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Evaluation of the Oral Acute Toxicity of Black Ginseng in Rats

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We studied the acute oral toxicity of black ginseng (BG) produced by heat process in rats. Single acute BG extract doses of 0, 5, 10, and 15 g/kg dissolved in saline were administered by oral gavage and the animals were kept under observation for 14 days. The single administration of BG extract up to 15 g/kg did not produce mortality, behavioral change or abnormal clinical signs in the rats. These results indicated that the oral LD_{50} of the BG extract in the rats is higher than 15 g/kg. Compared to the control group, no treatment-related biologically significant effects of BG extract were noted in the measurements of the body weight or food intake. At the end of the period, the biochemical parameters and hematological parameters were analyzed in the plasma and blood. A histopathological examination of the liver and kidney was also conducted. Only the blood nitrogen urea and potassium levels in the biochemical indices showed significant differences at 10 and 15 g/kg doses of BG extract compared to the control group. These changes were not considered to be due to the toxicity. None of the other clinical chemistry parameters were affected. Therefore, these results indicate that the BG by heat processing is virtually nontoxic.

Keywords: *Panax ginseng*, Black ginseng, Oral administration, Acute toxicity, Safety

INTRODUCTION

Ginseng (*Panax ginseng* C. A. Meyer, Araliaceae) has been used in Asian countries as a traditional herbal medicine for the treatment and prevention of many diseases. Ginseng has pharmacological activities, including antitumor, immunomodulation, hematopoiesis regulation, anti-aging, anti-diabetic, anti-stress, and anti-oxidative activity [1,2]. Raw ginseng is processed into white and red ginseng to improve its shelf life and efficacy. It has been reported that steamed ginseng shows more enhanced pharmacological effects than non-steamed ginseng. During the streaming treatment, the ratio of chemical constituents is changed with some newly produced components [3]. Recently, many studies have employed new technologies to maximize the herbal benefits of ginseng and conversion of the major constituent ginsenoside has been accomplished using heating, mild acids, alkaline solutions, and micro-organic enzymes [4-6].

Black ginseng (BG), a new ginseng product containing a high level of ginsenoside Rg_3 is produced by steaming at a high temperature, at which point the product becomes black in color. Ginsenoside Rg_3 is most likely produced by the loss of the glycosyl moiety at the C-20 position of protopanaxadiol type saponins of Rb_1, Rb_2, Rb_3, Rc, and Rd during the steaming process [7]. BG possesses better

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biological activity than red ginseng, inducing anti-stress, anti-cancer, anti-inflammatory, and free radical scavenging pharmacological effects [8-10].

However, during the heat processing procedure, chemical changes of macro- and micronutrient interaction and oxidation, glycation, and hydrolysis reactions occur in BG due to the characteristics of traditional heat processing [11]. Given that nutritional or toxicological changes such as the reduction of nutrients or carcinogenic polycyclic aromatic hydrocarbons can occur due to the heat process [12], it is clearly necessary to assess the safety of the BG heat processing procedure. Although there have been many studies of the pharmacological effects of BG, the toxicity of heat-treated BG has not been intensively studied. In mice, the LD_{50} for ginseng ranges from 10 to 30 g/kg, with a lethal oral dose of purified ginseng as high as 5 g/kg body weight [13]. We decided to a perform toxicological study to evaluate the safety of 80% ethanol extract from BG, which is extracted from the stems by the traditional heat processing, to build safety evidence for possible future clinical trials. This study was conducted on four groups of rats (a 0 mg/kg control group, a 5 g/kg low dose group, a 10 g/kg medium dose group, and a 15 g/kg high dose group) using histopathological and hematological examinations as well as biochemical parameters.

MATERIALS AND METHODS

Preparation of black ginseng extract

Raw ginseng (*Panax ginseng* C. A. Meyer) aged 4 years was obtained from a local ginseng center (Geumsan, Korea). To prepare the BG, the ginseng was manufactured by repeated steaming (9 times) at 98°C for 3 h followed by drying at 60°C for 18 h. The BG was ground and extracted by ultrasonication 3 times using 80% ethanol at 50°C for 1 h. After filtration, the filtrate was concentrated with a rotary evaporator followed by lyophilization. The yield of the BG extract (BGE) was 46.7% (w/w).

