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# Diet of Red Ginseng-*Cheonggukjang* Improves Streptozotocin-induced Diabetes Symptoms and Oxidative Stress

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**Abstract** Protective effects of *cheonggukjang* fermented with 20% red ginseng (RC) were observed in streptozotocin (STZ)-induced diabetic rats by measuring levels of blood glucose, serum lipid profiles, and hepatic reactive oxygen species generating and scavenging enzyme activities. RC diet was prepared by mixing with AIN-76 diet at the final concentration 2%, and it was fed to STZ-induced diabetic rats for 3 weeks. The RC diet was significantly improved body weight, feed efficiency ratio, levels of serum glucose, and serum and hepatic lipids in diabetes. The significantly elevated O type activity of xanthine oxidase in diabetes was also greatly decreased by the RC diet. The treatment of RC showed the improved hepatic glutathione S-transferase activities in the diabetic animals. The present study indicates that *cheonggukjang* fermented with red ginseng could ameliorate STZ-induced diabetic symptoms such as aggravated blood glucose levels, serum lipid profiles, and even the conditions of oxidative stress.

**Key words:** red ginseng, *cheonggukjang*, anti-diabetes

## Introduction

The incidence of diabetes in Korean has been increased rapidly in the past 10 years and it became the 4th leading cause of death (1). In diabetes patients, the metabolism of carbohydrate, protein, and lipid due to the imbalance of hormones is abnormal and thus hyperglycemia and hyperlipidemia are developed. Ultimately, it causes complications including cardiac circulatory diseases, peripheral nerves impairment, and renal damages etc (2,3). Generally, diet control, exercise, and/or drug therapy are recommended in combination as treatment for diabetes. The purposes of the treatments are to maintain normal blood glucose levels, so the development of diabetic complications is to prevent or delay (4,5). However, therapeutic modes for the complete normalization of diabetes have not been established so far. Therefore, continuous studies on food and drugs that could improve hyperglycemia and hyperlipidemia are required. It has been reported that serum cholesterol and triglyceride levels are lower in bean protein treated animals than in casein (6). And there are the reductions of the blood glucagons/insulin ratio or the increase of the blood levels of thyroxine by bean protein (7). Recently, it has been reported that isoflavone and the hydrolyzed low molecular peptides derived from soybean are effective in lowering blood pressure and thromboylysis (8). In addition, soybeans are consumed as various forms in Korea. Particularly, *cheonggukjang* is a food fermented for short term in comparison with the other soybean fermented foods, and highly nutritious food containing a high level of essential amino acids that are easily absorbed (9,10). Therefore, during the fermentation process of *cheonggukjang*, proteins are hydrolyzed to low molecule peptides and isoflavones (8) could be produced by microorganisms, so new biological functions are anticipated.

Ginseng, a Korean traditional galenical, has been evaluated to be good health food or medicine because of its pharmacological actions such as a nutritive tonic, anticancer, and antioxidative action, etc (11,12). It have been reported that ginseng has improved the blood circulation via dilation of blood vessels and suppressed the development of arteriosclerosis owing to protection of vascular endothelial cell damages (13). Futhermore, ginseng saponins have been reported to stimulate the lipid metabolism and reduce the production of cholesterol in tissue and serum, to prevent the formation of atheroma induced by hyperlipidemia (14,15). It has been known that maltol (2-methyl-3,3-hydroxypyrone) is a specific substance generated during preparing of red ginseng (16). The antioxidative effects of red ginseng in aging-accelerated mice are resulted from the activation of antioxidative enzymes (17,18).

*Cheonggukjang* fermented by *Bacillus* species that have a superior capacity to degrade carbohydrate and protein. In preliminary studies during fermentation together with red ginseng, acceleration of the degradation of cell wall polysaccharides in red ginseng and soybean by stimulating bacterial growth has been observed, and the effect of new ginsenosides derived from red ginseng-*cheonggukjang* are anticipated (11). Therefore, we studied the effect of red ginseng-*cheonggukjang* on serum blood glucose, lipid profiles, and hepatic reactive oxygen species generating and scavenging enzymes activities in STZ-induced diabetic rats.

## Materials and Methods

**Materials** Soybeans (*Glycine* max Enha cultivar) were purchased from the Agricultural Cooperative Federation

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(Daegu, Korea) and stored at 4°C until used. Red ginseng powder (100 mesh) from 6 years old was purchased from the Geumsan Agricultural Cooperative Federation (Kumsan, Chungnam, Korea).

**Preparation of *cheonggukjang* and red ginseng-*cheonggukjang*** Red ginseng-*cheonggukjang* was prepared as follow; Soybean (800 g) were washed with water, drained, soaked in water for 8 hr, and autoclaved at 121°C for 1 hr. Red ginseng powder (200 g) was mixed with the autoclaved soybeans and the seed culture of *Bacillus licheniformis* isolated from traditional *cheonggukjang* was inoculated at 2% concentration. The mixture was fermented at 40°C for 48 hr, freeze-dried, and then ground to obtain 100 mesh powder. General *cheonggukjang* was prepared as the same as the red ginseng-*cheonggukjang* without red ginseng.

