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Hypolipidemic and antioxidant effects on hypercholesterolemic rats of polysaccharide from *Salicornia bigelovii* seed

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Abstract−The hypolipidemic and antioxidant effects of dietary supplementation with polysaccharide isolated from *Salicornia bigelovii* seed (SBS) were evaluated in hypercholesterolemic rats. Following administration of SBS polysaccharide at 200 mg/kg BW/day (HPS group), total cholesterol (TC), and low density lipoprotein cholesterol (LDLC) levels were 32.45% and 58.09% lower than those in negative controls (NC) group, respectively. However, in the case of high density lipoprotein cholesterol (HDLC), it was 36.37% higher than that of NC group. The arteriosclerosis and cardiac risk indices were 64.23% and 50.53% lower than in the NC group. However, the HTR (HDLC/TC) value was higher 2.05-fold than that of the NC group. Hydroxy-3-methylglutaryl CoA reductase activity in HPS group was 55.1% lower than that of the NC group. Activities of alanine transferase, aspartate aminotransferase, and alkaline phosphatase in HPS group were 29.71%, 26.49%, and 32.24%, lower than that of the NC group. SOD, CAT, and GSH-Px activities in HPS group were 31.05%, 49.29%, and 54.62% higher than that of the NC group. These results indicate that SBS polysaccharide has both hypolipidemic and antioxidant effects and shows potential for ameliorating hypercholesterolemia.

Keywords: Hypolipidemic Activity, Antioxidant Activity, Polysaccharide, *Salicornia bigelovii* Seed

INTRODUCTION

Salicornia L. (Chenopodiaceae) is a genus of annual, apparently leafless halophytic herbs with articulated, succulent stems, known as glasswort. *Salicornia* species are salt tolerant and frequently occur in saline areas [1]. The Salicornioideae family comprises approximately 15 genera and 117 species. The commonly found *Salicornia* species are *S. ramosissima*, *S. indica*, *S. brachiata*, *S. bigelovii*, *S. herbacea*, *S. perenis*, *S. fragilis*, *S. nitens*, *S. pusilla*, and *S. disarticulate* [2]. *Salicornia* species have been used not only as food but also in traditional medicine. In particular, they contain high levels of dietary fiber and many bioactive substances, such as phytosterols, polysaccharides, and phenolic compounds, mainly flavonoids and phenolic acids, which may have beneficial effects for treatment of cardiovascular disease and diabetes [3,4]. *S. herbacea* and *S. bigelovii* are distributed in tidelands on the southern and western coast of Korea, and are known there as "tungtungmadi" or "hamcho." These species are eaten as a seasonal vegetable and are used as a folk medicine to treat a range of diseases, such as hypertension, diabetes, hemorrhoids, and cancer [6]. For example, *S. herbacea* extract inhibits tyrosinase activity [7], scavenges free radicals, and shows skin-whitening effects [8]. *S. herbacea* has also been reported to inhibit the growth of cancer cells and show immunomodulatory effects in macrophages [9]. *S. herbacea* extract also showed preventive effects on diabetes, arteriosclerosis, hyperlipidemia, and fatty liver disease [10]. Kang et al. [11] investigated the antioxidant and antineuroinflammatory effects of *S. bigelovii* extract (SBE) in lipopolysaccharide (LPS)-stimulated BV-2 microglial cells. SBE significantly inhibited DPPH-generated free radicals, showing maximum inhibition at 40 μg/mL. SBE also inhibited the LPS-induced increase in inducible nitric oxide synthase and cyclooxygenase-2 expression. Wang et al. isolated nortriterpenoid saponins, bigelovii A, bigelovii B, pfaffine B, and boussingoside from *S. bigelovii* and evaluated them for cytotoxicity against the HL-60 (promyelocytic leukemia), MCF-7 (breast carcinoma), HepG2 (liver carcinoma), and A549 (lung carcinoma) cell lines. The results showed that bigelovii A and pfaffine B were moderately active against HL-60, MCF-7, and HepG2 [12].

The biological activities of seeds of *Salicornia* species have attracted much recent attention. Glenn et al. reported that *S. bigelovii* seed is an important halophyte that is used as a conventional oil seed crop for direct seawater irrigation in coastal areas of arid regions. It has shown promise for commercial applications because it colo-

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nizes through the prolific production of seed composed of 30% oil (similar to safflower oil), 35% protein, and less than 3% salt. The seeds produce oil that is high in polyunsaturated fats [13]. The seeds also contain a high degree of unsaturated acids, mainly linoleic acids, and high values of beta-carotene, ascorbic acid, and total chlorophyll, making the plant a good source of vitamin A and C [14]. Previously, we reported methods for extracting oil from *Salicornia* seed and for manufacturing biodiesel from the oil [15,16]. We also performed physicochemical characterization of the stability and antioxidant activity of *S. herbacea* seed oil for industrial applications. This study suggested that *S. herbacea* seed oil is a source of powerful antioxidants, such as phenols and tocopherols, which could be used against diseases related to oxidative stress, for cosmetic and dermatological applications, as well as food supplements [17]. Although many studies have examined the seed of *Salicornia* species, the biological effects of the polysaccharides obtained from *S. bigelovii* seed have not been reported.

In this study, we examined the hypolipidemic and antioxidant effects of polysaccharides from *S. bigelovii* seed (SBS) in hypercholesterolemic rats.

MATERIALS AND METHODS

1. Preparation of SBS Polysaccharide Extract

S. bigelovii seed (SBS) was collected from the Gusan coastal area at Jeonbuk, South Korea in 2014. Samples were dried in a cool, windy, and dry location. The seed was ground finely in a grinder to a particle size of 0.05-0.1 mm. The powdered seed was defatted with petroleum ether. The defatted powder was extracted with double-distilled water at 100 °C for 4 h in several batches. The extracts were combined, filtered, and concentrated to approximately one-third of the original volume. Chilled ethanol was added at five-times the original volume and then samples were kept at 4 °C for 24 h. Samples were centrifuged and the precipitate was collected, redissolved in distilled water, treated with Sevag's reagent several times to remove protein, and then dialyzed against deionized water for 24 h at 4 °C. Polysaccharides were again precipitated with ethanol and the precipitate was lyophilized. The lyophilized polysaccharide was dissolved in water, reprecipitated with an equal volume of cetyltrimethylammonium hydroxide, and kept overnight. The supernatant was precipitated with chilled ethanol. After centrifugation, the polysaccharide extract (0.2 g) was dissolved in distilled water, dialyzed against distilled water, and applied to a DEAE-cellulose ion-exchange column (5×50 cm). Stepwise elution was performed with distilled water and a linear gradient of NaCl solution (0-2 M) in Tris-HCl buffer (pH 8.5). The polysaccharides were collected, lyophilized, and stored at 15 °C for further analyses.

