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Soybean Oligosaccharide Reduces Oxidative Stress in Streptozotocin-injected Rats

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This study was conducted to investigate the effect of oligosaccharide on the reduction of oxidative stress. Sprague- Dawley rats were fed an AIN-93G diet or a diet containing 5% soybean oligosaccharide for 6 weeks. Each group was divided into two sub-groups after streptozotocin (STZ) injection and fed the control diet or the diet containing oligosaccharide for the next 12 days. The number of fecal bifidobacteria increased significantly in groups fed oligosaccharide diet. Elevated blood glucose concentration after STZ injection declined faster in the oligosaccharide fed group. Liver thiobarbituric acid reactive substance concentration, as an indicator of oxidative stress, did not increase in groups fed the oligosaccharide diet after the STZ injection. In addition, these groups had significantly higher glutathione peroxidase activity both in the plasma and the liver than groups fed the control diet. The results of this study suggest that soybean oligosaccharide has a beneficial effect in reducing oxidative stress in streptozotocin-injected rats.

Key words : soybean oligosaccharide, bifidobacteria, streptozotocin, oxidative stress, blood glucose, glutathione peroxidase

INTRODUCTION

Oligosaccharides are carbohydrates with a low degree of polymerization.[1)] They are readily soluble and exhibit some sweetness. These oligosaccharides include fructo-, isomalto-, soybean-, galacto-oligosaccharides and inulin.[2)] They also have been reported to be effective bifidus factors in the colon.[3-5)]

The soybean oligosaccharide, one of the biologically functional oligosaccharides, is found in soybean milk, soybean powder and cooked beans.[6)] The effective components of soybean oligosaccharide have been identified as stachyose (Gal-Gal-Glc-Fuc) and raffinose (Gal-Glc-Fuc).

Soybean oligosaccharide is indigestible in the small intestine, but is efficiently utilized by microflora in the large intestines of rats.[7)] It has beneficial effects on increasing fecal moisture and in improving the color of the feces. In a study with human volunteers, supplementation of soybean oligosaccharide increased the number of intestinal bifidobacteria at the expense of other putrefactive anaerobes.[8,9)]

Because of its indigestibility in the small intestine, oligosaccharide is considered as a kind of dietary fiber.[10,11)] The beneficial effects of dietary fiber on postprandial glucose response and on the fasting blood glucose level in diabetes mellitus patients are well known.[12,13)] However, whether soybean oligosaccharide has such a regulatory effect on blood glucose concentration has not been investigated.

Oxidative stress is defined as a disturbance in the balance between the production of reactive oxygen species and antioxidant defenses.[14)] Increased oxidative stress has been implicated in the pathogenesis of diabetes.[15-17)] A decreased efficiency of enzymatic antioxidant defenses seems to correlate with the severity of pathological tissue changes in diabetes.[18,19)]

This study was designed to investigate the effect of soybean oligosaccharide on a diminution of oxidative stress produced by streptozotocin injection. The effect of the oligosaccharide diet before and after streptozotocin injection was also compared. Malondialdehyde was used as a parameter of lipid peroxidation. Glutathione peroxidase, catalase and superoxide dismutase activities were analyzed as antioxidant enzyme parameters.

MATERIALS AND METHODS

Male Sprague-Dawley rats, weighing 180 to 200g, were housed in individual, suspended, stainless steel

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wire-mesh cages in a room with a controlled temperature (20 to 22℃). The animals were fed a commercial diet for a two-week adaptation period. Rats were given free access to distilled water and experimental diets. The animal care and use committee of Ewha Womans University reviewed and approved the use of experimental animals in this study.

Control diet (Table 1) was formulated to contain all nutrients in quantities adequate for growth (AIN-93G diet).[20] Soybean oligosaccharide, which was extracted from soybean whey (Hyundai Drug Co., Korea), was substituted for sugar to make a 5% (w/w) soybean oligosaccharide diet. The composition of soybean oligosaccharide was stachyose 23%, raffinose 7%, sucrose 44%, water 3%, and others 23%. The composition of the experimental diet is given in Table 1.