Experimental animals

Male Sprague-Dawley rats, 8 weeks of age, were purchased from Daehan Biolink Co. (Eumseong, Korea). They were individually housed in plastic cages with grated stainless steel floors and acclimatized for one week at 22±3°C, 55±5% humidity, and a 12 h light/12 h dark cycle. The rats had *ad libitum* access to water and food. Animal care was in conformity with the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals, and all experiments were approved by the Chungnam National University Animal Experiments Ethics Committee.

Treatment of animals

Animals were assigned to 4 groups of 5 animals each. BGE doses (5, 10, and 15 g/kg) were administered by oral gavage in a single dose at a volume 10 mL/kg body weight after the rats were fasted for 18 h. The control animals received a vehicle solution only. All rats were monitored continuously for 10 h after dosing for signs of toxicity. For the remainder of the 14-day study period, the animals were monitored daily for any additional behavioral or clinical signs of toxicity. The body weights of the animals were measured prior to dosing and on day 7 and 14. At the end of the study, all animals were induced to fast for 12 h. Selected organs for weighing were the liver, kidneys, spleen, brain, heart, lung, and testes. The liver and kidney were preserved in 10% buffered formaldehyde solution. They were processed into paraffin blocks, sectioned at a nominal 5 μm, mounted on a glass microscope slides and stained with hematoxylin and eosin.

Hematological and biochemical analyses

Blood samples were collected from the postcava and transferred into tubes containing Na^+ ethylenediaminetetraacetic acid for hematological and biochemical analyses. A hematological analysis was done using an automated hematology analyzer (Sysmex XE 2100, Kobe, Japan). The hematological study included the red blood cell count (RBC), white blood cell count (WBC), hemoglobin concentration (Hb), hematocrit, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) concentration, mean corpuscular hemoglobin concentration delete, platelet count, and white blood cell differential count.

Blood samples for the biochemical analysis were centrifuged at 3000 ×g for 10 min and the plasma was analyzed for alkaline phosphatase, asparate aminotransferase, alanine aminotransferase, lactate dehydrogenase, creatinine, blood nitrogen urea, cholesterol, glucose, bilirubin, protein, sodium, potassium, and chloride. Biochemical parameters were determined by an ADVIA 1650 chemistry system (Bayer, Tarrytown, NY, USA).

Urinary analysis

Urine was collected from the metabolic case for 2 d in the last week. Urine tests were performed using a urine analyzer (AX 4030; Arkray, Kyoto, Japan) for nitrite, protein, glucose, ketone, bilirubin, and blood.

Statistical analysis

All of the results were expressed as means±SD. Data were analyzed by one-way ANOVA followed by Duncan's test for multiple comparisons. A difference of $p<0.05$ was regarded as being statistically significant.

RESULTS AND DISCUSSION

Body weights, food intake, and organs weights

The present study found that a single administration of BGE via the oral route up to a dose of 15 g/kg did not produce behavioral changes or abnormal clinical signs in the rats as compared to the vehicle-treated control group. All animals survived the 14-day observation period. Generally, a reduction in body weight gain and the weights of internal organs is a simple and sensitive index of toxicity after exposure to a toxic substance [14]. As shown in Table 1, the daily food intake levels of rats treated with 5, 10, and 15 g/kg doses of BGE were similar to that of the control group. The food efficiency ratio of the BGE-treated groups was also similar to that of the vehicle-treated control group. The oral lethal dose (LD_{50}) of BGE for male rats is higher than 15 g/kg. Therefore, it can be concluded that BGE is virtually nontoxic.

After the 14-day experimental period, all of the rats were sacrificed and the internal organs were removed. The relative organ weights of the rats treated with BGE are shown in Table 2. In the present study, the relative internal organ weights were not altered by oral administration of BGE. Moreover, no pathological features were observed in either the control or treated groups according to the histological analysis of the liver and kidney (Fig.1).