2. Animals and Diets

Thirty female Sprague-Dawley albino rats (101±4.2 g, six weeks old, purchased from Central Lab Animal Inc., Seoul, Korea) were used. All animals were acclimated to the animal room for one week. The rats were housed in an animal room at 23±2 °C under a 12 h/12 h dark/light cycle (17:00-5:00 h) and a relative humidity of 50-60%. Rats were divided into five feed groups: a normal (N) group without administration of cholesterol, a negative control (NC) group with administration of cholesterol, a low polysaccharide (LPS) group with administration of cholesterol and polysaccharide (100 mg/kg BW), a high polysaccharide (HPS) group with administration of cholesterol and polysaccharide (200 mg/kg BW), and a positive control (PC) group with administration of cholesterol and lovastatin (2 mg/kg BW).The compositions of the experimental diets are presented in Table 1, and the rats were fed for six weeks.

3. Analysis of Biochemical Parameters

At the end of the experimental period, overnight-fasting animals were sacrificed under injectable anesthetic (Zoletil 50; Virbac Laboratories, Carros, France). Blood samples were collected with a disposable plastic syringe into heparinized tubes. Serum was prepared by centrifugation at 2,493 ×*g* for 10 min at 4 °C. Triglyceride (TG) concentration was measured enzymatically using the glycerophosphate oxidase assay. Total cholesterol (TC), high-density lipoprotein cholesterol (HDLC), low-density lipoprotein cholesterol (LDLC), very low-density lipoprotein cholesterol (VLDLC), and phospholipid (PL) levels were measured enzymatically using the cholesterol oxidase assay using commercially available assay kits (Sekisui Medical Co., Ltd., Tokyo, Japan). Albumin, total bilirubin, creatinine, blood urea nitrogen, uric acid, and glucose levels were

Table 1. Composition of the experimental diet (g/kg)

Composition	N group	NC group	LPS group	HPS group	PC group
Casein	200.0	200.0	200.0	200.0	200.0
L-methionine	3.0	3.0	3.0	3.0	3.0
Beef tallow	100.0	100.0	100.0	100.0	100.0
Choline chloride	2.0	2.0	2.0	2.0	2.0
Corn starch	446	436	435.9	435.8	436
Sucrose	200.0	200.0	200.0	200.0	200.0
Cholic acid	2.0	2.0	2.0	2.0	2.0
Vitamin mixture(AIN 93)	10.0	10.0	10.0	10.0	10.0
Mineral mixture (AIN 93)	35.0	35.0	35.0	35.0	35.0
SBS Polysaccharide			0.1	0.2	
Lovastatin					0.002
Soybean oil	2	2	2	2	2
Cholesterol	0.0	10	10	10	10

measured by standard methods using an auto analyzer (Hitachi 7600-210; Hitachi, Tokyo, Japan). The activities of aspartate aminotransferase (AST) and alanine transferase (ALT) were determined by using the kinetic method. The oxoacids formed in the transaminase reactions were measured indirectly by enzymatic reduction to their corresponding hydroxyacids. The accompanying change in NADH concentration was measured at 340 nm with a spectrophotometer (Optizen POP; Mecasys Co. Ltd., Daejeon, Korea). Alkaline phosphatase (ALP) activity was determined using 4-nitrophenyl phosphate. ALP catalyzes the hydrolysis of 4-nitrophenyl phosphate, forming phosphate and free 4-nitrophenol, which is colorless in dilute acid solutions. However, under alkaline conditions, 4-nitrophenol is converted to the 4-nitrophenoxide ion, which gives an intense yellow color. The absorbance of this colored compound was measured spectrophotometrically at 420 nm to determine ALP activity. The arteriosclerosis index (AI), HTR, cardiac risk factor (CRF), and VLDLC were calculated by the following equations: AI=(9TC−HDLC)/HDLC, HTR=HDLC/TC, CRF=TC/HDLC, VLDLC=[TC−(HDLC+LDLC)].

4. Activities of HMG-CoA Reductase and ACAT

Hydroxy-3-methylglutaryl CoA (HMG-CoA) reductase activity was determined by using a modified version of the method described by Hulcher et al. [18]. The mixture was incubated at 37 °C for 30 min in potassium phosphate buffer containing 1.0 mg of microsomal protein, 150 nM of HMG-CoA, 2 μM of NADP, 20 mM of glucose 6-phosphate, 2 units of glucose-6-phosphate dehydrogenase, 100 μL of test sample, and 20 μL of sodium arsenite (10 mM). The solution was stayed for 1 min and incubated at 37 °C for 10 min after adding 0.1 mL of citrate buffer (2 mM, pH 3.5) including 3.0% sodium tungstate to stop the reaction. One milliliter of supernatant, centrifuged at 15,000 rpm, was added to 0.2 mL of Tris buffer (2 M, pH 8.0). To this solution, 50 mL of sodium arsenite (0.4 M) was added, followed by 5 min of incubation, then 1 mL of the reaction mixture was added to 20 μL of 5,5-dithio-bis (2-nitrobenzoic acid (3 mM), and the CoA-SH concentration was measured by a spectrophotometer at 420 nm.

HMG-CoA reductase activity was defined as the amount of enzyme that catalyzes the oxidation of 1 mol of NADPH per minute. Acyl-CoA: cholesterol acyltransferase (ACAT) activity was determined by using a modified version of the method described by Gillies et al. [19]. Cholesterol (6 mg) was dissolved in 6 mL of acetone, added to 600 mg of Triton WR-1339, and dried under nitrogen gas. This cholesterol mixture was dissolved in 20 mL of distilled water. The resultant cholesterol solution was prepared in a final concentration of 300 μg/mL. Oleoyl-CoA was diluted to 200 μg/mL, divided into several vials, and stored at −70 °C. Cholesterol solution (20 μL) was added to a mixture of 20 μL of potassium phosphate (1 M, pH 7.4), 10 μL of BSA (0.6 mM), 10 μL of microsomes, and 120 μL of distilled water and mixed well. The enzyme reaction was initiated by the addition of 20 μL (5.62 nmol) of [^{14}C]-oleoyl-CoA (specific activity; 15,000 cpm/nmol) to the above mixture in a final volume of 200 μL. After a 30-min incubation at 37 °C, the reaction was stopped by the addition of 500 μL of isopropanol : heptane (4 : 1, v/v) solution, 300 μL of heptane, and 200 μL of potassium phosphate (0.1 M, pH 7.4). The phase separated after a few minutes, and an aliquot of the upper phase (200 μL of the supernatant) was obtained and counted by scintillation counting. Corrections were made by multiplying the calculated value by 2. ACAT activity was expressed as nmol of cholesterol oleoate formed per min per mg of microsomal protein.

