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Original Article

Inhibition of Phenylhydrazine-induced Hematotoxicity by Bioconverted Ginsenosides

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ABSTRACT

Aim: To identify the inhibition of Phenylhydrazine (PHZ)-induced hepatotoxicity and anemia by bioconverted ginsenosides. **Materials and Methods:** We examined the potential benefits of bioconverted ginsenosides on anemia in a phenylhydrazine-induced rat model and described the prolonging effect of erythrocyte life span by focusing on compound K that was confirmed through *ex vivo* experiments. **Results:** Hematological analysis demonstrated that all groups treated with bioconverted ginsenosides showed significant recovery results. In the *ex vivo* experiments, compound K was treated in stored blood, and the erythrocytes half-life was 45 days, 15 days longer than when not treated in blood. In the ICP-OES assay, PHZ-induced erythrocyte hemolysis was significantly inhibited by bioconverted ginsenosides. **Conclusion:** Based on the present results, it has been demonstrated that bioconverted ginsenoside aids in prolonging the erythrocyte life span and increasing erythrocyte count *in vivo*. Thus, this material has the potential to develop as a promising drug candidate in the future.

Keywords: Anemia, Erythrocyte, Ginsenosides, Hemolysis, Phenylhydrazine.

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INTRODUCTION

Anemia is defined as a condition characterized by low hemoglobin concentration or low erythrocyte count. According to the World Health Organization (WHO), the hemoglobin levels are less than 13 g/dl and Hematocrit (HCT) ratios are less than 41% in men, while in women, the hemoglobin levels are about 12 g/dl and HCT less than 36%.[1] Statistically, 1.93 billion people (27% of the world population) showed signs of anemia in 2013.[2] Anemia can lead to many symptoms (Eg. pallor, heart failure, depression, retinal damage, menorrhagia).[3] Long-term anemia induces major problems in the nervous and immune systems.[4,5]

The main ingredients of ginseng (*Panax ginseng* C.A. MEYER) are ginsenosides (also called ginseng saponins), which have been identified in about 50 types of it.[6,7] The ginsenosides have various pharmacological effects, including anti-tumor, antioxidant, anti-inflammatory, anti-aging, central nervous system, cardiovascular, and erythrocyte protection.[8-11] Ginsenosides are divided into minor ginsenosides and major ginsenosides based on the sugar moiety. Minor ginsenosides (sugar moiety poor) are generally non-existent and consist of Rh1, F2, Rg3, and compound K.[12] Minor saponins, which are deglycosylated, have high absorption from the intestine into the bloodstream. Minor ginsenosides are bioconverted by heat treatment, microbacterial conversion, and enzymatic hydrolysis. Hydrolysis of the sugar moiety gives the highest yield by the enzymatic method.[13] Minor ginsenosides exhibit increased bioactivity *in vivo* and possess the potential to develop as important drugs in the future.[14]

In the present study, we examined the potential benefits of Bioconverted Ginsenosides (BCG) on anemia in a phenylhydrazine-induced rat model and described the prolonging effect of erythrocyte life span by focusing on compound K that was confirmed through *ex vivo* experiments.

MATERIALS AND METHODS

Materials

Ginseng was supplied by Geumsan Ginseng (Geumsan-gun, Chungcheongnam-do, Korea). Ginsenosides were purchased from ChemFaces (Wuhan, Hubei, China). Rapidase was obtained from DSM Food Specialties (MA Delft, Netherlands). The following chemicals were obtained from Sigma-Aldrich (St. Louis, MO, USA): Polyoxyethylene sorbitan monooleate (Tween 80), L-lysine, $CaCl_2$ and ethylene diamine tetra acetic acid (EDTA). Phenylhydrazine-hydrochloride was purchased from TCI (Kita-ku, Tokyo, Japan).

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Animals

Male SD rats were obtained from Hyochang Science (Daegu, Korea). All animals were housed in a temperature-controlled environment (23±2°C) and humidity (50±10%) with a 12 hr light-dark cycle and provided with food and water *ad libitum*. All experiments were performed in accordance with the WHO guidelines and the Institutional Review Board of Kyungsung University for the evaluation of safety. The study was approved by the committee of Kyungsung University (No. Study-19-002A).

Enzymatic Bioconversion of Ginsenosides (BCG) by ginseng extraction

Ginseng was prepared by steaming at 121°C for 15 min, followed by grinding a total of 100 g, and it was extracted about 5 times using 500 mL of 95% ethanol. The supernatant was harvested and filtered to remove precipitates. The dried ginseng saponin (20 g) was re-suspended in water (2,000 mL) to form a bulk active PPD. The suspension material was prepared in a minor ginsenoside component in order to decompose the sugar moiety of the extracts by a rapidase enzyme solution (340 mL), using a peristaltic pump (50 mL/min) and kept overnight at 4°C to form a clump. The wild type, steamed (121°C for 15 min), and bioconverted ginsenosides were collected by centrifugation at 15,000 × g for 10 min, and then the supernatant was diluted in ethyl alcohol (400 mL) and filtered. The filtrate was evaporated using a rotary evaporator and an analysis of ginsenoside contents was conducted as described by Ha *et al.*[15] The 22 major ginsenosides were analyzed using a LaChromUltra U-Series apparatus (Hitachi-High Technologies, Ibaraki, Japan), which consists of a pump (L-2160u), an eluant reservoir, an auto injection system, and an ultraviolet detector (L-2400U) at an absorbance of 203 nm. A LaChromUltra C_{18} short-length column (Hitachi-High Technologies, 2 mm × 50 mm, 2 mm) and a LaChromUltra C_{18} middle-length column (Hitachi-High Technologies, 2 mm × 100 mm, 2 mm) were used to analyze the ginsenosides. The gradient conditions were as follows: solvent A (20% acetonitrile), solvent B (80% acetonitrile); gradient, solvent A of the mobile phase was maintained at approximately 10 min, linearly to 25%, B for 30 min, 70%, B for 10 min, 100%, B for 30 min and returned to 0%, B for 5 min with an injection volume of 5 µL.