Hematological and biochemical analyses

The status of bone marrow activity and intravascular effects was monitored in a hematological examination as shown in Table 3. The data show that RBC, WBC, HCT, Hb, MCV, MCH, and the WBC components did not differ significantly compared to those of the control group. The plasma biochemical indices of the control group and the BGE-treated groups are presented in Table 4. There were no treatment-related biologically significant adverse effects of BGE on the plasma chemistry indices in rats. However, some parameters showed statistical differences compared to the control group. A significant decrease in blood urea nitrogen (BUN) was observed in rats treated with the 10 and 15 g/kg doses in the BGE groups. Urea and creatinine are nitrogenous end products of metabolism. The levels of BUN and creatinine reflect the renal glomerular function. However, there are many factors besides renal disease that can cause BUN alternation, including protein breakdown, hydration status, and

Table 1. Body weights and food intake levels of rats exposed to acute toxicity of black ginseng extract (BGE)

Parameters	Control	BGE (g/kg)		
		5	10	15
Initial weight (g)	215.2±10.7	220.0±13.2	218.6±7.6	214.5±11.7
Final weight (g)	292.0±12.1	287.4±10.6	303.5±6.7	293.0±5.3
Food intake (g/d)	25.3±1.3	23.4±1.8	24.8±2.2	25.5±2.4
Food efficiency ratio[1)] (%)	21.7±6.4	20.2±7.1	24.5±3.6	22.0±4.8

Values are expressed as means±SD (n=5).
[1)] Body weight gain/food intake×100.

Table 2. Internal organs weights of rats in exposed to acute toxicity of black ginseng extract (BGE)

Parameters (g/100 g b.w.)	Control	BGE (g/kg)		
		5	10	15
Liver	3.13±0.15	2.91±0.16	2.94±0.29	2.92±0.11
Kidney	0.82±0.03	0.78±0.02	0.79±0.02	0.79±0.04
Lung	0.83±0.12	1.11±0.2	1.00±0.14	0.94±0.15
Heart	0.43±0.12	0.41±0.07	0.39±0.05	0.38±0.07
Spleen	0.26±0.02	0.25±0.01	0.23±0.02	0.25±0.01
Brain	0.79±0.13	0.86±0.19	0.82±0.10	0.76±0.14
Testes	1.19±0.12	1.08±0.23	1.20±0.18	1.05±0.20

Values are expressed as means±SD (n=5).

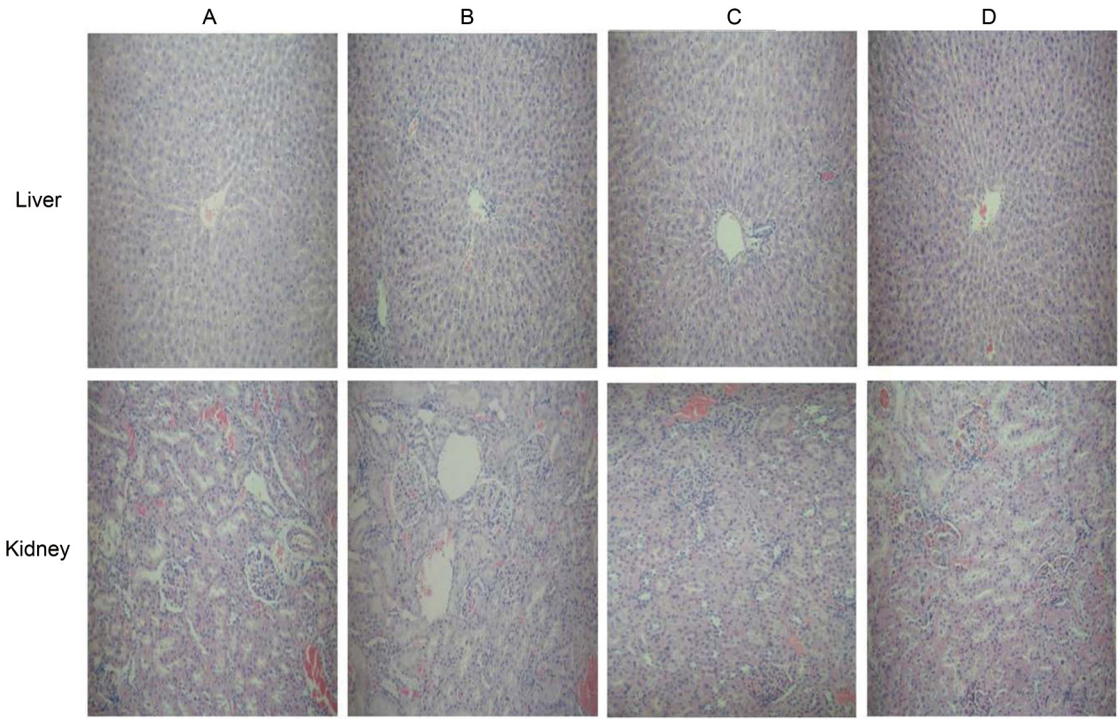

Fig. 1. Histopathological images of the liver and kidney of rats treated orally with black ginseng extract (BGE) by hematoxyline-eoxin staining (x100). (A) Control group, (B) BGE-treated group at a dose of 5 g/kg, (C) BGE-treated group at a dose of 10 g/kg, (D) BGE-treated group at a dose of 15 g/kg.