5. Activities of Catalase, Superoxide Dismutase, and Glutathione Peroxidase

To determine the catalase (CAT) activity, a 0.1 ml aliquot of surfactant was added to 50 mM potassium phosphate buffer (pH 7.0) and 10.5 mM H_2O_2. The reaction was allowed to take place for 30 s at 25 °C. The amount of enzyme activity required to decompose 1 μmol H_2O_2/s was defined as one unit of activity. Superoxide dismutase (SOD) activity was determined by recording the inhibition of ferricytochrome C reduction with EDTA. In each sample, the amount of enzyme sufficient to inhibit the rate of cytochrome C reduction by 50% was determined. To analyze glutathione peroxidase (GSH-Px) activity, 500 μL of a mixture of potassium phosphate buffer (0.1 M, pH 7.0) containing 1×10^{-3} M sodium azide, 1 mM EDTA, 10 μL of enzyme solution, 100 μL of glutathione reductase (2.768 U/mL), and 100 μL of glutathione (1×10^{-2} M) was mixed and precultured for 10 min at 37 °C. Next, an aliquot of $NaHCO_3$ (0.1%) containing 100 μL of NADPH (1.5×10^{-3} M) and 100 μL of H_2O_2 (1.5×10^{-3} M) was added to the reaction mixture. The absorbance was measured for 1 min at 340 nm.

6. Glutathione and Malondialdehyde Level

One milliliter of supernatant was added to 0.1% picric acid and centrifuged at 3,000 rpm for 15 min. The supernatant was added to phosphate buffer (0.1 M, pH 7.5) containing 0.2 mM NADPH, 0.6 mM DTNB, 5 mM EDTA, and GSH reductase. Changes in absorbance were measured at 412 nm for 3 min at 3-s intervals. The malondialdehyde (MDA) level was measured using the 2-thiobarbituric acid (TBA) method and expressed as nmol/mg protein in serum.

7. Statistical Analysis

All experiments were performed in triplicate and the results expressed as mean±SEM. One-way analysis of variance (ANOVA) was performed, followed by Duncan's multiple comparison test. Statistical Package for the Social Sciences (SPSS, Version 19.0, SPSS Inc., Chicago, IL, USA) was used for all analyses. A p-value<0.05 was considered to indicate statistical significance.

RESULTS AND DISCUSSION

Various polysaccharides were isolated from *S. herbacea* and their biological activities determined. For example, Im et al. examined the immunomodulatory activity of polysaccharides isolated from *S. herbacea* in a mouse monocytic cell line. They found that the polysaccharide activated RAW cells to produce cytokines such as tumor necrosis factor (TNF)-α and interleukin (IL)-1β, as well as nitric oxide (NO), in a dose-dependent manner [20]. In addition, the immunomodulatory effects of crude and refined polysaccharides of *S. herbacea* on BALB/c mice splenocytes were investigated [21]. Lee et al. reported that *S. herbacea* polysaccharides significantly induce NO production and inducible NO synthase transcription through the activation of nuclear factor-kB/Rel [22]. Lee et al. investigated the effect of physiologically active polysaccharides isolated from *S. herbacea* on STZ-induced diabetic rats. This

polysaccharide exhibited substantial antihyperglycemic activity in diabetic rats [23]. Ryu et al. investigated the anti-proliferative effect of crude and fine polysaccharides from *S. herbacea* on HT-29 human colon cancer cells. They suggested that both polysaccharides have anti-cancer activity in human colon cancer cells [24]. Although many studies have examined the effects of *S. herbacea* polysaccharides, the hypolipidemic and antioxidant effects of polysaccharides obtained from *S. bigelovii* seed (SBS) have not been reported.

In this study, we investigated the hypolipidemic and antioxidant effects of polysaccharides isolated from SBS on lipid levels, biochemical function, and activities of HMG-CoA reductase, ACAT, ALT, AST, and ALP, and antioxidant enzymes in hypercholesterolemic rats.

1. Effect of SBS Polysaccharide on Body Weight, Feed Intake, and Feed Efficiency

The effect of SBS polysaccharide administration on body weight, feed intake, and feed efficiency in rats fed a high-cholesterol diet is shown in Fig. 1. The body weight gain in the N group (without oral administration of high cholesterol) was 3.73 g/day. In the NC group (with oral administration of high cholesterol), it increased to 4.54 g/day. However, the weight gain in rats fed a high-cholesterol diet combined with oral administration of SBS polysaccharide (200 mg/kg body weight) or lovastatin (2.0 mg/kg body weight) was 3.90 or 3.91 g/day, respectively, approximately 86% of that in the NC group. Feed intake was decreased in all rats fed a high-cholesterol diet, compared to the N group. When the high-cholesterol diet was administered in combination with SBS polysaccharide (200 mg/kg BW) or lovastatin, feed intake was 22.91 or 23.82 g/day, respectively. In the NC group, the feed intake was 21.42 g/day. The feed efficiency ratio was 0.21 in the NC group. However, in the HPS and PC groups, the feed efficiency ratio was 0.17 and 0.16, respectively, approximately 81.0 and 76.20% of that observed in the NC group.

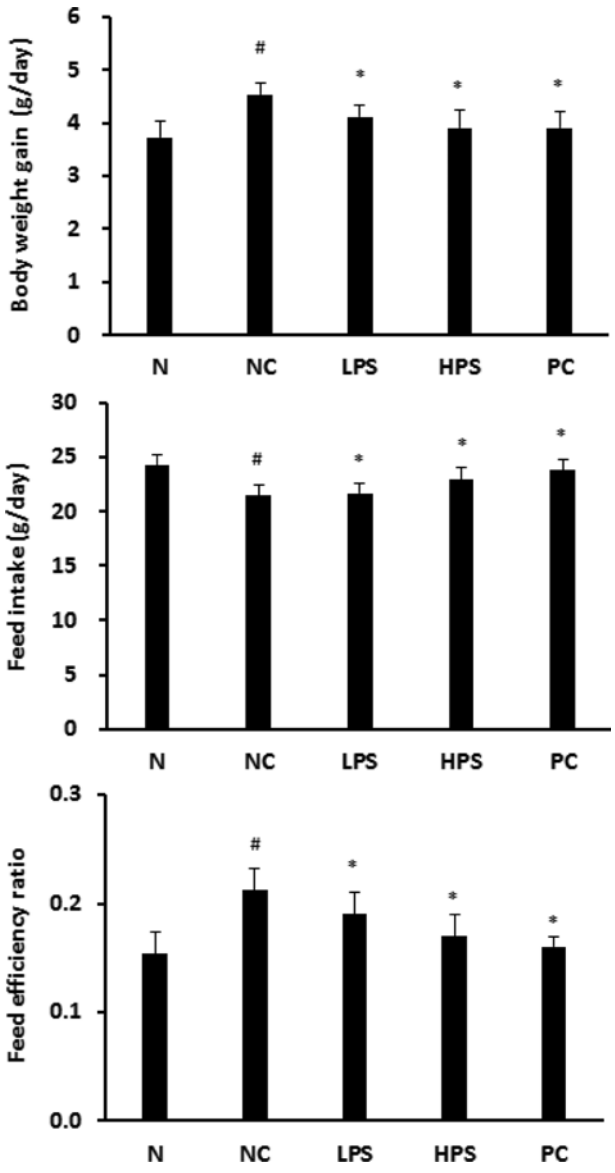

Fig. 1. Effect of SBS polysaccharide on body weight, feed intake, and feed efficiency in rats.