Cell viability (MTT assay) and measurement of NO production

RAW 264.7 cells (American Type Culture Collection, Rockville, MD, USA) were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 1% streptomycin and penicillin (Lonza, Walkersville, MD, USA), 10% (v/v) heat-inactivated fetal bovine serum (Performance Plus, Grand Island, NY, USA) at 37°C and 5% CO_2 in humidified air conditions.[16] Cell viability was determined using 3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) solution. RAW 264.7 cells (5×10^5 cells/mL) were seeded in 24-well plates. After incubation for 24 hr, the cells were treated with diverse concentrations of BCG (100, 50, 25, 12.5, and 6.25 µg/mL) for 18 hr. Thereafter, the cells were incubated with MTT solution (0.5 µg/mL) for 3 h. The medium was removed and the cells were dissolved in 350 µL DMSO for cell lysis for approximately 15 min. The results were measured with a vehicle-treated control to compare the absorbance at 570 nm.[17]

The RAW 264.7 cells were cultured in another 24-well plate with various concentrations of BCG (100, 50, 25, 12.5, and 6.25 µg/mL), lipopolysaccharide (LPS; 1 µg/mL), and ascorbic acid (250 µg/mL) for 18 hr. After incubation, the supernatant was harvested and added to an equal volume of Griess reagent (1% sulphanilamide, 0.1% N-1-naphthyl ethylenediamine) for 15 min. The NO level was calculated by measuring the absorbance at 540 nm.[18]

Changes in blood counts of stored whole blood and morphological deformation

Whole blood was collected from two different groups of SD rats (Male, 4 months old) in 14% CPDA (citrate-phosphate -dextrose-adenine, Green Cross Co. Ltd., Korea) tubes. The blood was divided into two groups (n = 5) [0.9% saline solution and compound K (20 nM)-treated solution]. The treated samples of 1 mL each were packed and stored at 2 - 6°C under standard blood bank conditions for up to 30 days. The samples were taken for analysis of blood count using a Complete Blood Count (CBC) analyzer (Advia 2120*i,* Siemens, Tarrytown, NY, USA) and observation of erythrocytes morphology was done by Giemsa staining on 0, 10, 20, 30, 40, and 50 days.

Protective effects of BCG on PHZ-induced hemotoxicity by ICP-OES (Inductively Coupled Plasma Optical Emission Spectroscopy)

For calcium, iron and zinc analysis, the whole blood was added with two concentrations (BCG 20 µg/mL and 20 ng/mL), 0.9% saline solution (control), and PHZ only at 37°C, and pre-incubated for about 1 h. After incubation, PHZ was added at a final concentration of 4 mM. The plasma samples were collected from whole blood at 1300 g, 10 min, and 4°C. The experiment was conducted according to the method described by James *et al.*[19] For the digestion method, 2 mL of each serum was dispensed into a microwave digestion vessel. For samples, the vials were treated with 2 mL concentrated HNO_3, 1.5 mL concentrated HCl, and 1 mL concentrated H_2O_2 solution. Deionized water was made up to a final volume of 8 mL, and then microwaved (Milestone Ethos Plus MR, Sorisole, Italy). The chemical compositions were determined using inductively coupled plasma – optical emission spectroscopy (OPTIMA 2100DV, Perkin Elmer, USA).

Animal grouping and experimental design

Five SD rats (male, 8 weeks old) were randomly selected as the normal control group (w/v, 0.9% saline only; CON), and the

other fifteen rats were injected with PHZ to induce the acute anemia model. The rats were injected with 20 mg/kg of PHZ once a day by intraperitoneal administration for 3 days. The anemic rats were divided into 3 groups (n = 5 per group): the positive control group (PHZ only; PCON), the BCG-Low group (0.8 mg/kg), and the BCG-High group (1.6 mg/kg). After 14 days of administration, all animals were anesthetized with diethyl ether in order to collect whole blood (in a container containing 14% CPDA) from an abdominal vein. Complete blood cell counts were evaluated using a hematology analyzer.

Histopathological study

The PHZ-induced damages to the spleen and liver were measured by hematoxylin and eosin staining.[20] Firstly, the organs were fixed in 10% formalin. Secondly, the alcohols were used for dehydration. The samples were then embedded in paraffin. Thirdly, the paraffin samples were cut into 4 μm sections and stained with hematoxylin (0.1%) and eosin (1%). Finally, the cells were observed under a light microscope (BOT-DS-5M-L1, Nikon, Japan).

