMA can be used as a specific marker to assess the activation of HSCs, which were stained red when the reaction was positive. As shown in Fig. 7, no visible positive reaction was observed in normal controls, indicating that no HSCs were activated. Compared with the control group, the other CCl₄-treated group showed a significant increase in the number of activated HSCs. The CCl₄ group showed the highest positive response, indicating that the number of activated HSCs was significantly higher than it was in the GE-treated groups. The results showed that treatment with silymarin as well as LGE, MGE, and HGE reduced the number of HSCs activated by CCl₄.

Fibrosis and cirrhosis of the liver can be observed following the accumulation of extracellular matrix, which leads to the formation of excessive collagen by activation of cells such as HSCs and fibroblasts [48]. In pathological progress, the activation of HSCs could

Fig. 5. Pathological examination of effects of ginseng essence on liver inflammation in rats with carbon tetrachloride (CCl₄)-induced liver injury after 8-week treatment. Livers were stained with hematoxylin and eosin and visualized at 100× magnification. HGE, high-dose ginseng essence; LGE, low-dose ginseng essence; MGE, medium-dose ginseng essence.
increase the synthesis of extracellular matrix proteins, which changes the structure of liver sinusoid endothelial cells causing necrosis [49]. Although the mechanism mediating hepatic fibrosis is still not fully understood, maintaining the shape of HSCs may prevent or mitigate the development of liver fibrosis. It has been shown that treatment of rats with CCl4 can cause collagen accumulation in the liver and increase the expression of \( \alpha \)-SMA, which implies that HSCs have a tendency to induce liver fibrosis. Therefore, a decrease in the positive reaction of \( \alpha \)-SMA could indicate the inactivation of HSCs and mitigation of fibrosis. In summary, the results showed that GE might have effectively inhibited liver fibrosis by reducing the activation of HSCs in CCl4-treated rats.

In conclusion, we found that GE contains ginsenosides including Rg1, Re, Rb1, Rc, Rd, and Rg3, which could exert hepatoprotective effects. Furthermore, the results of the animal experiments demonstrate that GE significantly reduced the liver injury induced by CCl4 in rats by ameliorating the oxidative stress, reducing inflammation, and inhibiting the activation of HSCs. Therefore, GE could be a promising hepatoprotective herbal formulation for future development of phytotherapy.
Confl icts of interest

All authors have no conflicts of interest to declare.

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