2. Effect of Polysaccharides on Lipid Levels

Hypercholesterolemia is regarded as a major risk factor for cardiovascular diseases such as arteriosclerosis, myocardial infarction, heart attack, and cerebrovascular disease, which are the leading causes of death in developed countries. Reducing circulating cholesterol levels can reduce the risk of these diseases. Hypercholesterolemia is related to increased levels of oxidative stress and lipid metabolism, and low-density lipoprotein (LDL) generation has been identified as a major contributor to the vascular damage induced by high cholesterol levels [25]. Generally, LDL cholesterol is the main carrier of blood cholesterol and is linearly related to levels of serum cholesterol. Circulating high density lipoprotein (HDL) cholesterol is regarded as "good cholesterol," and it carries cholesterol from peripheral cells to the liver for metabolic conversion into bile acids. This pathway is crucial for maintaining cholesterol homeostasis between blood and peripheral tissues. HDL cholesterol protects against coronary heart disease [26]. Fig. 2 shows the effects of SBS polysaccharides on levels of triglyceride (TG), total cholesterol (TC), HDL cholesterol (HDLC), LDL cholesterol (LDLC), and phospholipid (PL) in rats fed a high-cholesterol diet. TG, TC, and LDLC levels were significantly higher in the NC group than in the N group, whereas HDLC level was lower. When the quantity of SBS polysaccharide fed was increased from 100 to 200 mg/kg BW, the TG level decreased from 64.34 to 62.44 mg/dL, which represents a 17.31% reduction compared to the NC group. TC decreased from 89.22 to 76.45 mg/dL, which represents a 32.45% reduction compared to the NC group. Compared to the PC group, the TC level showed an increase of 1.49%. When the quantity of SBS polysaccharide fed was increased from 100 to 200 mg/kg BW, the HDLC level increased from 28.42 to 32.96 mg/dL, which represents a 36.37% increase compared to the NC group. This value is similar to that of the N group and about 97.7% of that of the PC group. LDLC decreased from 47.93 to 31.02 mg/dL, which represents a 58.09% decrease compared to the NC group, and an 8.67% increase compared to the PC group. The LDL/HDL ratio decreased from 1.69 to 0.94%, which represents a 17.50% increase compared to the PC group. The conversion of very-low-density lipoprotein (VLDL) to triglyceride-rich remnants by lipoprotein lipase interferes with chylomicron remnant clearance [45], resulting in hypertriglyceri-

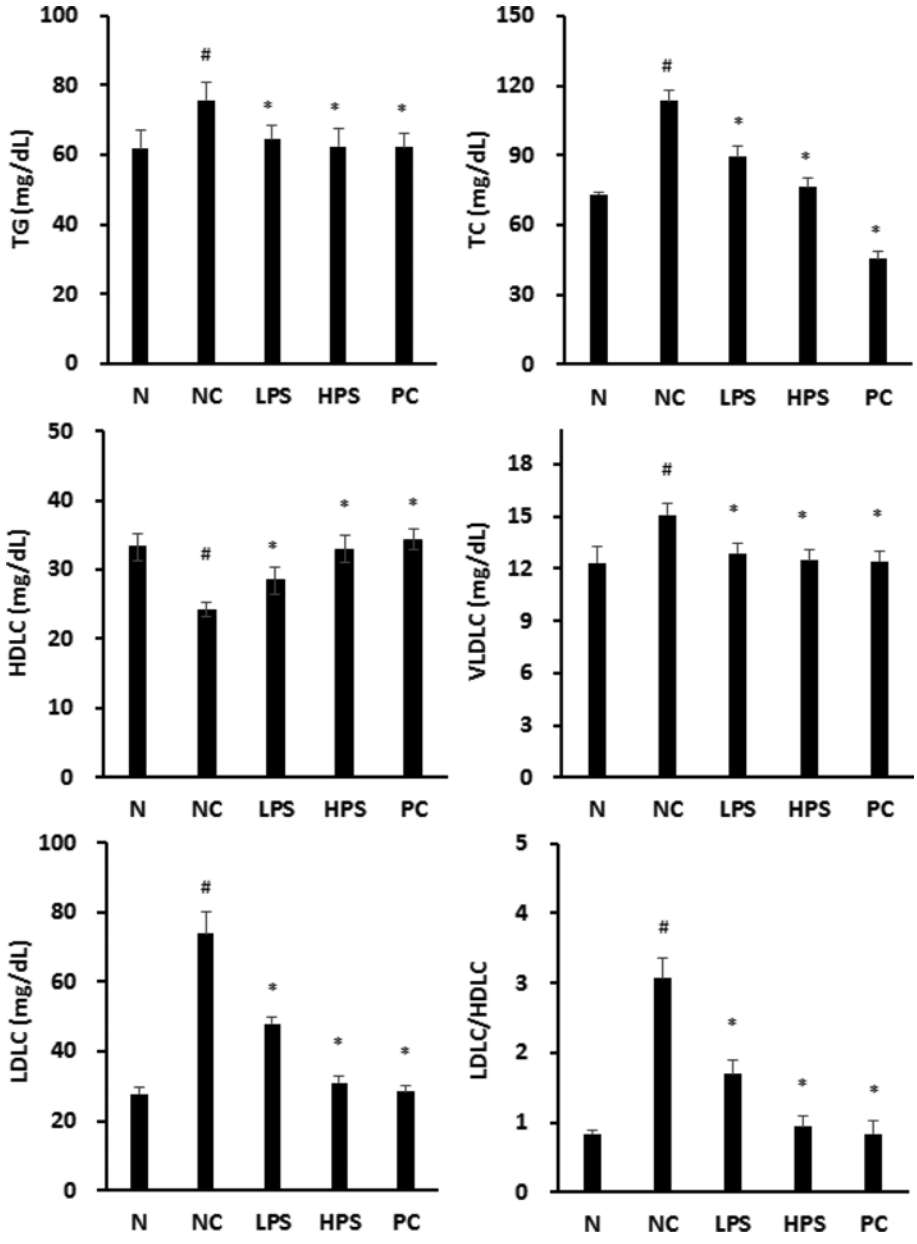

Fig. 2. Effect of SBS polysaccharides on the TG, TC, HDLC, VLDLC, LDLC, and LDLC/HDLC levels in rats. The results are expressed as mean±SEM (n=8). $^{\#}p<0.05$ compared with N group, $^{*}p<0.05$ compared with NC group (ANOVA followed by Duncan's multiple comparison test).