Table 1. Composition of Experimental Diets

(g/kg diet)

	Control diet	Oligosaccharide diet
Corn Starch	529.486	529.486
Casein	200.000	200.000
Sucrose	100.000	50.000
Soy-oligosaccharide	-	50.000
Soybean oil	70.000	70.000
Fiber	50.000	50.000
Mineral mixture[1)]	35.000	35.000
Vitamin mixture[2)]	10.000	10.000
L-Cystine	3.000	3.000
Choline bitartarate	2.500	2.500
t-butylhydroquinone	0.014	0.014

1) Mineral mixture: AIN-93G mineral mixture (g/kg mix)
Calcium carbonate, anhydrous 357.00; Potassium phosphate, monobasic 196.00; Potassium citrate, tri-potassium, monohydrate 70.78; Sodium chloride 74.00; Potassium sulfate 46.60; Magnesium oxide 24.00; Ferric citrate 6.06; Zinc carbonate 1.65; Manganous carbonate 0.63; Cupric carbonate 0.30; Potassium iodate 0.01; Sodium selenate, anhydrous 0.01025, Ammonium paramolybdate, 4 hydrate 0.00795; Sodium meta-silicate, 9 hydrate 1.45, Chromium potassium sulfate, 12 hydrate 0.275; Lithium chloride 0.0174; Boric acid 0.0815; Sodium fluoride 0.0635; Nickel carbonate 0.0318; Ammonium vanadate 0.0066; Powdered sucrose 221.026

2) Vitamin mixture: AIN-93 Vitamin mixture (g/kg mix)
Nicotinic acid 3.000; Ca Pantothenate 1.600; Pyridoxine-HCl 0.700; Thiamin-HCl 0.600; Riboflavin 0.600; Folic acid 0.200; D-Biotin 0.020; Vitamin B_{12} (cyanocobalamin) 2.500; Vitamin E (all-rac-α-tocopheryl acetate, 500 IU/g) 15.000; Vitamin A (all-trans-retinyl palmitate, 500,000 IU/g) 0.800; Vitamin D_3 (cholecalciferol, 400,000 IU/g) 0.250; Vitamin K (phylloquionone) 0.075; Powdered sucrose 974.655

Rats were divided into two groups and fed a control diet or a diet containing 5% soybean oligosaccharide for six weeks. Each group was divided into two sub-groups after streptozotocin (STZ) injection and fed the control diet or the diet containing 5% oligosaccharide. Streptozotocin (Sigma Chemical Co., MO., USA, 50 mg/kg/BW in 0.1 M citric acid buffer, pH 4.5) was injected into the femoral muscle at week seven. Following are the experimental diabetic groups: CC (control diet ⇒ STZ injection ⇒ control diet), CO (control diet ⇒ STZ injection ⇒ oligosaccharide diet), OC (oligosaccharide diet ⇒ STZ injection ⇒ control diet), and OO (oligosaccharide diet ⇒ STZ injection ⇒oligosaccharide diet). The normal group (N) was given a control diet throughout the experiment and a placebo (physiological saline solution) was injected instead of STZ. Ten rats per group were used. Two weeks after STZ injection, the rats were sacrificed and heparinized blood, liver, thymus and spleen were collected.

At eight weeks, fresh fecal contents were collected by direct stimulation of the anus and serially diluted with a sterilized phosphate buffer solution (0.2 M Na_2HPO4, 0.2 M NaH_2PO_4, pH 7.0, 0.1% polypeptone). Bifidobacterium species were enumerated from diluted samples and inoculated onto petri dishes containing a selective differential medium, trypticase phytone yeast extract medium (TPY medium), in an anaerobic environment. The medium composition (g/L) was as follows: trypticase, 10; phytone, 5; glucose, 5; yeast extract, 2.5; tween 80, 1 ml; cysteine hydrochloride, 0.5; K_2HPO4, 2; $MgCl_2$ $6H_2O$, 5; $ZnSO_4{\cdot}7H_2O$, 0.25; $CaCl_2$, 0.15; $FeCl_3$, trace; Agar, 15. Colony counts were made after 48 hours of incubation at 37°C in an anaerobic jar and colony-forming units (CFU) per gram of wet sample were calculated. At 4, 8 and 12 days after STZ injection, blood glucose (mg/dl) was measured by using automatic glucose analyzer (Life Scan, USA) to sample tail blood.