**Animals, experimental groups, and induction of diabetes** Male Sprague-Dawley rats were purchased from Oriental Co., Ltd. (Busan, Korea). The rats were fed a standard rodent pellet chow (Purina Co., Seoul, Korea) and acclimatized to their environment for 1 week before commencement of the experiments. Eight groups of male Sprague-Dawley rats weighing 195±5 g (10 animals/group) were fed for 3 weeks with the following: nondiabetic control (NC); STZ-treated diabetic (DM); 0.16% *cheonggukjang*-treated (CH); 0.40% red ginseng-treated (RG); 2.00% red ginseng-*cheonggukjang* (*cheonggukjang* fermented with 20% red ginseng)-treated (RC); diabetic CH (DM-CH); diabetic RG (DM-RG); diabetic RC (DM-RC) (Table 2).

The experimental diets were prepared to contain 1.6-2.0% of the respective freeze dried powder in rodent AIN-76 basic diet. In order to make the final diets isocalories, the contents of protein, lipid, non-fibrous carbohydrate, fiber, and ash in the powders (CH, RG, or RC) were analyzed by AOAC method (19) and their contents (Table 1) except ash were subtracted from casein, corn oil, corn starch, and cellulose in the diets, respectively (Table 2).

The rats were individually housed in stainless steel wire bottom cages in a room maintained at 20±2°C and 60±5% relative humidity. The room was exposed to alternating 12-hr periods of light and dark. To confirm hyperglycemia, blood (12 hr after feed withdrawal) was sampled from the tail end, and plasma glucose level was determined using Smart ScanTM (Inc 2000, IFD/KR/HK/TW/OB/SMT; Lifescan, Milpitas, CA, USA) on the 3rd day after STZ (Sigma-Aldrich Chemical Co., St. Louis, MO, USA) intramuscular injection (55 mg/kg BW in 0.1 M citrate buffer, pH 4.3). The control rats received an isovolumetric dose of citrate buffer as a vehicle. Rats with above 300 mg/dL plasma glucose concentration were used. All rats were allowed free access to diets and drinking water. The experimental protocols were conducted in accordance with internationally accepted principles for laboratory animal use and care as found in Korea Food and Drug Administration (KFDA) guidelines.

**Preparation of samples** After 3 weeks on the experimental diets, rats were fasted for 24 hr, and blood was collected from the abdominal aorta under anesthetized with ether. The liver was exhaustively perfused with cold physiological saline solution through the portal vein and quickly removed. The liver was homogenized with 4°C volumes of 0.25 M sucrose, centrifuged at 1,000×g for 10 min and the supernatant was recentrifuged at 10,000×g for 20 min. The pellet was resuspended with 0.25 M sucrose to use as

**Table 1. General components of freeze-dried *cheonggukjang* (CH), red ginseng (RG), and red ginseng-*cheonggukjang* (RC)**

%(w/w)

| General components | CH | RG | RC |
|---|---|---|---|
| Protein | 36.66±1.89 | 6.95±0.20 | 30.37±0.72 |
| Lipid | 21.18±0.53 | 0.72±0.01 | 16.64±0.39 |
| Carbohydrate | 26.12±0.62 | 71.94±1.79 | 36.02±0.93 |
| Cellulose | 11.08±0.23 | 19.99±0.43 | 12.95±0.34 |
| Ash | 4.96±0.09 | 0.40±0.01 | 4.02±0.09 |

**Table 2. Compositions of experimental diets**

(g/kg diet)

| Ingredients | Normal[1)] | | | | Diabetes [2)] | | | |
|---|---|---|---|---|---|---|---|---|
| | NC | CH | RG | RC | DM | DM-CH | DM-RG | DM-RC |
| Casein | 200 | 194.13 | 199.72 | 193.93 | 200 | 194.13 | 199.72 | 193.93 |
| Corn starch | 150 | 145.82 | 147.12 | 142.80 | 150 | 145.82 | 147.12 | 142.80 |
| Sucrose | 500 | 500 | 500 | 500 | 500 | 500 | 500 | 500 |
| Cellulose | 50 | 48.23 | 49.20 | 47.41 | 50 | 48.23 | 49.20 | 47.41 |
| Corn oil | 50 | 46.61 | 49.97 | 46.67 | 50 | 46.61 | 49.97 | 46.67 |
| AIN mineral mixture[3)] | 35 | 35 | 35 | 35 | 35 | 35 | 35 | 35 |
| AIN vitamin mixture[4)] | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| DL-Methionine | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 |
| Choline bitartarate | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 |
| *Cheonggukjang* | - | 16 | - | - | - | 16 | - | - |
| Red ginseng | - | - | 4 | - | - | - | 4 | - |
| Red ginseng- *cheonggukjang* | - | - | - | 20 | - | - | - | 20 |