Statistical analysis

All of the experimental results are expressed as the mean ± SD. Statistical comparisons among the experimental groups were performed by one-way ANOVA and Duncan`s multiple range test using the R software program (version 3.5.2) and visualized by using the ggplot2 package in R into boxplots. Statistical significance was considered at p values <0.05.[21]

RESULTS

Analysis of enzymatic bioconverted ginsenosides by ginseng extraction by ultra-high-performance liquid chromatography

The wild type, steamed, and bioconverted ginsenosides by ginseng extraction were analyzed using ultra-HPLC. The total ginsenoside content of wild-type ginseng was about 1.98 mg/g, that of steamed ginseng was 15.06 mg/g, and that of the bioconverted ginsenosides were 126.16 mg/g. Rd, F_2, Rg_3, and compound K comprised 92.35% of the total ginsenosides in the bioconverted ginsenosides. The pectinase enzyme made the sugar moiety poor and increased the minor ginsenoside content (Table 1).

Cell viability and inhibitory effect of NO production by bioconverted ginsenosides

The cytotoxic effects of bioconverted ginsenosides on the cell (RAW 264.7) were confirmed by MTT assay. BCG was treated at various concentrations (6.25, 12.5, 25, 50, and 100 μg/mL), and the results showed an IC_{50} density of 100 μg/mL in this study. The cell viability was observed to be about 90% or more at a concentration of 50 μm or less (Figure 1A).

NO production was achieved using cell culture supernatant with Griess reagent. The negative control group which was treated with ascorbic acid (250 μg/mL) produced NO at 2 μM. The positive group treated only with LPS (1 μg/mL) produced NO at approximately 10 μM. In the case of the bioconverted ginsenosides treated with various concentrations, most concentrations significantly inhibited NO production (Figure 1B).

Complete blood cell counts of stored blood with compound K and visualization of aging-erythrocyte morphological deformation

Erythrocytes were packed with either compound K or vehicle treatment. Compound K was treated at a final concentration of 20 nM, as previously described.[22] To compare the erythrocyte half-life span, a CBC analyzer was used. The control (30 days, from 5.07 to 1.94 × 10^6 cells/μL), compound K (30 days, from 5.39 to 4.1 × 10^6 cells/μL), and the compound K-treated blood groups had an erythrocyte half-life-span of 15 days longer. Hemoglobin (HGB) concentrations were higher in the compound K group (30 days, from 9.8 to 9.6 g/dL) than 0.9% saline solution group (30 days, from 9.7 to 5.8 g/dL). HCT levels were higher in the compound K group (30 days, from 27% to 19%) than 0.9% saline solution group (30 days, from 26.7% to 9.2%) (Table 2).

The packed blood samples were observed using post-Giemsa staining under a microscope. At 30 days, the control group showed a large number of debris cells. However, the compound K-treated blood groups maintained many of the most stable red blood cells (Figure 2).

Protective effects of BCG on PHZ-induced hemotoxicity by ICP-OES

The selected elements are indicative of the status of red blood cells: for calcium at a wavelength of 317 nm and calculated value (control: 56.3 mg/L, PHZ only; 108.2 mg/L, BCG 20 μg: 97.2 mg/L, BCG 20 ng: 92.2 mg/L), for iron at a wavelength of 238 nm and measured value (control: 5 mg/L, PHZ only: 10.2 mg/L, BCG 20 μg: 8.2 mg/L, BCG 20 ng: 8.9 mg/L), for zinc at a wavelength of 206 nm and measured value (control: 3.8 mg/L, PHZ only: 6.6 mg/L, BCG 20 μg: 5.5 mg/L, BCG 20 ng: 5.1 mg/L (Figure 3).

Hematological analysis

Intraperitoneal administration of bioconverted ginsenoside to SD-rats induced with anemia with PHZ showed a recovery effect (Table 3). Compared to the number of White Blood Cells (WBC) in the CON group, the number in PCON group decreased by 66%, the BCG-Low (0.8 mg/kg) group by 87%, and the BCG-High (1.6 mg/kg) group recovered by 84%. Red Blood Cell (RBC) numbers also decreased by 79% in the PCON group and the BCG-High groups significantly recovered by about 91%. Decreased RBC levels contributed to lower HGB and HCT levels. Mean Corpuscular Volume (MCV); PCON was 25% higher than

Table 1: Ultra-HPLC analysis of wild type, steamed, and Bioconverted Ginsenosides (BCG).

Ginsenosides	Materials (mg/g)		
	Wild type	Steamed	BCG
Rg1	0.24	2.09	0
Re	0.89	2.58	0
Rf	0.1	0.61	0.47
Rh1(S)	0	0	2.13
Rg2(S)	0.09	0.41	0
Rg2(R)	0	0.06	0
Rh1(R)	0	0.04	0.31
Rb1	0.23	3.15	0.11
Rc	0.12	1.72	0
F1	0	0	0
Rb2	0.11	1.62	2.63
Rb3	0	0.22	0.59
Rd	0.16	1.48	29.87
F2	0	0.86	15.91
Rg3(S)	0.02	0.13	13.51
Rg3(R)	0	0.07	9.76
PPT (S)	0	0	0
PPT(R)	0	0.02	2.21
Compound K	0	0	47.55
Rh2(S)	0	0	1.11
Rh2(R)	0	0	0
PPD	0.02	0	0
Total	1.98	15.06	126.16