Plasma thiobarbituric acid reactive substances (TBARS) was measured using a luminescence spectrophotometer (Perkin Elmer, LS 50) and the result was expressed as a nanomole of malondialdehyde (MDA) per milliliter of plasma.[21] Liver TBARS was analyzed by modifying Buckingham's method using spectrophotometer at 532 nm.[22] Plasma and liver glutathione peroxidase activities were determined by the coupled assay of Paglia and Valentine.[23] The reaction mixture contained 50 mM potassium phosphate buffer at pH 7.0, 1 mM EDTA, 1 mM NaN_3, 0.2 mM NADPH, 1 U/ml glutathione, 0.1 mM H_2O_2, and 0.05-0.1 ml of diluted sample in a total volume of 1 ml. One unit of glutathione peroxidase activity was defined as 1 micromole NADPH oxidized per min. In the liver, the activity of SOD was determined by the inhibition of xanthine- and xanthine-oxidase- catalyzed reduction of ferricytochrome C.[24] Johansson and Hankan Borg's method was used to measure catalase activity.[25] Protein contents of liver and blood were determined by Lowry's method.[26]

Statistical analysis was carried out with SAS software.[27] Values reported are means ± standard

deviation. The comparison of means among the four groups was made by two-way ANOVA, followed by Duncan's multiple range tests. The comparison between normal (N) and each diabetic group (CC, CO, OC, OO) was made using Dunnett's multiple range tests.

RESULTS

1. Body weight and fecal bifidobacteria in the experimental animals

Body weight and fecal bifidobacteria of the experimental animals at the end of experiment are shown in Table 2. Streptozotocin injection had a strong negative effect on body weight. That is, the CC, CO, OC and OO groups had significantly lower mean body weights than the normal group (N) at the end of experiment. The number of fecal bifidobacteria at the end of experiment was significantly higher in the CO and OO groups than in the CC group. Therefore, recent intake of oligosaccharide seems to be important to maintain higher intestinal bifidobacteria.

2. Blood glucose concentration in the experimental animals

Blood glucose level before STZ injection was not different between the normal (0.96 ± 0.20 g/L) and oligosaccharide-fed (0.94 ± 0.21 g/L) groups. The change in blood glucose concentration after STZ injection is shown in Table 3. Streptozotocin injection drastically increased the blood glucose level of the experimental rats. On day 4, the OC group had a significantly lower blood glucose concentration than the CO group. On day 8, the OO group had a significantly lower blood glucose level than the CO group. On day 12, no difference in blood glucose level among the diabetic groups was found. The OO group had a trend toward lowering of blood glucose at day 12 and showed no significant difference in blood glucose compared to the normal group.

3. Lipid peroxidation and antioxidant enzyme activity in the plasma and liver of the rats

The degree of lipid peroxidation in the plasma and liver after STZ injection is shown in Table 4. TBARS concentration in the plasma was not different among the groups at the end of experimental period. In contrast, TBARS concentration in the liver was significantly lower in the CO and OO groups than in the CC and OC groups. Therefore, soybean oligosaccharide feeding after STZ injection reduced the lipid peroxidation of the liver.

Antioxidant enzyme activity in the plasma and liver are shown in Table 5. Groups fed oligosaccharide after streptozotocin injection (OO, CO) had significantly

Table 2. Body weight and fecal bifidobacteria number at the end of experiment

	Normal group	Diabetic group			
	N[1)]	CC	CO	OC	OO
Body weight (g)	424.2 ± 46.8[3)]	307.1 ± 26.6[*]	287.4 ± 33.6[*]	307.4 ± 39.9[*]	300.7 ± 32.7[*]
Number of *Bifidobacteria* (log cfu[2)]/ g wet feces)	8.67 ± 0.38	8.47 ± 0.81[b]	9.32 ± 0.23[a]	8.98 ± 0.37[ab]	9.32 ± 0.65[a]

1) Abbreviations: N = normal group with control diet, CC = control diet before and after streptozotocin (STZ) injection, CO =control diet before STZ injection and oligosaccharide diet after STZ injection, OC = oligosaccharide diet before STZ injection and control diet after STZ injection, OO = oligosaccharide diet before and after STZ injection
2) log colony forming unit
3) Values are Mean ± S.D.
* : Means in a row significantly different from normal group by Dunnett's multiple range test ($p<0.05$).
Means in a row with unlike superscript letters differ significantly among diabetic groups by Duncan's multiple range test ($p<0.05$).

