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Research Paper

Efficacy of ginseng adventitious root extract on hyperglycemia in streptozotocin-induced diabetic rats

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ABSTRACT

Ethnopharmacological relevance: Ginseng has various bioactive effects on human health including its potential activity of improving the glucose homeostasis and insulin sensitivity.
Materials and methods: Tissue culture raised mountain ginseng adventitious root (TCMGARs) extract enriched with ginsenosides was used as experimental material. Streptozotocin-induced diabetic 'Sprague Dawley' male rats were used as experimental systems and were fed with Tissue culture raised mountain ginseng adventitious root extract. Field cultivated Korean ginseng root extract fed rats were used as positive control and several indices such as body weight, blood glucose level and other serological indicators were tested.
Results: Chemical profile showed TCMGARs were rich in varied ginsenosides especially Rb1, Rb2, Rc, Rd, Rg3, and Rh2 when compared to field cultivated Korean ginseng. TCMGARs extract at dosage levels of 250 and 500 mg/kg body weight significantly lowered the blood glucose, total cholesterol and triglyceride content in streptozotocin-induced diabetic rats.
Conclusion: The data of in vivo experiments on anti-glycemic effects of TCMGARs proves their efficacy and also their use as dietary supplement.

1. Introduction

Diabetes mellitus is a metabolic disease resulting due to the reduced insulin secretion by pancreas or due to a low biological activity of the insulin secreted and is classified as insulin-dependent (type 1) and insulin independent type (type 2) (Geroge and Ludvik, 2000). *Panax ginseng* C.A. Meyer (Korean ginseng) is traditionally used for treating hyperglycemia including diabetes mellitus (Ivorra et al., 1989; Attele et al., 1999) and it is recognized officially as one of the herbal drug ingredients in China for treating diabetes mellitus (Jia et al., 2003). Ginseng is available in commercial market in two forms namely red ginseng and white ginseng. The roots of *Panax ginseng* is steamed and dried to prepare red ginseng, while the peeled roots dried without steaming are designated as white ginseng. The major components of ginseng are triterpenoidal dammarane glycosides (saponins) called ginsenosides. More than 30 kinds of ginsenosides have been reported and they have been named as 'Rx' based on their mobility on thin layer chromatographic plates, with polarity decreasing from index '*a*' to '*h*'. They differ from one another by the type of sugar moieties, their number and the site of attachment (Park et al., 2005).

Ginsenosides are classified into three groups by their structure i.e., Rb group (protopanaxadiols including Rb1, Rb2, Rc and Rd, etc.), the Rg group (protopanaxatriols including Rg1, Re, Rf, and Rg2, etc.) and the Ro group (Oleanolic acid; Park et al., 2005). It was reported that diol-type ginsenosides such as Rb1, Rb2, Rc, Rd, Rg3 and Rh2 are having anti-diabetic activities (Suda et al., 2000) and ginsenoside Rh2 increases insulin secretion in streptozotocin-induced diabetic rats to decrease the blood glucose concentration (Lai et al., 2006). Recently, microbial fermentation methods have been introduced and red ginseng powder was fermented using micro-organisms such as lactic acid bacteria to transform ginsenosides such as Rb1, Rb2, RC and Rd into readily absorbable forms (Bae et al., 2004; Trinh et al., 2007; Kim et al., 2010) and these fermented red ginseng powders and extracts were

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also made available in the market. Simultaneously, a group of scientists have induced adventitious roots from 100 year old mountain ginseng and called them as 'tissue cultured mountain ginseng adventitious roots' (TCMGARs), and also adopted methyl jasmonate elicitation strategy to overproduce pharmacologically useful ginsenosides (Paek et al., 2009). TCMGARs were reported to be rich in diol-group of ginsenosides (Sivakumar et al., 2005) and were proved as 'biosafe' through toxicological evaluations (Sivakumar et al., 2006).

The aims of the study were to investigate the efficacy of TCMGARs, the evaluation of antidiabetic effect of TCMGARs following the administration of TCMGARs extracts in streptozotocin (STZ) induced diabetic rats and the examination of body weight, blood glucose level and other serological indicators. Chemical profiling of ginsenosides present in TCMGARs as well as field cultivated Korean ginseng was also carried out before their administration for antidiabetic evaluations.