[1)]NC, normal control; CH, *cheonggukjang* fed-group; RG, red ginseng fed-group; RC, red ginseng *cheonggukjang* fed-group.
[2)]DM, STZ-induced diabetes control group; DM-CH, diabetes-CH fed-group; DM-RG, diabetes-RG fed-group; DM-RC, diabetes-RC fed group.
[3,4)]AIN mineral and vitamin mixture was prepared according to AIN-76 (Harlan Teklad, Madison, WI, USA), respectively.

**Table 3. Effect of red ginseng-*cheonggukjang* on the weight gain, food and water intakes, feed efficiency ratio (FER), and urine and feces amounts of STZ-induced diabetic rats fed for 3 weeks**

| Groups[1)] | Weight gain (g/day) | Feed intakes (g/day) | FER[2)] | Water intakes (mL/100 g BW) | Urine (mL/100 g BW) | Feces (g/100 g BW) |
|---|---|---|---|---|---|---|
| NC | 6.0±0.6$^{b,3)}$ | 27.0±0.8$^{ab}$ | 0.22±0.08$^{b}$ | 10.1±0.4$^{c}$ | 2.6±0.3$^{b}$ | 2.5±0.3$^{b}$ |
| CH | 8.2±0.7$^{a}$ | 25.8±0.7$^{b}$ | 0.32±0.08$^{ab}$ | 10.7±0.5$^{c}$ | 2.2±0.3$^{b}$ | 2.4±0.3$^{b}$ |
| RG | 9.2±0.6$^{a}$ | 28.2±0.7$^{a}$ | 0.33±0.02$^{a}$ | 11.0±0.6$^{c}$ | 2.5±0.2$^{b}$ | 2.5±0.3$^{b}$ |
| RC | 6.5±0.8$^{b}$ | 27.2±0.6$^{ab}$ | 0.24±0.08$^{ab}$ | 10.6±0.5$^{c}$ | 2.5±0.2$^{b}$ | 2.7±0.2$^{b}$ |
| DM | -0.9±0.1$^{c}$ | 28.0±1.4$^{a}$ | -0.03±0.02$^{c}$ | 69.8±6.9$^{a}$ | 15.3±0.6$^{a}$ | 3.5±0.2$^{a}$ |
| DM-CH | -1.6±0.2$^{d}$ | 28.0±1.5$^{a}$ | -0.06±0.03$^{c}$ | 68.3±7.3$^{a}$ | 15.6±0.5$^{a}$ | 3.7±0.2$^{a}$ |
| DM-RG | -0.7±0.1$^{c}$ | 28.0±1.6$^{a}$ | -0.03±0.01$^{c}$ | 58.9±8.5$^{a}$ | 16.6±0.7$^{a}$ | 3.5±0.2$^{a}$ |
| DM-RC | 5.2±0.8$^{b}$ | 26.0±1.1$^{b}$ | 0.20±0.05$^{b}$ | 33.7±7.4$^{b}$ | 6.5±0.2$^{c}$ | 2.9±0.2$^{b}$ |

[1)]NC, normal control; CH, *cheonggukjang* fed-group; RG, red ginseng fed-group; RC, red ginseng *cheonggukjang* fed-group; DM, STZ-induced diabetes control group; DM-CH, diabetes-CH fed-group; DM-RG, diabetes-RG fed-group; DM-RC, diabetes-RC fed group.
[2)]Feed efficiency ratio = weight gain/feed intakes.
[3)]Values are means±SD of 10 rats; different superscripts within a column (a-c) indicate significant differences ($p<0.05$).

mitochondrial fraction, and the supernatant was used as postmitochondrial fraction. The collected blood was centrifuged at 2,000×g for 10 min at room temperature and the serum separated was kept frozen at −70°C. Urine and fecal were collected, and their volume and weight were measured everyday.

**Measurement of the biochemical parameters** Body weight, feed, and water intakes were measured everyday. Feed efficiency ratio (FER) was calculated by dividing the weight gains into feed intakes by a day. Content of triglyceride, total cholesterol, and high-density lipoprotein (HDL)-cholesterol in serum were measured by using kit reagents (AM 157S-K; Asanpharm Co., Seoul, Korea). And the content of low-density lipoprotein (LDL)-cholesterol was calculated by method of Friedewald *et al.* (20). Serum insulin level was measured by radioimmuno assay using Insulin-IRMA (Biosource Co. San Diego, CA, USA).