demia and elevated lipoproteins enriched with triglycerides, which are susceptible to hepatic lipase and cause a decrease in HDL cholesterol [46]. The VLDLC level in the NC group increased to 15.11 mg/dL. However, it decreased to 12.49 mg/dL in the HPS group. This value is similar to that of the N and PC groups. Phospholipid (LP) synthesis is generally affected by a high-lipid diet. The LP level increased to 84.51 mg/dL in the NC group, which represents an increase of 86.02% compared to the N group (data not shown). When the SBS polysaccharide concentration was increased from 100 to 200 mg/kg BW, the LP level decreased from 76.13 to 50.22 mg/dL, which represents a 40.58% reduction compared to the NC group (data not shown). These results suggest that SBS polysaccharide improved the composition of blood lipid profiles in rats fed a high-cholesterol diet, and therefore shows potential for development as an antiobesity ingredient in oriental medicine compounds.

In the standards published by the US National Institute of Health, HTR (HDLC/TC) value, arteriosclerosis index (AI, atherogenic index: TC-HDLC)/HDLC), and cardiac risk index (CRF, cardiac risk factor: TC/HDLC) are used for determination of the level of risk of cardiovascular diseases [27]. Therefore, the HTR value, AI, and CRF were calculated and these are shown in Fig. 3. The AI in the NC group increased to 3.26, which was about 3.29 times higher than that of the N group. However, when the SBS polysaccharide concentration was increased from 100 to 200 mg/kg BW, the AI value decreased from 2.14 to 1.32, which represents a 64.23% reduction compared to the NC group. Compared to the PC group (ad-

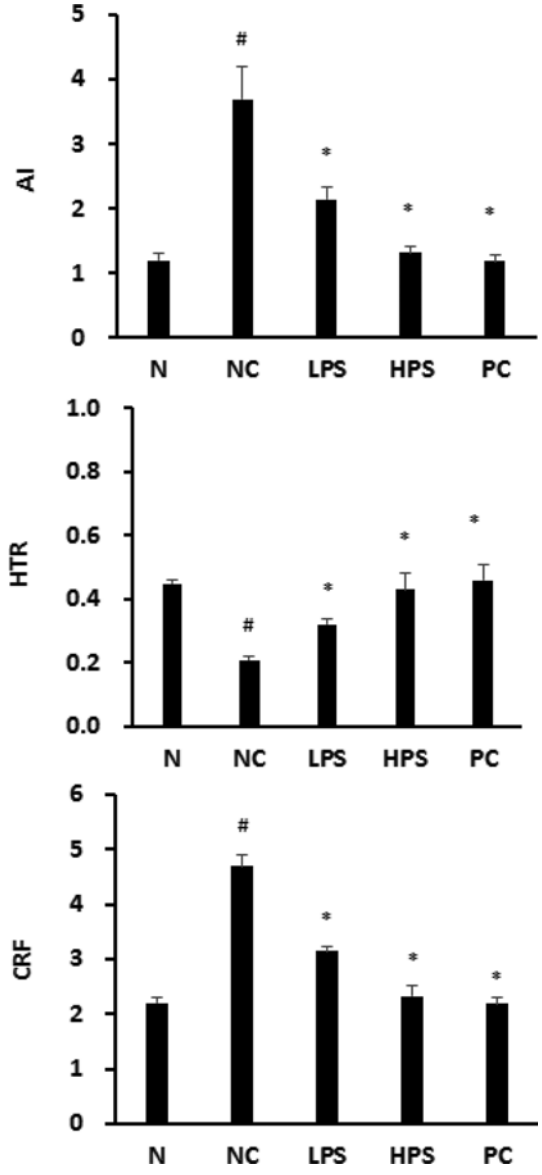

Fig. 3. Effect of SBS polysaccharide on the AI, HTR, and CRF in rats. The results are expressed as mean±SEM (n=8). $^{\#}p<0.05$ compared with N group, $^{*}p<0.05$ compared with NC group (ANOVA followed by Duncan's multiple comparison test).

ministration of lovastatin at 2.0 mg/kg body weight), it represents an increase of 10.92%. This result indicates that SBS polysaccharide may reduce the risk of arteriolosclerosis. The CRF increased significantly to 4.69, in the NC group. However, when the SBS polysaccharide concentration was increased from 100 to 200 mg/kg BW, the CRF decreased from 3.14 to 2.32, which represents a decrease of 50.53% compared to the NC group. The PC group showed a similar CRF. In contrast, the HTR value decreased to 0.21 in the NC group. However, when the SBS polysaccharide concentration was increased from 100 to 200 mg/kg BW, the HTR value decreased from 0.32 to 0.43, approximately 2.05 times that of the NC group. Compared to the PC group, it represents a decrease of 6.52%.

3. Effect of SBS Polysaccharide on Biochemical Function

Table 2 shows the effects of SBS polysaccharide on levels of glucose, albumin, creatinine, blood urea nitrogen, uric acid, and total bilirubin. The glucose level in the NC group increased to 135.12 mg/dL, which was a 16.23% increase compared to the N group. When the SBS polysaccharide concentration was increased from 100 to 200 mg/kg BW, the glucose level decreased from 125.41 to 117.86 mg/dL, which represents a 12.77% reduction compared to the NC group. This value is similar to that of the PC group. These results indicate that SBS polysaccharides exhibited a considerable hypoglycemic effect in rats fed a high-cholesterol diet, and may prove to be useful for the management of hyperlipidemia. Albumin levels were similar in all groups. Creatinine, blood urea nitrogen, uric acid, and total bilirubin levels were significantly higher in the NC group than in the N group. The creatinine level was 0.82 mg/dL in the NC group, which was about 12.34% higher than that of the N group. In the HPS group, the creatinine level was 3.50 mg/dL, which was similar to that of the PC group. The blood urea nitrogen level in the HPS group was 15.84 mg/dL, which was 12.56% lower than that of the NC group. This value was similar to that of PC group. The uric acid level in the HPS group was 2.63 mg/dL, which was about 26.64% lower than that of the NC group. The total bilirubin level in the HPS group was 0.15 mg/dL, 6.67% lower than that of the NC group.