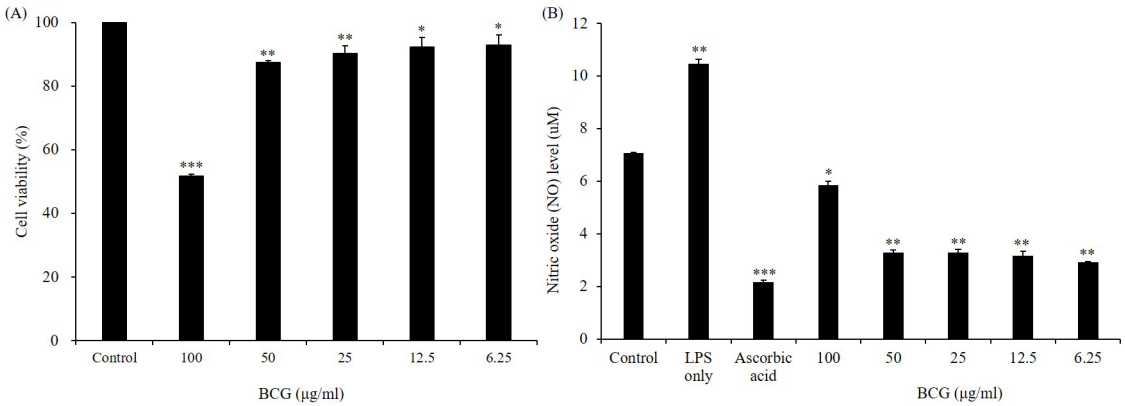

Figure 1: Effect of BCG on cell viability and nitric oxide production in RAW 264.7 cells. (A) Results of the MTT assay. (B) Inhibition of nitric oxide levels in LPS (1 µg/mL)-induced RAW 264.7 cells with ascorbic acid (250 µg/mL) and BCG (6.25, 12.5, 25, 50 and 100 µg/mL). Data are shown as the mean ± SD for three independent experiments ($n = 3$). *$p<0.1$, **$p<0.01$, ***$p<0.001$.d

the CON group, showing that the average red blood cell size was larger. Red Blood Cell Distribution Width (RDW) was also lower than PCON, but it was not noticeable. The most notable significant result was the Platelet (PLT) level. Both experimental groups returned to the CON level, but decreased to 80% for PCON. Macro RBCs were present in 18% of PCON and decreased in proportion to the concentration of experimental groups.

Table 2: Comparison of CBC values of stored blood by the BCG effect. The blood was divided into two groups (0.9% saline solution and Compound K (20 nM)-treated groups). The results are presented as mean ± SD for 5 rats. *p<0.01 versus 0.9% saline solution group.

	Day 0		Day 30	
Parameters	**0.9% saline solution group**	**Compound K group**	**0.9% saline solution group**	**Compound K group**
RBC (10^9 cells/ml)	5.07±0.01	5.39±0.01	1.9±0.3	4.1±0.3*
HGB (g/dL)	9.7±0.01	9.8±0.01	5.8±0.3	9.6±0.01*
HCT (%)	26.7±0.01	27±0.01	9.2±0.3	19±0.3*

RBC: Red blood cell; HGB: Hemoglobin; HCT: Hematocrit.

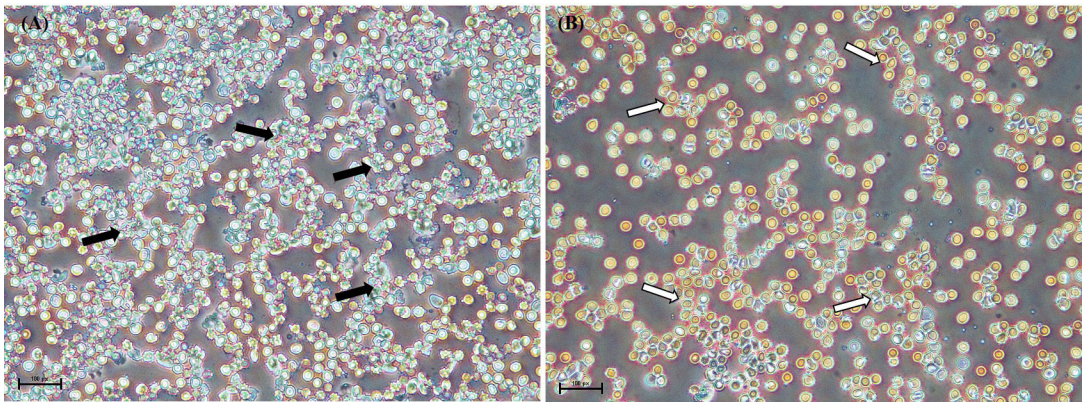

Figure 2: Representative observation of morphological changes [(A) 0.9% saline solution group and (B) compound K-treated blood groups] of rat RBCs at 30 days in storage (n = 10) using a fluorescence microscope (X400, Nikon ECLIPSE Ti-s, Tokyo, Japan) by Giemsa staining. Black arrow: debris cells and white arrow: stable red blood cells. Scale bar: 100 µm.