Table 3. Changes in blood glucose concentration (g/L) after streptozotocin injection

	Normal group	Diabetic group			
	N[1)]	CC	CO	OC	OO
4 days	0.88 ± 0.25[2)]	2.30 ± 0.45[ab,*]	2.38 ± 0.22[a,*]	1.65 ± 0.70[b,*]	2.70 ± 0.73[a,*]
8 days	0.84 ± 0.19	2.19 ± 0.26[ab,*]	2.61 ± 0.59[a,*]	2.31 ± 0.61[ab,*]	1.96 ± 0.56[b,*]
12 days	1.08 ± 0.16	2.11 ± 0.53[*]	2.37 ± 0.26[*]	2.31 ± 0.97*	1.77 ± 0.83

1) Abbreviations: NC = normal group with control diet, CC = control diet before and after streptozotocin (STZ) injection, CO =control diet before STZ injection and oligosaccharide diet after STZ injection, OC = oligosaccharide diet before STZ injection and control diet after STZ injection, OO = oligosaccharide diet before and after STZ injection
2) Values are Mean ± S.D.
Means in a row with unlike superscript letters differ significantly among diabetic groups by Duncan's multiple range test ($p<0.05$).
* : Means in a row significantly different from normal group by Dunnett's multiple range test ($p<0.05$)

Table 4. Thiobarbituric acid reactive substance concentration at the end of experiment

	Normal group	Diabetic group			
	N[1)]	CC	CO	OC	OO
Plasma TBARS (nmol/dl)	0.11 ± 0.03[2)]	0.11 ± 0.03[ns]	0.14 ± 0.03	0.11 ± 0.02	0.11 ± 0.03
Liver TBARS (nmol/g)	0.06 ± 0.03	0.08 ± 0.02[a,*]	0.04 ± 0.02[b]	0.07 ± 0.01[a]	0.05 ± 0.01[b]

1) Abbreviations: NC = normal group with control diet, CC = control diet before and after streptozotocin (STZ) injection, CO =control diet before STZ injection and oligosaccharide diet after STZ injection, OC = oligosaccharide diet before STZ injection and control diet after STZ injection, OO = oligosaccharide diet before and after STZ injection
2) Values are Mean ± S.D.
NS: not significantly different at <0.05.
Means in a row with unlike superscript letters differ significantly among diabetic groups by Duncan's multiple range test (p <0.05).
* : Means in a row significantly different from normal group by Dunnett's multiple range test (p<0.05)

Table 5. Antioxidant enzyme activities in rat plasma and liver at the end of experimental period

		Normal group	Diabetic group			
		N[1)]	CC	CO	OC	OO
Plasma	GSH-Px (U)[2)]	3.0 ± 1.9[5)]	2.3 ± 1.1[c]	8.6 ± 3.3[a,*]	2.2 ± 1.5[c]	5.7 ± 2.3[b,*]
Liver	GSH-Px (U)[2)]	35.0 ± 9.0	26.2 ± 5.6[b,*]	41.9 ± 5.7[a]	27.1 ± 7.3[b,*]	41.6 ± 5.6[a]
	SOD (U)[3)]	18.0 ± 3.8[ns]	17.1 ± 5.1	14.1 ± 3.4	16.5 ± 5.4	18.0 ± 3.8
	Catalase (U)[4)]	15.2 ± 5.2[ns]	13.9 ± 6.3	8.9 ± 5.9	19.7 ± 12.4	10.8 ± 7.4