4. Effect of SBS Polysaccharide on HMG-CoA Reductase and ACAT Activity

It is now well established that inhibitors of hydroxy-3-methylglutaryl CoA (HMG-CoA) reductase, the rate-limiting enzyme in cholesterol biosynthesis, lower plasma triglycerides but not plasma cholesterol [28]. Therefore, many attempts have been made to reduce cholesterol levels by inhibiting the activity of HMG-CoA reductase. Qureshi et al. [29] reported that the activity of HMG-CoA reductase was decreased in pullets fed solvent extracts of garlic acid and consequently reduced plasma cholesterol levels. The activity of HMG-CoA reductase was decreased by 3% by intake of dietary garlic and was significantly reduced by supplementation with gar-

Table 2. Effect of SBS polysaccharide on levels of glucose, albumin, creatinine, blood urea nitrogen, uric acid, and total bilirubin

Composition	N group	NC group	LPS group	HPS group	PC group
Glucose (mg/dL)	116.25	135.12	125.41	117.86	115.41
Albumin (g/dL)	3.53	3.52	3.44	3.5	3.54
Creatinine (mg/dL)	0.73	0.82	0.75	0.74	0.72
Blood urea nitrogen (mg/dL)	15.42	17.85	16.22	15.84	15.21
Uric acid (mg/dL)	2.52	3.49	3.13	2.63	2.41
Total bilirubin (mg/dL)	0.13	0.15	0.13	0.14	0.14

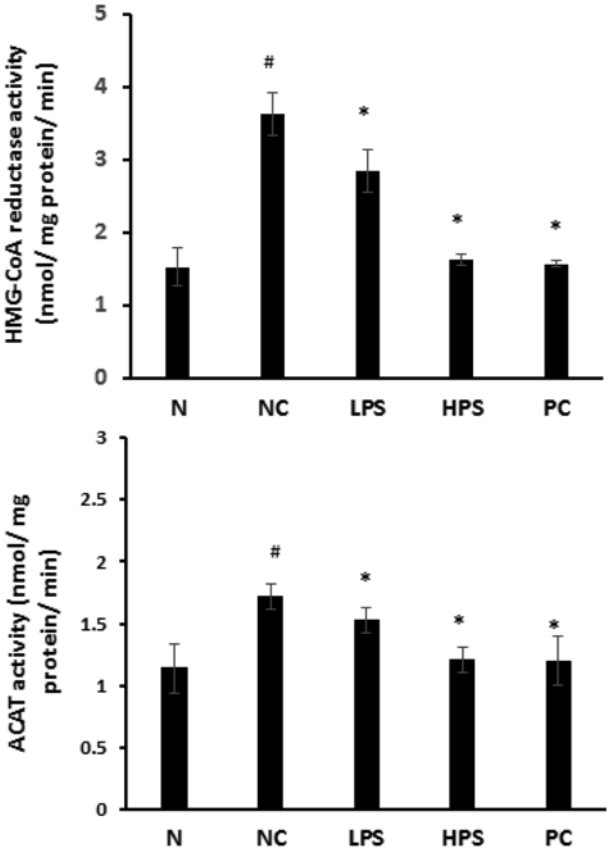

Fig. 4. Effect of SBS polysaccharide on activities of HMG-CoA reductase and ACAT in rats. The results are expressed as mean± SEM (n=8). $^{\#}p<0.05$ compared with N group, $^{*}p<0.05$ compared with NC group (ANOVA followed by Duncan's multiple comparison test).

lic extract [30]. Evans et al. [31] have reported that galactomannans produced a substantial reduction of hepatic synthesis of cholesterol in rats; moreover, the magnitude of the reduction was proportional to lowering of plasma cholesterol. Cheung [32] has demonstrated that polysaccharides of *V. volvacea* reduce the activity of HMG-CoA reductase. Fig. 4 shows the effect of SBS polysaccharide on the activities of HMG-CoA reductase and ACAT. HMG-CoA reductase activity was 1.52 nmol/mg protein/min in the N group. In the NC group, HMG-CoA reductase activity was 3.63 nmol/mg protein/min. However, when SBS polysaccharides were administered to the high-cholesterol diet group, HMG-CoA reductase activity decreased as the SBS polysaccharide concentration increased. In the LP group, HMG-CoA reductase activity was decreased to 3.04 nmol/mg protein/min, which was 16.3% lower than that of the NC group. In particular, in the HP group, HMG-CoA reductase activity was significantly decreased to 1.63 nmol/mg protein/min, which was approximately 55.1% lower than that of the NC group. Compared to the PC group, it was about 96.3%. These results suggest that one of the main reasons for the cholesterol-lowering effect of SBS polysaccharide was the reduction of cholesterol synthesis via inhibition of hepatic HMG-CoA reductase activity in rats fed a high-cholesterol diet. Therefore, it appears that the reduction of HMG-CoA reductase activity is one of the major mechanisms by which SBS polysaccharide lowers total plasma levels of cholesterol. ACAT catalyzes the esterification of cholesterol. ACAT activity has been identified in a variety of tissues such as the intestine, liver, and artery wall, which are the major organs involved in cholesterol metabolism and atherosclerosis. Thus, accumulation of esterified cholesterol is a major metabolic change in atherosclerotic lesions [33,34]. Therefore, inhibition of ACAT would be expected to decrease cholesterol concentrations through the suppression of intestinal cholesterol absorption and hepatic VLDL secretion, thereby delaying or preventing atherosclerosis. ACAT activity decreased as the polysaccharide concentration increased, when polysaccharide was administered in the high-cholesterol diet group. In the LPS group, ACAT activity decreased to 1.53 nmol/mg protein/min. In the HPS group, ACAT activity was 1.21 nmol/mg protein/min, which was 30% lower than in the NC group. Levels were similar to those observed in the PC group. These results indicate that SBS polysaccharide can be expected to contribute to inhibition of cholesterol esterification in tissue.

5. Effect of SBS Polysaccharide on the Activities of Alanine Aminotransferase, Aspartate Aminotransferase, and Alkaline Phosphatase

Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) are enzymes that synthesize amino acids. AST can generally be found in the liver, cardiac muscle, kidneys, brain, pancreas, lungs, leukocytes, and erythrocytes, whereas ALT is present at highest concentration in the liver. High levels of cholesterol or alcohol lead to damage by causing degeneration of lipids in the liver, and ALT and AST activities are used as an index for liver damage [35]. The alkaline phosphatase (ALP) level in the plasma represents the total amount of ALPs released from tissues such as the liver, bones, kidneys, intestinal layers, and muscle, where ALP is synthesized, into the blood [36]. The effects of polysaccharide on the activities of ALT and AST and ALP are shown in Fig. 5. ALT activity was 41.37 U/mL in the N group. In the NC group, it was 63.61 U/mL. However, when polysaccharide was administered in the high-cholesterol diet group, ALT activity decreased as the polysaccharide concentration increased. In the LPS group, ALT activity decreased to 58.44 U/mL. In particular, in the HP group, ALT activity was 44.71 U/mL, 29.71% lower than the level in the NC group. AST activity was 70.81 U/mL in the N group and 98.52 U/mL in the NC group. However, when polysaccharide was administered in the high-cholesterol diet group, AST activity decreased as the polysaccharide concentration increased. ALT activity was 44.71 U/mL in the HPS group, which represents a decrease of 26.49% compared to the NC group. ALP activity in the N group was 25.73 U/mL. In the NC group, ALP activity increased significantly to 39.54 U/mL. However, when polysaccharide was increased from 100 to 200 mg/kg of BW/day in the high-cholesterol diet group, ALP activity significantly decreased from 33.64 to 26.83 U/mL, which represents a decrease of 32.24% compared to the NC group. These results indicate that ALT, AST, and ALP activities were strongly affected by polysaccharide, and that polysaccharide is useful for prevention and treatment of damage to liver cells in rats fed a high-cholesterol diet.