Spleen and Liver Histopathology

In the hematoxylin and eosin staining of representative liver, the black arrows show vacuoles in the cytoplasm. The nucleus of hepatocytes in the CON group was clear and round without vacuoles. Various sizes and many vacuoles were present in the PCON group hepatocytes. It also showed that the nucleus was lost and the cells contracted. In contrast, the number of vacuoles was significantly reduced in the BCG-High group (Figure 4A).

In the hematoxylin and eosin staining of the representative spleen, the Periarterial Lymphatic Sheath (PALS) length of splenocytes was measured. PALS was widespread around the central artery in PCON. In the BCG treatment group, the spread of PALS was significantly reduced, which was dependent on the concentration of BCG (Figure 4B).

DISCUSSION

In the present study, *ex vivo* and *in vivo* experiments demonstrated the inhibition of PHZ-induced hematotoxicity and the therapeutic effects of anemia through bioconversion of ginsenosides. The transformation of ginsenosides can be categorized into three groups: physical, chemical, and enzymatic methods.[23] The enzymatic method, one of the bioconversion methods, is the most preferred method and is promising owing to its short reaction time and low contamination.[24] As the result of ginsenosides analysis after enzymatic treatment using rapidase, sugar moiety poor group (Rd, F_2, Rg_3, compound K) was increased and its contents occupied over 90%. In the *in vitro* results of BCG, the IC_{50} was 100 µg/mL and the cell survival rate was over 90% at 50 µg/mL. In the normal range, it regulates blood vessels and defenses, but high concentrations of NO produce harmful substances and cause inflammatory reactions.[24] Therefore, the NO production inhibition effect of BCG was excellent even at a low concentration of 6.25 ng/mL.

Ex vivo experiments were conducted to study the erythrocyte lifespan. Previous studies have shown that Rg2 and Rh1 have erythrocyte protective effects, but no reports on compound K have been reported.[25] The compound K concentration was based on 20 nM, referring to a previous study by Bae *et al.*[22] A comparison of the packed blood sample with compound K and non-treatment groups showed that the half-life was 30 days in the untreated group, but the half-life extended to 45 days in the treated group (data not shown). A microscopic examination of the erythrocyte morphology revealed that the compound K group significantly reduced the occurrence of debris and aggregation. Although the exact mechanism of compound K on erythrocytes is not

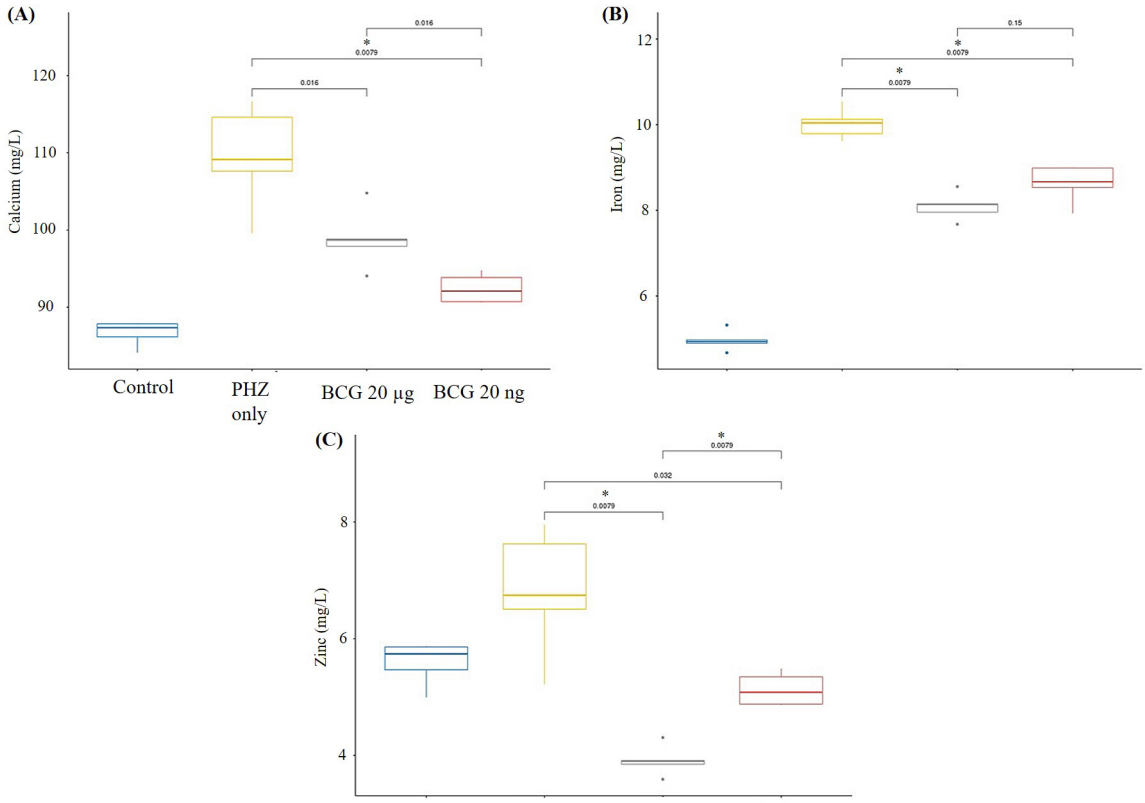

Figure 3: Comparison of (A) Ca (317 nm), (B) Fe (238 nm), and (C) Zn (206 nm) ionic elements in plasma by ICP-OES (n = 10) of PHZ only, PHZ treated with BCG 20 µg and BCG 20 ng groups. *p<0.01.

