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Hepatoprotective activity of ginsenosides from *Panax ginseng* adventitious roots against carbon tetrachloride treated hepatic injury in rats

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ABSTRACT

Ethnopharmacological relevance: Ginseng (*Panax ginseng* C. A. Meyer) has a beneficial role in the treatment of various diseases including liver disorders like acute/chronic hepatotoxicity, hepatitis, hepatic fibrosis/cirrhosis and hepatocellular carcinoma.

Materials and methods: Tissue culture raised mountain ginseng adventitious root (TCMGARs) extract with ginsenosides in abundance was used as an experimental material. 'Sprague–Dawley' male rats were used as experimental systems and were fed with TCMGARs extracts at doses of 30, 100, 300 mg/kg body weight for two weeks to test the effect on carbon tetrachloride (CCl_4) induced acute liver damage. Field cultivated Korean ginseng root extract fed rats (100 mg/kg) were used as positive control. Plasma enzyme levels, alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) were assessed. Glutathione (GSH) and malondialdehyde (MDA) concentrations were also evaluated.

Results: TCMGARs extracts remarkably prevented the elevation of ALT, AST, ALP and liver peroxides in CCl_4-treated rats. Hepatic glutathione levels were significantly increased by the treatment with the extracts in experimental groups.

Conclusion: The TCMGARs rich in varied ginsenosides can afford protection against CCl_4-induced hepatocellular injury.

1. Introduction

Panax ginseng C. A. Meyer is an important herbal medicine in East Asian countries. It is used in traditional medicine to promote health and healing, as an adaptogen and a stimulant. The major components of ginseng are triterpenoidal dammarane glycosides called ginsenosides. Pharmacological effects of ginseng have been demonstrated in cancer, diabetes mellitus, cardiovascular system, immune system and central nervous system including anti-stress and anti-oxidant activities (Park et al., 2005). Wild ginseng is a scanty and expensive commodity. To cultivate ginseng in fields, it needs 5–7 years till harvesting stage during which the plant may suffer from various diseases and pests. Adventitious roots have been induced from 100 year old ginseng and the roots have been cultivated in vitro in large scale bioreactors for the use of pharmaceutical industry (Paek et al., 2009). Toxicological evaluation has proved that the tissue cultured mountain ginseng adventitious roots (TCMGARs) are biosafe and possess higher amounts of ginsenosides when compared to cultivated ginseng (Sivakumar et al., 2006). Various products such as ginseng powder, sirup, wine, tablets, ginseng-based cosmetics and dietary supplements made out of TCMGARs are available in the market (Murthy et al., 2014a). Presently, our research work is focused on the analysis of efficacy tests of TCMGARs and recently, the anti-glycemic effect of TCMGARs is reported (Murthy et al., 2014b).

In the present study, we aimed to investigate the efficacy of TCMGARs on liver function and its hepatoprotective activity in mice against the carbon tetrachloride stress on hepatocytes.

2. Materials and methods

2.1. Material and preparation of extracts

Panax ginseng C. A. Meyer (Araliaceae) plant material was collected from Mt. Odaesan, Gangwon province, Republic of Korea and the herbarium is deposited with voucher number-2002-5761 in Korean Simmani Society, South Korea. The adventitious roots (tissue cultured mountain ginseng adventitious roots) and cultivated Korean ginseng roots (positive control) were powdered and soaked in 70% aqueous ethanol for 10 days at 25 °C and filtered.

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The solution evaporated in vacuo gave a semi-gelatinous extract and the yield of crude ginsenosides was 128.51 mg/g and 100.08 mg/g from ginseng adventitious roots and cultivated ginseng roots respectively.

2.2. Determination of ginsenoside content

The chemical profiling of ginsenosides in these two materials was carried out by high pressure liquid chromatography (HPLC) in Korean Food Research Institute, Sungnam-Si, Republic of Korea (File no. AO2012-06-26-200). Extraction and analysis of ginsenosides were carried out by the method of Yu et al. (2002). The ginsenoside fraction was analyzed using an HPLC system (Shimadzu, Kyoto) consisting of 10AT pump, 10AXL autosampler, SPDM10A photodiode array detector, and CTO-10A column oven, 5 μM Lichrosorb column (250 × 4.6 mm^2) (Altech, Deerfild, IL), and a C18 guard column, at 40 °C. The eluted peaks were detected at 203 nm and quantified against external standards of ginsenosides Rf, Rb2, Rd, (Karl Roth, Germany), Re, Rg1, Rg2, Rh1, Rh2, Rb1, Rb3, Rc and Rg2 (Wako, Osaka, Japan). The mobile phase was a gradient elution of water (A) and acetonitrile (B), commencing with 20% B, rising to 22% B after 20 min then to 46% after 45 min and 55% B after 50 min.