6. Effect of SBS Polysaccharide on Antioxidant Activities and GSH and MDA Content

SOD, CAT, and GSH-Px constitute the antioxidant enzyme sys-

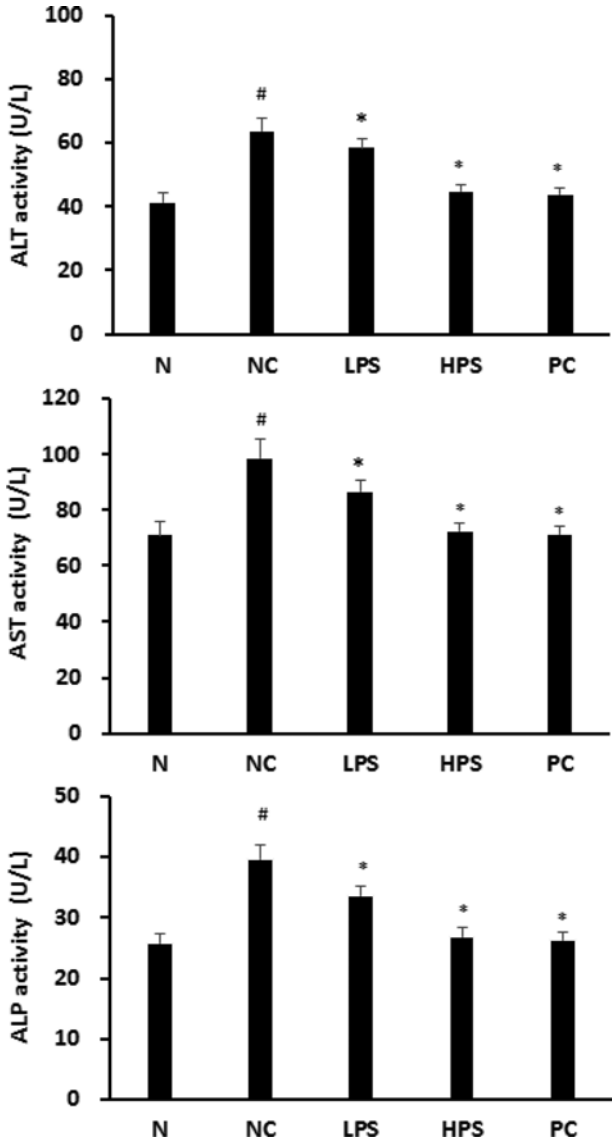

Fig. 5. Effect of SBS polysaccharide on the activities of ALT, AST and ALP in rats. The results are expressed as mean±SEM (n=8). $^{\#}p<0.05$ compared with N group, $^{*}p<0.05$ compared with NC group (ANOVA followed by Duncan's multiple comparison test).

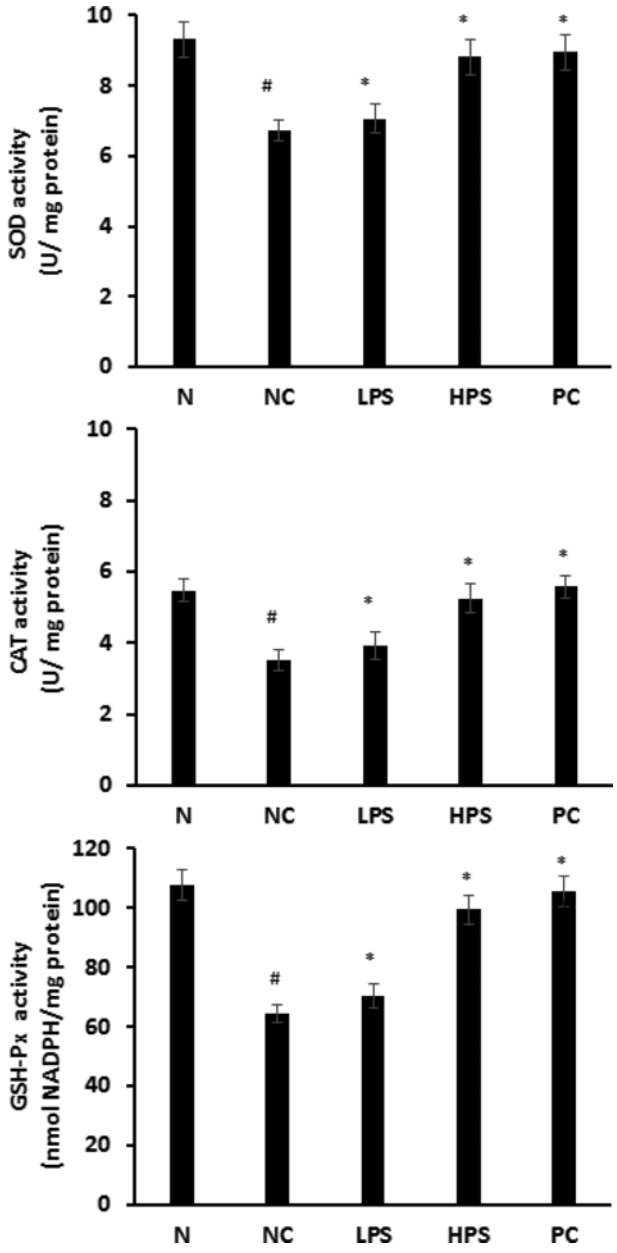

Fig. 6. Effect of SBS polysaccharide on SOD, CAT, and GSH-Px activities. The results are expressed as mean±SEM (n=8). $^{\#}p<0.05$ compared with N group, $^{*}p<0.05$ compared with NC group (ANOVA followed by Duncan's multiple comparison test).