Activities of xanthine oxidase (XOD) and glutathione S-transferase (GST) were determined by a method of Stirpe and Della Corte (21) and Habig *et al.* (22), respectively. Hepatic glutathione (GSH) content was determined by the method of Ellman (23). Lipid peroxide (LPO) content was determined by a method of Satho (24). Protein content was determined by a Lowry *et al.* (25) method with bovine serum albumin as a standard.

**Histological evaluation** Small pieces of pancreas taken from experimental animals were fixed in 10% neutral formalin, and then alcohol dehydration, paraffin-embedded and sectioned to a mean thickness of 4 μm. The sections were stained with hematoxylin-eosin (HE), and observed under a light microscope (26).

**Statistical analysis** The results were expressed as mean ±standard deviation (SD) of the 10 animals. Statistical comparison of differences between the different groups was carried out 2-way ANOVA test followed by Duncan's multiple range test using SPSS statistical software package (Version 12.0, SPSS Inc., Chicago, IL, USA).

## Results and Discussion

**Weight gain, feed and water intakes, FER, and urine and feces amounts** After the induction of diabetes, animals were fed with experimental diet for 3 weeks, and subsequently, body weight gain, the amount of feed intakes, and the FER were examined (Table 3).

Among the normal groups, the weight gain of CH and RG group was relatively higher than those of NC and RC group. Among the diabetes groups, DM-RC group was markedly higher in comparison with other diabetes groups. This result implicated that the weight reduction due to the development of diabetes was recovered by RC diet (27).

While, amount of feed intakes among all experimental groups were not significantly different, nonetheless, the DM-RC group showed a trend to be low. The amount of water intakes in the normal groups were not significant, on the other hand, among the STZ-induced diabetes groups, the DM-RC group showed significantly lower than those of the other diabetes groups. Similarly, the amounts of urine and feces in the DM-RC group were lower than other diabetes groups.

Park *et al.* (28) have reported that water intakes in STZ-induced diabetes rats fed red ginseng was reduced by approximately 29% in comparison with the diabetes control group. Nevertheless, in this study, such substantial difference was not detected. Our results suggest that alone *cheonggukjang* or red ginseng diet has no effect or a poor effect on the polyphagia and polyuria in diabetes (29).

**Levels of blood sugar and serum insulin** After the induction of diabetes, the change in blood sugar level is shown in Fig. 1. The control groups (NC, CH, RG, RC) were 121.7-150.3 mg/dL, and a significant difference was not shown. On the other hand, diabetes groups except DM-RC group were powerfully higher than those of NC group. However, serum glucose level in DM-RC group (171.3 mg/dL) was nearly recovery to NC group. This result implicated that red ginseng-*cheonggukjang* decreases blood sugar in diabetes animals, it may be due to the newly produced ginsenosides and isoflavones by the mixed

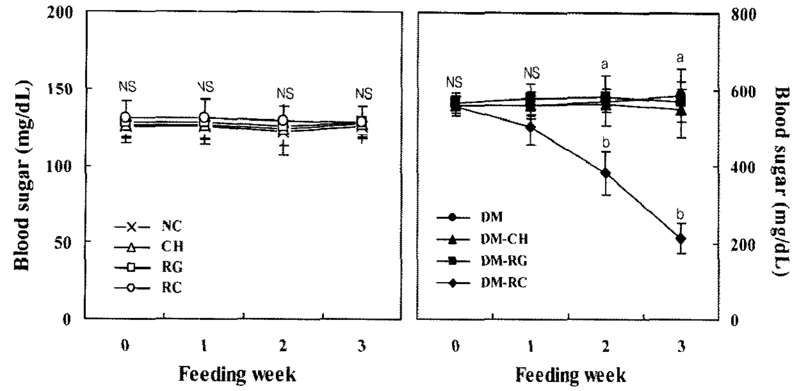


**Fig. 1. Changes in levels of blood sugar of STZ-induced diabetic rats during feeding of red ginseng-*cheonggukjang*.** NC, normal control; CH, *cheonggukjang* fed-group; RG, red ginseng fed-group; RC, red ginseng *cheonggukjang* fed-group; DM, STZ-induced diabetes control group; DM-CH, diabetes-CH fed-group; DM-RG, diabetes-RG fed-group; DM-RC, diabetes-RC fed group. NS, not significant. Values are means±SD of 10 rats; different superscripts (a-b) indicate significant differences ($p$<0.05).