Table 3: CBC results on blood toxicity recovery effect by BCG administration. The results are presented as mean ± SD for 5 rats. *p<0.05, **p<0.01, ***p<0.001 versus PCON group.

Parameters	Units	CON	PCON	BCG-Low	BCG-High
WBC	10^6 cells/mL	7.75 ± 1.97	5.90 ± 0.15	7.75 ± 1.68**	7.51 ± 1.70**
RBC	10^9 cells/mL	7.71 ± 0.07	6.16 ± 0.12	6.56 ± 0.06**	6.84 ± 0.10**
HGB	g/dL	15.17 ± 0.21	14.85 ± 0.14	15.25 ± 0.21*	14.85 ± 0.17
HCT	%	47.25 ± 0.61	46.73 ± 0.66	47.43 ± 0.77	47.45 ± 0.37
MCV	fL	60.63 ± 0.75	75.88 ± 0.80	71.65 ± 0.17*	68.68 ± 0.64**
MCH	pg	21.60 ± 0.37	24.33 ± 0.78	24.43 ± 0.29	22.73 ± 0.50**
RDW	%	11.19 ± 0.25	22.00 ± 0.81	20.33 ± 0.33*	20.93 ± 0.43*
Macrocyte	%	Not dectected	18.32 ± 2.51	13.99 ± 3.20	9.59 ± 2.19***
PLT	10^6 cells/mL	1,036.21±71.02	839.57 ± 96.74	1094.47 ± 58.88***	1,066.03±199.91

known, it supports the evidence that its erythrocyte protective effect is apparent as a result of prolonged erythrocyte life and morphological protection.

PHZ is a substance that causes hemolytic anemia and is widely used in anti-anemia research.[26] PHZ is one of the substances capable of inducing macro RBCs by cytotoxicity.[27] It mainly works as an oxidative stress to radicals and decomposes hemoglobin into methemoglobin, irreversibly, to break down the cytoskeletal proteins of erythrocytes.[28] In order to defend against hemolysis by PHZ, the cytotoxic protective effect of erythrocytes by BCG was observed through ICP-OES plasma (PHZ 4 mM) analysis. Many researchers have studied hemolysis using plasma and serum ion concentrations. In particular, 80% of the total zinc in the blood is inside the red blood cells. Therefore, it is studied as an important hemolysis marker.[29,30] As a result of mineral ion analysis through plasma data, it was shown that the BCG treatment group had a protection of erythrocytes by PHZ. Furthermore, these results were hypothesized to inhibit Phosphatidyl Serine (PS) exposure of erythrocyte membranes by ginsenoside, but had no significant effect. However, it was confirmed that the size of red blood cells was controlled by treatment with bioconverted ginsenosides by flow cytometry (data not shown).

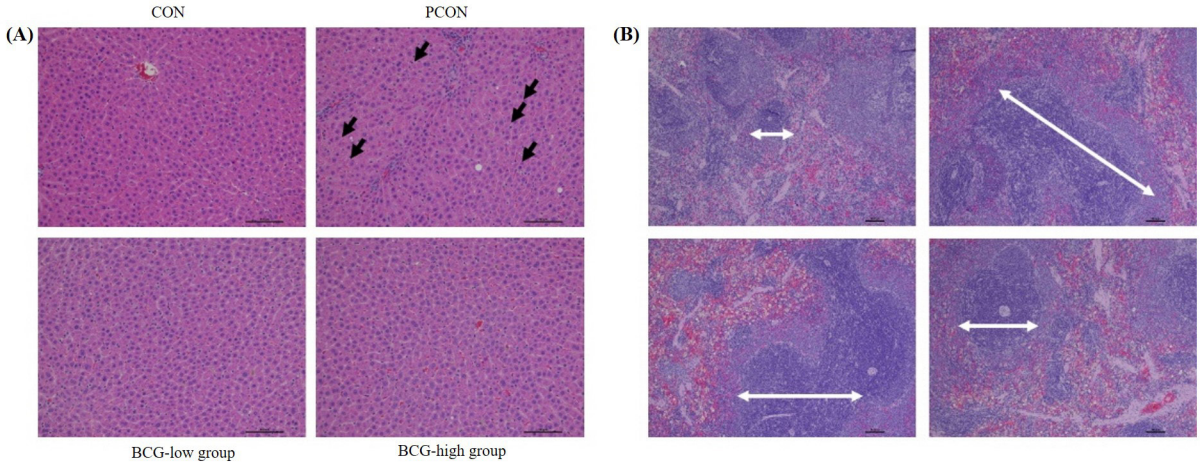

Figure 4: Histological sections of the liver (A) and spleen (B) in CON, PCON, BCG-low and -high groups. (x200, black arrows: vacuoles, white arrows: PALS length, scale bar: 100 µm).