tem in the human body. These proteins act in coordination to reduce the generation of active oxygen radicals, and prevent lipid peroxidation and intermediate products of metabolism from causing harm [37,38]. Oxidative stress, a disturbance in the balance between oxidative and antioxidative processes, plays an important role in the pathogenesis of atherosclerosis. A high-cholesterol diet results in increased lipid peroxidation by free radicals, followed by hypercholesterolemia, a major risk factor for atherosclerosis. During metabolism of dietary cholesterol, it is delivered to hepatocytes, where a substantial amount of reactive oxygen species is generated [39,40]. Therefore, antioxidant enzymes perform a valuable function. However, blood is the primary substance affected by oxidative stress when cholesterol is ingested in excess and assaying blood biochemical markers is of great clinical importance in the diagnosis of organ malfunction. Normally, erythrocytes and the liver contain sufficient scavengers such as SOD, CAT, and GSH-Px to protect against free radical injury. SOD plays a key role in protecting cells from oxidative damage by converting superoxide radicals into hydrogen peroxide, which is then further metabolized by CAT and GSH-Px. Therefore, the combined activities of SOD and CAT or GSH-Px may be necessary to reduce oxidative stress [41]. Thus, in this study, SOD, CAT, GSH-Px, GSH, and malondialdehyde (MDA) were selected to determine antioxidant effects *in vivo*. To investigate the effects of polysaccharides on the antioxidant activity in HCS in hypercholesterolemic rats, SOD, CAT, and GSH-Px activi-

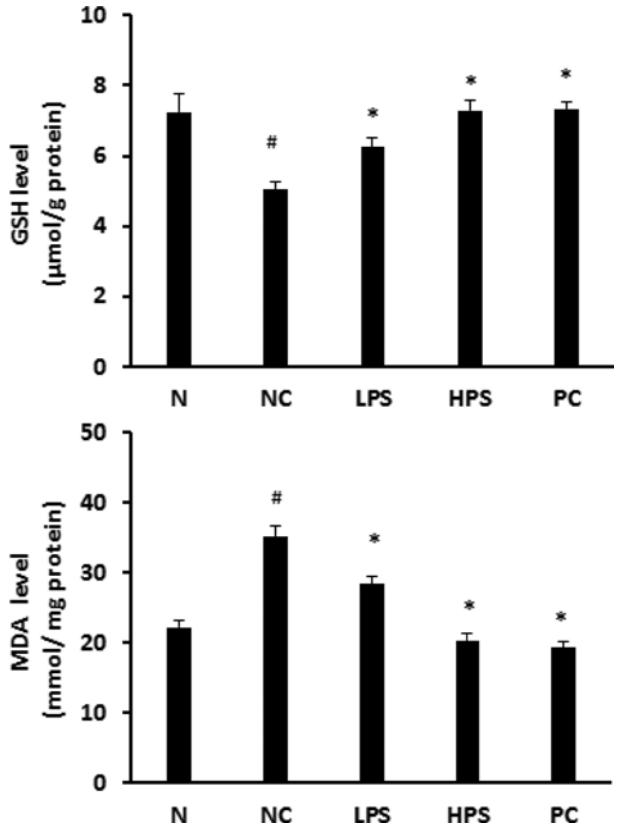

Fig. 7. Effect of SBS polysaccharide on GSH and MDA levels. The results are expressed as mean±SEM (n=8). $^{\#}p<0.05$ compared with N group, $^{*}p<0.05$ compared with NC group (ANOVA followed by Duncan's multiple comparison test).

ties and GSH and MDA content were measured. The results are shown in Figs. 6 and 7. Antioxidant activity was significantly higher in the HPS group than in the NC group. In particular, SOD, CAT, and GSH-Px activities in the NC group were decreased to 6.73, 3.51 U/mg protein, and 64.34 nmol NADPH/mg protein, respectively. These decreases compared to the N group may be related to the ability to scavenge singlet oxygen, superoxide anions, peroxyl radicals, and hydroxyl radicals in hypercholesterolemic rats. On the other hand, SOD, CAT, and GSH-Px activities were increased to 8.82 U/mg protein, 5.24 U/mg protein, and 99.48 nmol NADPH/mg protein in the HPS group, respectively, which was about 98.44, 94.07, and 94.16% of the levels observed in the PC group. In addition, antioxidant activities were significantly higher in the HPS group than in the LPS group. Thus, different doses yielded different effects. Moreover, the antioxidant activities in the group that was orally administered polysaccharide were found to increase in a dose-dependent manner.

Glutathione (GSH), which is widely distributed in most living cells, is a principal antioxidant and low-molecular weight non-protein thiol compound, and plays an important role in maintaining the intracellular thiol redox state. GSH is capable of preventing damage to important cellular components caused by reactive oxygen species such as free radicals, peroxides, lipid peroxides, and heavy metals [42]. GSH production decreased in the NC group, but in response to polysaccharide administration (200 mg/kg body), it was found to be approximately 1.4-fold higher than in the NC group. This result indicated that the antihypercholesterolemic effect of polysaccharides could be related to their antioxidative stress effect. Obesity, a globally important chronic disorder, is related to diverse factors and hence, there is a need to assess the hypoglycemic/antihypercholesterolemic effect and therapeutic potential of the polysaccharides. Lipid peroxides are products of chemical damage to the lipid components of cell membranes caused by oxygen free radicals. This oxidative damage is thought to be a basic mechanism underlying many diverse pathological conditions. Therefore, high lipid peroxide levels permit pathological effects of oxygen free radicals, the risk for degenerative processes, and the need for compensatory antioxidant supplementation. High lipid peroxide levels indicate excessive oxygen free radical lipid peroxidation [43]. Kwon et al. [44] reported that when lipid peroxide was introduced orally or intra-abdominally, the malondialdehyde (MDA) level increased significantly. In this study, it was increased to 35.26 mmol/mg protein in the NC group compared to the N group. However, the MDA level in the HP group was 20.24 mmol/mg protein, lower than in the NC group. These results indicate that SBS polysaccharide could preserve the activity of antioxidant enzymes in high-lipid organs, ameliorate metabolic disturbance of free radicals, maintain the dynamic balance between the oxidation and antioxidant systems, reduce the toxic side-effects of free radicals, and reduce lipid peroxidation damage to the cardiovascular system.

To conclude, our data suggest that polysaccharides isolated from SBS have significant health benefits for modulation of physiological functions, including various atherogenic lipid profiles and antioxidants in hypercholesterolemia. Therefore, it shows potential for use as a functional food, where it may act as a prophylactic against hypercholesterolemia and provide health benefits in terms of counteracting hyperlipidemia and its related complications; the antioxidant activity of the polysaccharides is likely one of the mechanisms underlying their hypolipidemic activities. However, further studies should be conducted to elucidate the exact mechanism underlying the lipid-lowering and antioxidant effects of SBS polysaccharide and the possible hypolipidemic and antioxidant compounds contained in polysaccharide isolated from SBS.

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