Table 3. Effect of black ginseng extract (BGE) on hematological values

Parameters	Unit	Control	BGE (g/kg)		
			5	10	15
Red blood cell	10^6/μL	7.7±0.28	8.1±0.40	8.7±0.13	9.06±0.35
White blood cell	10^3/μL	10.5±4.55	7.3±1.88	7.2±1.03	8.11±0.69
Hematocrits	%	53.4±0.78	53.0±3.68	59.1±0.57	60.6±3.32
Hemoglobin concentration	g/dL	15.6±0.64	16.1±0.64	17.5±0.21	18.1±0.71
Mean corpuscular volume	fL	69.4±1.56	65.8±1.27	68.1±0.42	66.9±1.06
Mean corpuscular hemoglobin	pg	20.3±0.07	20.0±0.21	20.2±0.07	20.0±0.05
Platelet	10^3/μL	1226±68	1034±107	995±25	945±116
Monocyte	%	2.3±0.71	3.9±1.27	3.8±0.14	4.85±0.92
Eosinophil	%	0.3±0.01	0.85±0.21	0.45±0.07	0.45±0.04
Lymphocyte	%	93.7±2.12	85.4±2.26	87.5±1.56	±1.13

Values are expressed as means±SD (*n*=5).

liver failure. In this study, the BUN levels in all groups were within the normal range (10.47–19.24 mg/dL) as described by Kang *et al*. [15], and the creatinine levels were not changed by BGE. Therefore, the decrease in the BUN values in the groups treated with BGE was not considered to be due to toxicity. Although the plasma potassium level increased in rats treated with 10 and 15 g/kg doses in the BGE groups, the plasma potassium levels in these two BGE groups were also within the normal ranges [16]. There were no treatment-related adverse effects on the urinalysis parameters in rats (data not shown). Thus, the noted differences in the BUN and potassium levels were not considered to be due to a toxicity effect. These results indicate that the administration of BGE up to 15 g/kg dose did not affect the parameters of the renal and hepatic functions. In summary, BGE was found to be fairly nontoxic when oral acute toxicity was examined in rats. However, a chronic toxicity study

Table 4. Blood chemistry values of rats exposed to acute toxicity of black ginseng extract (BGE)

Parameters	Unit	Control	BGE (g/kg)		
			5	10	15
ALP	U/L	310±30.4	290±24.5	283±25.4	291±21.3
AST	U/L	92.7±4.2	96.5±3.7	89.4±9.5	94.2±12.7
ALT	U/L	43.2±2.5	41.5±4.9	44.5±4.9	49.0±7.0
LDH	U/L	477.5±57.6	470.5±46.1	477.5±58.7	489.0±50.4
Creatinine	mg/dL	0.6±0.2	0.7±0.2	0.65±0.1	0.60±0.1
BUN	mg/dL	14.5±2.3	15.3±1.5	11.0±2.0*	11.7±1.4*
Cholesterol	mg/dL	68.5±6.4	57.0±9.0	69.0±7.0	55.7±8.6
Glucose	mg/dL	198.4±12.5	162.0±15.7	230.5±10.6	213.5±25.4
Bilirubin	mg/dL	0.1±0.01	0.1±0.01	0.1±0.01	0.1±0.01
Protein	g/dL	5.32±0.1	5.35±0.2	5.32±0.2	5.30±0.2
Na	mmol/L	142.5±8.6	139.5±12.2	144.5±10.4	146.7±14.2
K	mmol/L	5.5±0.3	5.6±0.3	6.8±0.6*	7.1±0.5*
Cl	mmol/L	103.4±10.7	102.5±8.2	104.7±7.3	107.0±10.6

Values are expressed as means±SD (n=5).
BGE, black ginseng extract; ALP, alkaline phosphatase; AST, aspartate aminotransferase; ALT, alanine aminotransferase; BUN, blood urea nitrogen; LDH, lactate dehydrogenase.
*p<0.05 compared with control group.

is needed for further support of the safe use of BGE.

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