In the present *in vivo* study of erythropoiesis, the effect of ginsenosides on reducing erythrocyte counts and anemia was induced by the administration of PHZ for 3 consecutive days to SD-rats. BCG injection was administered for 2 weeks to determine the recovery of anemia. As a result of CBC analysis, the damage of most positive control groups recovered by 80% compared to the normal control group. In contrast, the WBC, RBC, and PLT recovered to nearly normal levels in the BCG-High group. The MCV level gradually returned to normal, and the number of macroRBCs also decreased in the BCG group. Therefore, BCG was found to be effective in regulating erythrocyte size as well as in restoring and increasing erythrocyte numbers.

In histological studies, the positive control group of hepatocytes was found to have many vacuoles and the nuclei of hepatocytes disappeared, crumbled, and aggregated. The BCG-Low group did not recover to normal levels, but the BCG-High group showed an almost normal level in liver tissue. In the splenic histological study, the Periarterial Lymphatic Sheath (PALS) length, which spreads around the central artery, was measured. Pharmacological effects were significantly observed with BCG administration as the length of the arrow gradually decreased.

CONCLUSION

In this study, we studied the various pharmacological effects of BCG in prolonging the life of red blood cells, preventing and recovering from death. There are many types of ginsenosides including Rg_3 which causes hemolysis, and Rg_2 and Rh_1 have a protective effect on erythrocytes; however, there are still disagreements among scholars. This is the first study of the phenomena of erythrocytes in compound K, and it has been demonstrated that bioconverted mixtures also have a protective effect against erythrocytes.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

PHZ: Phenylhydrazine; **WHO:** World health organization; **HCT:** Hematocrit; **BCG:** Bioconverted ginsenosides; **DMEM:** Dulbecco's Modified Eagle Medium; MTT: 3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyl tetrazolium bromide; **LPS:** Lipopolysaccharide; **CPDA:** Citrate-phosphate-dextrose-adenine; **CBC:** Complete blood count; **HGB:** Hemoglobin; **WBC:** White blood cell; **RBC:** Red blood cell; **RDW:** Red blood cell distribution width; **PLT:** Platelet; **MCV:** Mean corpuscular volume; PS: phosphatidyl serine.

SUMMARY

- This study was investigated the inhibitory effects of Phenylhydrazine (PHZ)-induced hepatotoxicity and anemia using bioconverted ginsenosides.
- The bioconverted ginsenoside increases erythrocyte count and erythrocyte life span *in vivo* by the results of MTT assay, nitric oxide inhibition, and ICP-OES assay.
- The bioconverted ginseng extraction have the potential for drug substance.