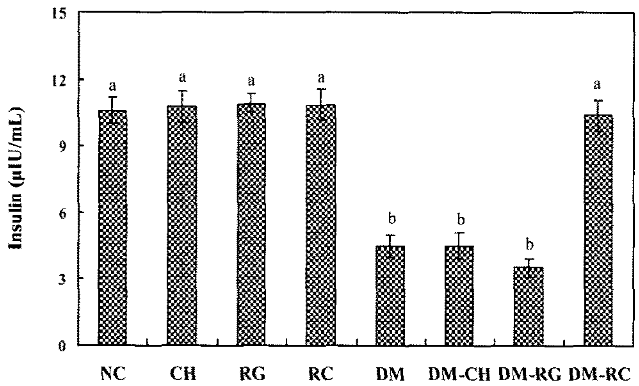


**Fig. 2. Levels of serum insulin of STZ-induced diabetic rats fed with red ginseng-*cheonggukjang* for 3 weeks.** NC, normal control; CH, *cheonggukjang* fed-group; RG, red ginseng fed-group; RC, red ginseng *cheonggukjang* fed-group; DM, STZ-induced diabetes control group; DM-CH, diabetes-CH fed-group; DM-RG, diabetes-RG fed-group; DM-RC, diabetes-RC fed group. Values are means±SD of 10 rats; different superscripts on the same bars indicate significant differences ($p$<0.05).

fermentation of *cheonggukjang* and red ginseng (11).

Figure 2 showed the levels of serum insulin in normal and experimental groups. Serum insulin levels were significantly decreased in all STZ-treated groups except DM-RC group. However, the decreased insulin levels by the treatment of STZ were nearly recovered to the NC group by the supplementation of the red ginseng-*cheonggukjang*. These results suggest that decreasing of the blood sugar and increasing of the insulin may provide strong evidence for antihyperglycemic effects of the red ginseng-*cheonggukjang* due to prevention from pancreatic damage by STZ. As above mentioned, these effects of red ginseng-*cheonggukjang* on STZ-induced DM may be due to increasing of insulin secretion via alleviation of pancreatic β-cell damages by the newly produced ginsenosides and isoflavones from mixed fermentation process. However, in this experiment, although there was not significant effect in the *cheonggukjang* or red ginseng supplemented diet group, it has been reported that the *cheonggukjang* or red ginseng contains bioflavonoids and gensenosides which have been proved to be effective on hyperlipidemia, hyperglycemia, and oxidative stress (14,15). We considered that these discrepancies between our results and the others (30,31) may be due to differences of experimental sample preparations, administration route, doses, animal species, and/or experimental durations etc.

**Level of serum lipids** After the induction of diabetes, the levels of serum triglyceride, total cholesterol, HDL-, and LDL-cholesterol are shown in Table 4. The triglyceride of normal groups was not significant difference. However, the serum triglyceride in DM-CH, DM-RG, and DM-RC groups were lower than that of DM group. Especially, the triglyceride of the DM-RC group (112.1 mg/dL) was strikingly lower than that of the DM group (153.1 mg/dL). And serum total cholesterol in normal groups (CH, RG, and RC) showed a slightly lower than that of the NC group, and a significant difference among them was not detected. All STZ-induced diabetes groups except DM-RC group were not significantly lower than that of these groups. HDL-cholesterol content in normal groups did not show a significant difference, but diabetes groups were slightly lower than that of each control group. On the other hand, LDL-cholesterol content in CH, RG, and RC groups was lower than that of NC group. But serum LDL-cholesterol levels in diabetes groups were higher than those of normal groups. And LDL-cholesterol in DM-CH, DM-RG, and DM-RC groups were slightly lower than that of DM group.

It is well documented that high levels of serum TG, total cholesterol, and LDL-cholesterol and low level of HDL-cholesterol by hyperglycemia may contribute to the development of cardiovascular disease in diabetes (32,33). Therefore, above results indicate that the red ginseng-*cheonggukjang* showed the effect of the improvement of serum triglyceride and LDL-cholesterol due to increasing of insulin secretion by the synergistic effect of red ginseng (34) and *cheonggukjang* (35). Although the action mechanisms of hypoglycemic and hypolipidemic effects of the red ginseng-*cheonggukjang* are unknown in this study, these results suggest that red ginseng-*cheonggukjang* may regulate cardiovascular diseases caused by diabetic abnormal glucose and lipid metabolism, and its transport

**Table 4. Effect of red ginseng-*cheonggukjang* on the levels of triglyceride (TG), total cholesterol, HDL-cholesterol, and LDL-cholesterol in serum of STZ-induced diabetic rats fed for 3 weeks** (mg/dL)

| Groups[1)] | Triglyceride | Total cholesterol | HDL-cholesterol | LDL-cholesterol[2)] |
|---|---|---|---|---|
| NC | 74.9±11.7[d,3)] | 116.9±14.1[b] | 55.4±7.9[abc] | 46.5±8.7[d] |
| CH | 79.7±10.0[d] | 104.7±21.0[c] | 61.1±5.7[ab] | 27.7±8.9[e] |
| RG | 69.3±9.0[d] | 109.4±16.4[c] | 65.2±8.8[a] | 30.3±3.4[e] |
| RC | 74.0±12.1[d] | 106.1±19.7[c] | 62.3±6.3[a] | 29.0±5.6[e] |
| DM | 153.1±7.3[a] | 174.4±15.4[a] | 47.4±6.0[c] | 96.4±10.7[a] |
| DM-CH | 127.6±7.2[b] | 151.4±5.6[a] | 52.3±3.2[bc] | 73.6±5.1[bc] |
| DM-RG | 135.6±6.0[b] | 157.5±5.5[a] | 51.9±4.0[bc] | 78.5±8.4[ab] |
| DM-RC | 112.1±5.9[c] | 136.4±6.1[b] | 54.2±3.6[abc] | 59.8±8.8[cd] |

