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Research article

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

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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 (CCl_4)-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% CCl_4 (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 CCl_4-treated rats. Moreover, CCl_4-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 CCl_4-induced hepatic inflammation and fibrosis. Furthermore, immunohistochemical staining of alpha-smooth muscle actin indicated that CCl_4-triggered activation of hepatic stellate cells was reduced.

Conclusion: These findings demonstrate that GE improves CCl_4-induced liver inflammation and fibrosis by attenuating oxidative stress. Therefore, GE could be a promising hepatoprotective herbal formulation for future development of phytotherapy.

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.

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Medicine food homology (藥食同源 yào shí tong 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 (人參 rén shēn, *Panax ginseng*), American ginseng (西洋參 xī yáng shēn, *Panax quinquefolius*), lotus seed (蓮子 lián zǐ, *Nelumbo nucifera*), and the lily bulb (百合 bǎi 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], antidiabetic [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 (CCl_4) is a well-known hepatotoxin, which is widely used to induce acute toxic liver injury in animals. Numerous studies have shown that CCl_4 is metabolized by the cytochrome P_{450} 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 CCl_4-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 CCl_4-induced lipid peroxidation and hepatotoxicity by decreasing the metabolic activation of CCl_4 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 CCl_4-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 1st wk of treatment, rats were gavaged with 20% CCl_4 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 CCl_4-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); CCl_4 (negative control); CCl_4 with silymarin (CCl_4 + silymarin); and CCl_4 with low-, medium-, and high-dose GE (CCl_4 + LGE, CCl_4 + MGE, and CCl_4 + HGE, respectively). The CCl_4 + silymarin, CCl_4 + LGE, CCl_4 + MGE, and CCl_4 + 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 CCl_4 groups were orally treated with equal volumes of the vehicle (0.5% carboxymethyl cellulose). After 1 wk of treatment, rats in the CCl_4, CCl_4 + LGE, CCl_4 + MGE, and CCl_4 + HGE groups were further administered 20% CCl_4 (1.5 mL/kg bw, two times a week) for 8 wk to induce hepatic fibrosis, whereas rats in the control group were administered with 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 (CCl_4), and treatment groups (CCl_4 + silymarin, CCl_4 + LGE, CCl_4 + MGE, and CCl_4 + 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 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 modifications [24,25]. The HPLC procedure was carried out

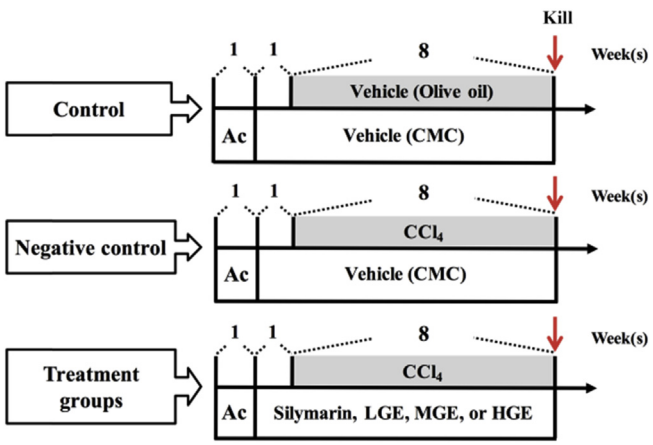

Fig. 1. Schematic diagram showing the design for studying protective activity of ginseng essence on carbon tetrachloride (CCl_4)-induced liver injury in rats. Treatments of animals are detailed in the "Materials and Methods" section. Ac = acclimatization; CMC = carboxymethyl cellulose; HGE = high-dose ginseng essence; LGE = low-dose ginseng essence; MGE = medium-dose ginseng essence.

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