1) Abbreviations: NC = normal group with control diet, CC = control diet before and after streptozotocin (STZ) injection, CO =control diet before STZ injection and oligosaccharide diet after STZ injection, OC = oligosaccharide diet before STZ injection and control diet after STZ injection, OO = oligosaccharide diet before and after STZ injection
2) 1 Unit = 1 nmol NADPH disappearance/minute/mg protein
3) 1 Unit = inhibition of cytochrome C reduction by 50% per mg protein
4) 1 Unit = μmol formaldehyde utilized as standard per mg protein
5) Values are Mean ± S.D.
NS: not significantly different at <0.05.
Means in a row with unlike superscript letters differ significantly among diabetic groups by Duncan's multiple range test (p <0.05).
* : Means in a row significantly different from normal group by Dunnett's multiple range test (p<0.05)

higher plasma and liver glutathione peroxidase (GPx) activity than groups fed a control diet (CC, OC). However, oligosaccharide feeding or STZ injection did not significantly affect superoxide dismutase or catalase activities in the liver.

DISCUSSION AND CONCLUSION

This study was performed to elucidate the anti-oxidative effect of soybean oligosaccharide in STZ injected rats. The dietary soybean oligosaccharide has been shown to reduce oxidative stresses in rats by repressing lipid peroxidation and by increasing glutathione peroxidase enzyme activity in the rat plasma and liver.

Feeding a 5% oligosaccharide diet showed a significant increase in fecal bifidobacteria. The significant increase in the number of fecal bifidobacteria was even found with two weeks of beginning the oligosaccharide diet in the CO group, but had disappeared within two weeks of returning to the control diet in the OC group. Similar changes in intestinal microflora by oligosaccharide intake have been reported both in human and animal experiments.[6-9, 28)] Dietary oligosaccharide has drawn interest as a functional food component, mostly because of its property to stimulate the growth of bifidobacteria in the colon.[29-32)] Gibson and Roberfroid[33)] explained the changes in intestinal microflora due to increased acid production by fermentation of oligosaccharide in the colon. Acid production results in a low pH. Therefore, it may prevent enteric colonization of potentially pathogenic microorganism and growth of putrefactive bacteria.

In this study, we tried to see whether soybean oligosaccharide had an effect on blood sugar regulation. The 5% soybean oligosaccharide diet had no effect on the blood glucose level of the rat under normal conditions. In contrast, the feeding of soybean oligosaccharide had a beneficial effect in normalizing the blood sugar of the streptozotocin-injected rats. There is a report that consumption of fructo-oligosaccharides significantly lowers fasting glycemia in subjects with non-insulin-dependent diabetes mellitus.[33-35)] Prolonged ingestion of fructo-oligosaccharide is known to decrease basal hepatic glucose production in humans. It was proposed that modification of glucose metabolism induced by fructo-oligosaccharide could be mediated by

the short-chain fatty acids produced in the colon.[36]

Among the short-chain fatty acids produced, propionate in particular was shown to improve hepatic glucose metabolism in rats and in healthy subjects.[37,38] Propionate has been shown to inhibit gluconeogenesis from lactate and to stimulate glycolysis in isolated hepatocytes.[39] Therefore, blood glucose regulation by long-term oligosaccharide intake, in this study, may also be partially due to fatty acids produced by oligosaccharide fermentation. Further study is necessary to elucidate the mechanism of blood glucose regulation by oligosaccharide.

Oxidative stress is increased and antioxidant defense is reduced in a diabetic condition.[40-42] It was reported that the lipid peroxide level significantly increased in the liver and kidneys of rats after streptozotocin administration.[43] In this study, we found that the oligosaccharide diet after STZ injection decreased the TBARS level of the rat liver. The diet also had an effect on antioxidative enzyme activity by increasing glutathione peroxidase activity in the plasma and liver. The decrease in oxidative stress was found both in the long-term (OO) and a short-term (CO) oligosaccharide fed groups. It is likely that soybean oligosaccharide has a direct effect in decreasing oxidative stress in rats.

In conclusion, the results of this study suggest that dietary soybean oligosaccharide has beneficial effects in reducing the oxidative stress of streptozotocin injected rats by decreasing lipid peroxidation and by increasing glutathione peroxidase activity. This antioxidative effect of soybean oligosaccharide seems to contribute to blood glucose regulation after streptozotocin administration.

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