[1)]NC, normal control; CH, *cheonggukjang* fed-group; RG, red ginseng fed-group; RC, red ginseng *cheonggukjang* fed-group; DM, STZ-induced diabetes control group; DM-CH, diabetes-CH fed-group; DM-RG, diabetes-RG fed-group; DM-RC, diabetes-RC fed group.
[2)]LDL-cholesterol=Total cholesterol−HDL-cholesterol−(TG/5).
[3)]Values are means±SD of 10 rats; different superscripts within a column (a-e) indicate significant differences ($p<0.05$).

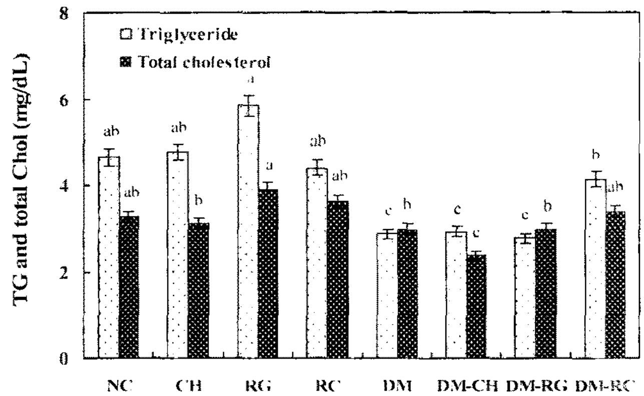


**Fig. 3. Levels of hepatic triglyceride (TG) and total cholesterol (Chol) of STZ-induced diabetic rats fed for 3 weeks.** NC, normal control; CH, *cheonggukjang* fed-group; RG, red ginseng fed-group; RC, red ginseng *cheonggukjang* fed-group; DM, STZ-induced diabetes control group; DM-CH, diabetes-CH fed-group; DM-RG, diabetes-RG fed-group; DM-RC, diabetes-RC fed group. Values are means±SD of 10 rats; different superscripts (a-c) indicate significant differences ($p<0.05$).

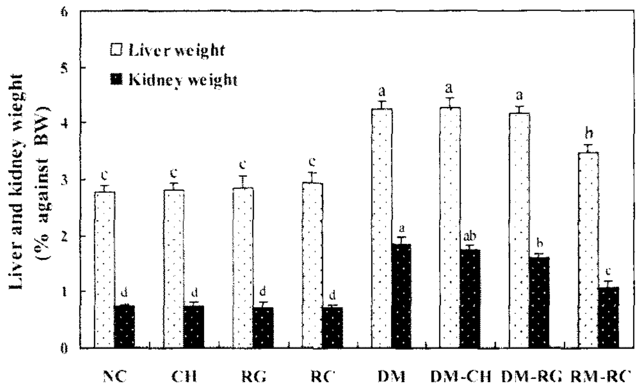


**Fig. 4. Effect of red ginseng-*cheonggukjang* on liver and kidney weight per body weight of STZ-induced diabetic rats fed for 3 weeks.** NC, normal control; CH, *cheonggukjang* fed-group; RG, red ginseng fed-group; RC, red ginseng *cheonggukjang* fed-group; DM, STZ-induced diabetes control group; DM-CH, diabetes-CH fed-group; DM-RG, diabetes-RG fed-group; DM-RC, diabetes-RC fed group. Values are means±SD of 10 rats; different superscripts (a-d) indicate significant differences ($p<0.05$).

via control of serum insulin level by the newly formed constituents from mixed fermentation of *cheonggukjang* and ginseng.

**Level of hepatic lipids** The result of hepatic triglyceride and total cholesterol levels in rats after the induction of diabetes is shown in Fig. 3. The levels of triglyceride in hepatic tissues of the normal groups were not significant difference. All STZ-induced diabetes groups except DM-RC group were significantly lower than those of the normal groups. However, the DM-RC group was nearly recovery to NC group. And hepatic total cholesterol in all other experimental groups except DM-CH group was not significantly different.