2.3. Experimental animals

Hepatotoxicity experiments were conducted in Biotoxtech Laboratories, South Korea on Sprague–Dawley male rats. All the animals involved in the experiment were maintained on a standard diet and kept in a room maintained under controlled conditions of 24 ± 1 °C temperature and 12 h light: 12 h dark cycles. The animals had free access to water and a standard diet [a normal laboratory commercial stock diet containing 16% protein, 56% carbohydrate, and 8% fat (w/w)]. All animal procedures were conducted in accordance with legal requirements appropriate to the species and with the approval by the local Ethical Committee with an ethical clearance number, CBNUR-188-1001.

2.4. Treatment of animals

In the treatment studies, animals were divided into six groups of seven animals each (G). G1 was served normal control; G2 was CCl_4 control and G3, G4 and G5 were treated with tissue cultured mountain ginseng extract at a dose of 30, 100 and 300 mg/kg body weight, respectively. G6 animals were treated with cultivated ginseng extract of 100 mg/kg body weight. All the grouped animals were maintained with above feeding schedule for 14 days. Further, the rats were administered with CCl_4, which is a model agent to induce hepatic lesions. A single dose of CCl_4 leads to centrizonal necrosis and steatosis (Pierce et al., 1987), while prolonged administration leads to liver fibrosis, cirrhosis, and hepato-cellular carcinoma (Perez, 1983). Acute oral dose of administration consisted of 0.5 ml/kg of 1:1 (v/v) mixture of CCl_4 and corn oil (Smile et al., 2001). Group 2–6 received single dose of CCl_4 on day 14. Normal control received equal amount of corn oil instead of CCl_4. All the rats were sacrificed after 24 h of CCl_4 administration.

2.5. Assessment of liver functions

Blood was collected from the blood vessels of neck region under mild ether anesthesia and kept for 30 min at 4 °C. Serum was separated by centrifugation at 2500 rpm at 4 °C for 15 min. The liver was removed rapidly and cut into separate portions for hepatic glutathione, lipid peroxidation estimation. Alanine aminotransferase (ALT) (Amador et al., 1963), aspartate aminotransferase (AST) (Bergmeyer et al., 1976), alkaline phosphatase (ALP) (Bergmeyer, 1980) assays were carried out. Glutathione (GSH) level was estimated as per the protocol of Moron et al. (1979). Lipid peroxidation in liver was measured by the formation of malondialdehyde (MDA) and it was estimated by using the procedures of Ohakawa et al. (1979).

2.6. Statistical analysis

All values are expressed as mean with standard error. The results were statistically analyzed by using Analysis of variance (ANOVA) to note the significance among different groups and further Student's *t*-test was carried out to find out the significant differences between two groups.

3. Results

3.1. Standardization of ginsenosides present in the extract

The high pressure liquid chromatography (HPLC) analysis data on ginsenosides of TCMGARs and Korean ginseng is presented in Table 1. The results revealed that Rb1, Rb2, Rb3, Rc, Rd, Re, Rg1, Rg2, Rg3, Rh1, Rh2 and Rf ginsenosides were present in the extract of TCMGARs. The ginsenosides present in the Korean cultivated ginseng root extract were Rb1, Rb2, Rc, Rd, Re, Rg1, and Rf. Ginsenosides such as Rb3, Rg2, Rg3, Rh1 and Rh2 which were not present in the Korean ginseng roots were abundant in the TCMGARs, the content of other ginsenosides are also at higher levels in TCMGARs when compared to Korean cultivated ginseng (Table 1; Fig. 1).

3.2. Evaluation of AST, ALT and ALP

In the rats, which were administered with CCl_4 showed an elevated level of serum alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) levels. Treatment with the extract of TCMGARs at a dose of 100 and 300 mg/kg as well as the treatment with the extract of cultivated ginseng at a dose of 100 mg/kg significantly ($p \leq 0.05$) provided the resistance against the CCl_4 stress in rats. Korean ginseng extract which was used as positive control also showed a remarkable protection towards CCl_4 intoxication (Table 2).

Table 1
Ginsenoside content of tissue cultured mountain ginseng adventitious roots in comparison with cultivated Korean ginseng.[a]

Ginsenoside	Content (mg/g dry weight)	
	TCMGARs	Korean ginseng
Rb1	4.1	0.05
Rb2	2.3	0.06
Rb3	0.9	–
Rc	2.0	0.03
Rd	4.2	0.06
Re	0.2	0.24
Rg1	0.19	0.17
Rg2	0.38	–
Rg3	11.2	–
Rh1	0.49	–
Rh2	3.8	–
Rf	1.64	0.03

[a] Analysis was carried out by Korea Food Research Institute, Sungnam-si, Republic of Korea.