REFERENCES

1. Report of a WHO scientific group. Nutritional anaemias. World Health Organ Tech Rep Ser. 1968;405:5-37. PMID 4975372.

2. Kassebaum NJ, GBD 2013 Anemia Collaborators. The global burden of anemia. Hematol Oncol Clin North Am. 2016;30(2):247-308. doi: 10.1016/j.hoc.2015.11.002, PMID 27040955.
3. Ludwig H, Strasser K. Symptomatology of anemia. Semin Oncol. 2001;28(2);Suppl 8:7-14. doi: 10.1016/s0093-7754(01)90206-4, PMID 11395846.
4. Yokusoglu M, Nevruz O, Baysan O, Uzun M, Demirkol S, Avcu F, *et al*. The altered autonomic nervous system activity in iron deficiency anemia. Tohoku J Exp Med. 2007;212(4):397-402. doi: 10.1620/tjem.212.397, PMID 17660705.
5. Ekiz C, Agaoglu L, Karakas Z, Gurel N, Yalcin I. The effect of iron deficiency anemia on the function of the immune system. Hematol J. 2005;5(7):579-83. doi: 10.1038/sj.thj.6200574, PMID 15692603.
6. World Health Organization. WHO Monogr Sel Med Plants. 1999;2.
7. Shinkai K, Akedo H, Mukai M, Imamura F, Isoai A, Kobayashi M, *et al*. Inhibition of *in vitro* tumor cell invasion by ginsenoside Rg3. Jpn J Cancer Res. *1996*;87(4):357-62. doi: 10.1111/j.1349-7006.1996.tb00230.x, PMID 8641966.
8. Bian Y, An GJ, Kim K, Ngo T, Shin S, Bae ON, *et al*. Ginsenoside Rg3, a component of ginseng, induces pro-thrombotic activity of erythrocytes via hemolysis-associated phosphatidylserine exposure. Food Chem Toxicol. 2019;131:110553. doi: 10.1016/j.fct.2019.05.061, PMID 31163221.
9. Attele AS, Wu JA, Yuan CS. Ginseng pharmacology: multiple constituents and multiple actions. Biochem Pharmacol. 1999;58(11):1685-93. doi: 10.1016/s0006-2952(99)00212-9, PMID 10571242.
10. Li GX, Liu ZQ. The protective effects of ginsenosides on human erythrocytes against hemin-induced hemolysis. Food Chem Toxicol. 2008;46(3):886-92. doi: 10.1016/j.fct.2007.10.020, PMID 18069111.
11. Zhang Y, Liu QZ, Xing SP, Zhang JL. Inhibiting effect of Endostar combined with ginsenoside Rg3 on breast cancer tumor growth in tumor-bearing mice. Asian Pac J Trop Med. 2016;9(2):180-3. doi: 10.1016/j.apjtm.2016.01.010, PMID 26919952.
12. Noh KH, Son JW, Kim HJ, Oh DK. Ginsenoside compound K production from ginseng root extract by a thermostable beta-glycosidase from Sulfolobus solfataricus. Biosci Biotechnol Biochem. 2009;73(2):316-21. doi: 10.1271/bbb.80525, PMID 19202288.
13. Park CS, Yoo MH, Noh KH, Oh DK. Biotransformation of ginsenosides by hydrolyzing the sugar moieties of ginsenosides using microbial glycosidases. Appl Microbiol Biotechnol. 2010;87(1):9-19. doi: 10.1007/s00253-010-2567-6, PMID 20376631.
14. Yang XD, Yang YY, Ouyang DS, Yang GPA. A review of biotransformation and pharmacology of ginsenoside compound K. Fitoterapia. 2015;100:208-20. doi: 10.1016/j.fitote.2014.11.019, PMID 25449425.
15. Ha J, Shim YS, Seo D, Kim K, Ito M, Nakagawa H. Determination of 22 ginsenosides in ginseng products using ultra-high-performance liquid chromatography. J Chromatogr Sci. 2013;51(4):355-60. doi: 10.1093/chromsci/bms148, PMID 22977120.
16. Shahid I, AlMalki WH, AlRabia MW, Mukhtar MH, Almalki SSR, Alkahtani SA, *et al. In vitro* inhibitory analysis of consensus siRNAs against NS3 gene of hepatitis C virus 1a genotype. Asian Pac J Trop Med. 2017;10(7):701-9. doi: 10.1016/j.apjtm.2017.07.011, PMID 28870347.
17. Uma Anitha KPG, Mythili S. Antioxidant and hepatoprotective potentials of novel endophytic fungus *Achaetomium* sp., from Euphorbia hirta. Asian Pac J Trop Med. 2017;10(6):588-93. doi: 10.1016/j.apjtm.2017.06.008, PMID 28756924.
18. Hanh TT, Hang DT, Van Minh C, Dat NT. Anti-inflammatory effects of fatty acids isolated from Chromolaena odorata. Asian Pac J Trop Med. 2011;4(10):760-3. doi: 10.1016/S1995-7645(11)60189-2, PMID 22014728.
19. Harrington JM, Young DJ, Essader AS, Sumner SJ, Levine KE. Analysis of human serum and whole blood for mineral content by ICP-MS and ICP-OES: development of a mineralomics method. Biol Trace Elem Res. 2014;160(1):132-42. doi: 10.1007/s12011-014-0033-5, PMID 24917052.
20. Lee Y, Kim KM, Kang JS. Preventive effect of *Angelica gigas* Nakai extract oral administration on dry eye syndrome. Asian Pac J Trop Med. 2018;11(6):369-75. doi: 10.4103/1995-7645.234764.
21. Wickham H. 3rd ed. Springer; 2016. p. ggplot2: elegant graphics for data analysis.
22. Bae SJ, Rho GJ, Kim KM, Kang JS. Pharmacological effects of active saponins from *panax ginseng* Meyer. Trop J Pharm Res. 2019;18(3):555-61. doi: 10.4314/tjpr.v18i3.16.
23. Zheng MM, Xu FX, Li YJ, XI XZ, Cui XW, Han CC, *et al*. Study on transformation of ginsenosides in different methods. BioMed Res Int. 2017;2017:8601027. doi: 10.1155/2017/8601027, PMID 29387726.
24. Ippoushi K, Itou H, Azuma K, Higashio H. Effect of naturally occurring organosulfur compounds on nitric oxide production in lipopolysaccharide-activated macrophages. Life Sci. 2002;71(4):411-9. doi: 10.1016/s0024-3205(02)01685-5, PMID 12044841.
25. Samukawa K, Suzuki Y, Ohkubo N, Aoto M, Sakanaka M, Mitsuda N. Protective effect of ginsenosides Rg(2) and Rh(1) on oxidation-induced impairment of erythrocyte membrane properties. Biorheology. 2008;45(6):689-700. doi: 10.3233/BIR-2008-0516, PMID 19065015.
26. Pandey S, Ganeshpurkar A, Bansal D, Dubey N. Hematopoietic effect of Amaranthus cruentus extract on phenylhydrazine-induced toxicity in rats. J Diet Suppl. 2016;13(6):607-15. doi: 10.3109/19390211.2016.1155685, PMID 27027824.
27. Banerjee M, Ray AK. The role of thyroid hormone on phenylhydrazine hydrochloride mediated inhibitory effects on blood acetylcholinesterase: an *in vivo* and *in vitro* study. J Biochem Mol Toxicol. 2002;16(4):162-8. doi: 10.1002/jbt.10039, PMID 12242684.
28. Shukla P, Yadav NK, Singh P, Bansode F, Singh R. Phenylhydrazine induced toxicity: a review on its haematotoxicity. Int J Basic Appl Med Sci. 2012;2(2):86-91.
29. Aggett PJ, Comerford JG. Zinc and human health. Nutr Rev. 1995;53(9 Pt 2):S16-22. PMID 8577412.
30. Leecharoenkiat K, Lithanatudom P, Sornjai W, Smith DR. Iron dysregulation in beta-thalassemia. Asian Pac J Trop Med. 2016;9(11):1035-43. doi: 10.1016/j.apjtm.2016.07.035, PMID 27890361.

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