It has been reported that the hepatic triglyceride and total cholesterol content in diabetes rats was lower than that of the normal control group (36,37). In present experiments, a similar result was observed, which as explained due to the increase of consumption of lipid instead of glucose by diabetes. However, Koh (38) has reported that the soybean diet significantly decreased hepatic triglyceride and total cholesterol in diabetes rats, which is contradictory to our results

**Organ weight** The liver and kidney weight per body weight of experimental animals after STZ treatment is shown in Fig. 4. All diabetes groups showed significantly higher levels than the normal groups. However, the DM-RC group showed significantly lower value than the other diabetes groups. Generally, the liver and the kidney in diabetes rats induced by STZ are enlarged due to abnormal glucose metabolism and the accumulation of lipid caused by the reduction of insulin (39,40). The results that decreased weight of organs in diabetes rats by supplementation of RC diet were considered to be an amelioration of abnormal metabolism of glucose and lipid in diabetes.

**Levels of hepatic GSH and LPO, and activities of XOD and GST** The levels of hepatic GSH and LPO, and activities of XOD and GST in the experimental rats after induction of diabetes by STZ are shown in Table 3. The mean levels of the hepatic GSH and LPO of all STZ-

**Table 5. Effect of red ginseng-*cheonggukjang* on the levels of hepatic GSH and LPO, and activities of XOD and GST in rats fed for 3 weeks[1)]**

| Groups[2)] | GSH | LPO | XOD | | | GST |
|---|---|---|---|---|---|---|
| | | | Total (T) | O type (O) | O/T (%) | |
| NC | 4.01±0.17[b,3)] | 10.92±0.49[b] | 4.59±0.47[NS] | 1.12±0.79[b] | 39.22±2.77[b] | 379.66±40.39[a] |
| CH | 4.34±0.31[b] | 10.37±0.99[bc] | 4.44±0.45 | 1.95±0.75[ab] | 43.33±12.96[b] | 271.34±43.10[bc] |
| RG | 4.26±0.58[b] | 10.44±0.65[bc] | 4.42±0.77 | 2.02±0.42[ab] | 43.25±6.08[bc] | 351.02±63.43[ab] |
| RC | 3.94±0.10[b] | 9.43±0.72[c] | 4.99±0.74 | 1.93±0.29[b] | 40.67±5.29[bc] | 379.08±79.72[a] |
| DM | 4.49±0.89[ab] | 12.49±2.07[ab] | 4.34±1.02 | 3.11±0.84[a] | 71.26±6.06[a] | 314.11±74.00[ab] |
| DM-CH | 4.91±0.21[a] | 11.11±1.08[b] | 4.39±0.58 | 1.67±0.16[b] | 38.25±1.30[b] | 202.93±26.93[c] |
| DM-RG | 4.93±0.91[ab] | 13.46±0.19[a] | 4.83±1.02 | 1.97±0.64[ab] | 40.07±5.83[bc] | 299.87±59.72[ab] |
| DM-RC | 4.20±0.30[b] | 10.93±0.25[b] | 4.60±0.55 | 1.52±0.30[b] | 35.64±0.47[c] | 362.35±41.13[a] |

[1)]GSH, glutathione (μmoles/g of tissue); LPO, lipid peroxide (MDA nmoles/g of tissue); XOD, xanthine oxidase (uric acid nmoles/mg protein/min); GST, glutathione S-transferase (dinitrophenol-glutathione conjugate nmoles/mg protein/min).
[2)]NC, normal control; CH, *cheonggukjang* fed-group; RG, red ginseng fed-group; RC, red ginseng *cheonggukjang* fed-group; DM, STZ-induced diabetes control group; DM-CH, diabetes-CH fed-group; DM-RG, diabetes-RG fed-group; DM-RC, diabetes-RC fed group.
[3)]Values are means±SD of 10 rats; different superscripts within a column (a-e) indicate significant differences ($p<0.05$).

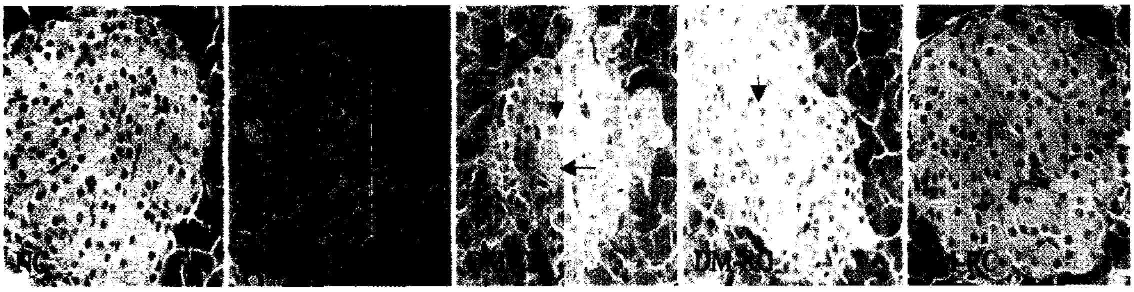

**Fig. 5. Photomicrographs of rat pancreas (HE stain, 400×).** NC, normal control; CH, *cheonggukjang* fed-group; RG, red ginseng fed-group; RC, red ginseng *cheonggukjang* fed-group; DM, STZ-induced diabetes control group; DM-CH, diabetes-CH fed-group; DM-RG, diabetes-RG fed-group; DM-RC, diabetes-RC fed group.

induced diabetic rats showed a trend to be slightly higher than those of each normal group.