2. Materials and methods

2.1. Material

Tissue cultured mountain ginseng adventitious roots (TCMGARs) and freshly harvested field cultivated ginseng roots (positive control) were dried (forced air drying at 50 °C), powdered and soaked in 50% aqueous ethanol for 10 days at 25 °C and filtered. The solution evaporated in vacuo gave a semi-gelatinous extract and yielded the crude ginsenosides of 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) at the Korean Food Research Institute, Sungnam-Si, Republic of Korea (File no. AO2012-06-26-200). Extraction and analysis of ginsenosides were carried out using the protocol by Yu et al. (2002). The ginsenoside fractions were analyzed using 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

Experiments were carried out in Biotoxtech Laboratory, South Korea on Sprague Dawley male rats. All the experimental animals were supplemented with a standard diet and were maintained in a room kept under environmentally controlled conditions of 24 ± 1 °C and 12:12 h light/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)].

2.4. Oral glucose tolerance test

Blood glucose levels of normal rats kept under fasting for at least 12 h were determined. Then the rats were orally administered TCMGARs (125, 250 and 500 mg/kg body weight) or field cultivated Korean ginseng extract (250 mg/kg body weight) dissolved in distilled water. For the control group, an equal amount of normal saline was given. Subsequently, oral dose of 40% glucose was administered to all the experimental group of rats at 1 g/kg rate and later blood samples were collected at 30, 60 and 120 min post-glucose administration to record the variation in blood glucose levels.

2.5. Induction of diabetes in experimental rats

In the treatment studies, animals were divided into six groups of seven animals in each (G). All animals were of seven weeks old and the average body weight was 305 g. Group G1 consisted of normal rats and which served as a control and the remaining five groups consisted of the rats induced with diabetes. The G2 was streptozotocin control (diabetes control) and G3, G4 and G5 groups were included with diabetic rats which were fed with diet containing tissue cultured mountain ginseng extract at a dose of 125, 250 and 500 mg/kg body weight respectively. Group G6 had diabetic rats which were fed with a diet containing field cultivated Korean ginseng extract of 250 mg/kg body weight. All rats were kept on observation for four weeks and further used for biochemical analysis. For the induction of diabetes, rats that had undergone one week adjustment period were kept under fasting for a minimum of 12 h, and the intra-peritoneal injection of streptozotocin (STZ) diluted in 0.01 M citrate buffer was administered at 50 mg/kg of body weight. In the normal control group, the same concentration of normal saline (without streptozotocin) was used for intra peritoneal injection. Blood collected from the veins of tail region was used to confirm the induction of diabetes, and the rats with fasting blood glucose levels of about 200 mg/dL were used for further experiments.

2.6. Analysis of body weight, blood glucose, and other biochemical tests

Increase in body weight was measured every week at the same time in a study period of four weeks. Alterations in blood glucose levels during the feeding period were measured at weekly intervals using a blood glucose monitoring system (ACCU-CHEK Sensor; Roche Diagnostics GmbH, Mannheim, Germany), and blood was collected from the veins of tail region of the rats which were kept on fasting for over 12 h. The total triglyceride level and cholesterol contents were measured using a triglyceride measuring kit and a total cholesterol content measuring kit respectively (Asan Pharmaceuticals, Whasung, Korea).

2.7. Statistical analysis

The results were statistically analyzed by using analysis of variance (ANOVA) followed by a Student's *t*-test and all the values are expressed as mean with standard errors.

3. Results and discussion

3.1. Ginsenoside content in TCMGARs and Korean ginseng

The details of ginsenosides present in TCMGARs and field cultivated Korean ginseng are presented in Fig. 1 and Table 1. The TCMGARs possessed higher amounts of Rb1, Rb2, Rc, Rd, Re, Rg1and Rf ginsenosides when compared to field cultivated Korean ginseng, additionally, ginsenosides such as Rb3, Rg2, Rg3, Rh1 and Rh2 which were lacking in the extracts of field cultivated Korean ginseng were abundant in the TCMGARs. The higher accumulation of ginsenosides and other novel ginsenosides is obvious because of the influence of methyl jasmonate elicitation during the cultivation of TCMGARs but it was not observed with field cultivated Korean ginseng (Paek et al., 2009). It is reported that methyl