While the total activity of XOD in all experimental groups were not significantly different, O type activity of XOD in DM group was powerfully higher than that of NC group, and in DM-CH, DM-RG, and DM-RC groups showed decreased activity. The O/T ratios (%) of the normal groups were 39.22-43.33%, and among the diabetes groups, the DM-RC group (35.64%) was lower than DM group (71.26%) and the other diabetes groups (38.25-40.07%).

GST activities of the NC, RG, and RC group were 351.02-379.66 units, and a significance was not detected, nevertheless, the CH group (271.34 unit) was significantly lower ($p<0.05$). Among the diabetes groups, the DM-CH group (202.93 unit) was low in comparison with other diabetes groups (299.87-314.11 units) ($p<0.05$), and the DM-RC group was recovered to the NC groups.

GSH has been known to be a substance that converts $H_2O_2$ together with glutathione peroxidase and catalase to $H_2O$ (41,42). LPO is generated by the increases of oxidative stress and reduces the antioxidants (43). And red ginseng-*cheonggukjang* did not mediate a significant effect on them, nonetheless, considering their mean value, it was estimated to mediate a substantial effect.

XOD is a non-specific enzyme involved in the metabolism of purine, pyrimidine, aldehydes, and heterocyclic compounds, and *in vivo*, it oxidizes primarily hypoxanthine via xanthine into uric acid.

It has been reported that XOD activity of diabetes rats is increased in hepatic tissue and plasma (44). In this study, we also observed increased XOD O type activity in DM group, but increased XOD O type activity by STZ treatment was inhibited by RC-diet.

Generally, in the case with low XOD O type activity show the generation decrease of reactive oxygen species (ROS) such as superoxide, hydroxyl radical, and $H_2O_2$ (44,45). And, GST is involved in the elimination of electrophilic toxic substances and organic hydroperoxides using GSH in hepatic tissues (46). Based on the above results, the red ginseng-*cheonggukjang* may have the hypoglycemic and hypolipidemic effects due to the increasing insulin secretion by their synergistic action. In addition, it suggests that the ROS and LPO were likely to be reduced by antioxidative components in the fermented red ginseng-*cheonggukjang* through inhibition of XOD type O activity and increasing of GST activity.

**Histopathological findings of pancreas** Figure 5 shows the light microscopic histopathological findings of pancreas in normal (NC) and experimental groups (DM, DM-CH, DM-RG, DM-RC). Islands around blood vessels were normal and not detected any damages in pancreas of NC animals. However, cells of peri-central regions in pancreas of DM, DM-CH, and DM-RG animals were found remarked swelling (←) and some necrotic cells (↓). On the other hand, STZ-induced pancreatic tissue damage could ameliorate by *cheonggukjang* or red ginseng supplemented diets (DM-CH or DM-RG). Especially, red ginseng-*cheonggukjang* supplemented diet fed animals (DM-RC) was shown to increase the numbers of β-cell. These results suggest that serum level of insulin and histopathological findings may provide strong evidence for cytoprotective effects of red ginseng-*cheonggukjang*.

Meanwhile, it is well established that insulin play a central role of normal transport and metabolism of carbohydrate (47), lipid (48), and protein (49,50), imbalance of insulin caused by pancreatic β-cell damages, and/or insulin resistance caused by various factors such as hyperlipidemia (51,52) and abnormal adipokinins (53,54) lead to hyperglycemia and diabetes. Furthermore, it is well documented that impairment of glucose and lipid metabolism in tissues due to insufficient insulin action via insulin resistance in cell membranes leads to metabolic syndrome such as obesity, hyperlipidemia, atherosclerosis, and hypertension (55,56). Thus, it is very important to maintain the blood glucose and lipid levels within normal range by normal insulin action in the living organisms.

Therefore, these results support that red ginseng-*cheonggukjang* may modulate metabolic syndrome such as diabetes and hyperlipidemia via recovered secretion of insulin by alleviation of STZ-induced pancreatic β-cell damage.

In conclusion, although the precise action mechanisms of the hypoglycemic and hypolipidemic effects of the experimental diets in this paper are unknown, our study provides experimental evidence that red ginseng-*cheonggukjang* may regulate hyperglycemia via increasing of insulin secretion due to recovery and/or prevention from STZ-induced pancreatic β-cell damage by a newly formed some antioxidative constituents such as ginseosides and isoflavones from mixed fermentation *cheonggukjang* and red ginseng. But further study is needed in this field.

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