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Effects of Functional Extracts Made from Fermented Plants on Serum Glucose and Lipids Level in Streptozotocin-Induced Diabetic Rats

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Abstract

This study was performed to explore the effects of the fermented functional extracts (FE) on blood glucose and lipid levels in diabetes. FE were created by mixing 9 kinds of plants with sea water and then allowing the mixture to ferment for 1 year. FE were supplemented in the feed of streptozotocin (STZ)-induced diabetic rats at 1%, 3% and 5%. The 1% feeding group showed the lowest weight loss of the three experimental groups. The blood glucose and glycosylated hemoglobin level were significantly decreased in the FE fed rats compared to the diabetic control (DMC) group. The lipid levels in serum were decreased in 1% and 3% FE fed rats in comparison to the DMC group, and there was no significant difference in triglyceride levels due to the FE concentration. The HDL-C level was significantly increased in rats with FE supplemented diets, compared to the DMC group. The levels of lipid peroxides in liver tissue were significantly decreased in FE fed diabetic rats, and the hepatic glycogen content was increased in rats receiving supplements. As a result of these studies, we believe 1% FE may be the optimum level for controlling blood glucose and alleviating hyperlipidemia in STZ-induced diabetic rats.

Key words: functional extracts, antidiabetic, hypolipidemia

INTRODUCTION

Diabetes is a disease in which an excess of glucose is excreted in urine due to an increase of blood glucose content. There is also a metabolic disorder of carbohydrates, which then causes disturbances of albumin, lipid and electrolyte metabolism (1). This is classified as Type 1 diabetes, which is characterized by leading hyperglycemia due to insulin deficiency resulting from destruction of pancreatic β-cell. Type 2 diabetes is characterized by an increase of resistance of peripheral tissues to insulin due to obesity, lack of exercise and life styles such as smoking and drinking (2).

In Korea, the rate of people getting type 2 diabetes has continuously increased, in accordance with an increase in the rate of the obese population. Statistical data regarding causes of death in 2007 reported that diabetes ranked fifth, behind cancer, heart and cardiovascular diseases (3). It is highly possible that an prevalence rate of type 2 diabetes will be gradually increased due to the Westernization of Koreans' dietary style and an increase of aged population. Type 2 diabetes requires continuous control of blood glucose to prevent complications, but complete recovery from Type 2 diabetes is impossible because there is no known principle treatment. Approximately 70% of patients in Korea with diabetes have depended more on alternative medicine using plants with antidiabetic properties rather than commercial medicine (4). According to the World Health Organization, at least 1200 classes of plants have been used for diabetic therapy all over the world (5). In controlling diabetes, it is considered important to reduce lipid peroxides and oxidative stress, as well as blood glucose level, to reduce onset of diabetes complications. For this reason, it is necessary to continuously search for plant materials that decrease high blood glucose levels and oxidative stress. A natural approach to treating Type 2 diabetes may also help to reduce the adverse reactions of therapeutic agents, such as oral hypoglycemic agent.

This study was performed to investigate an optimum level of functional extracts (FE) that decrease blood glucose and lipid levels in STZ-induced diabetic rats.

MATERIALS AND METHODS

Materials

Functional extracts (FE), used in this experiment, were made by mixing 100 g each of mistletoe, araliaceous

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shrub, bush clover, birch, unhulled barley, onion, beet, apple and persimmon in 10 L of water from the depth sea, for a year, thereby fermenting the mixture in the dark at room temperature. After a year of fermentation, the mixture was filtered and extracts prepared. The completed FE were donated from Mineral 21 Co. Ltd. (Seoul, Korea), who have filed a patent application (No. 10-2010- 0040177) covering the manufacturing protocol. The FE were stored at 4°C, and added to the diet of diabetic rats.

Animals

Male albino Sprague-Dawley (SD) rats were purchased from Samtako Bio Korea Inc. (Osan, Korea). The antidiabetic studies were performed on these healthy rats, aged 5 weeks and weighing 150±10 g for this experiment. All rats were housed individually in polypropylene cages and maintained under standard conditions of 22±2°C, relative humidity of 50±5% and a constant 12 hr (07:00～19:00) light and dark cycle. To help them adapt to the laboratory conditions, the rats were fed a standard pellet diet (SuperFeed Inc., Korea) with water *ad libitum* the first week prior to the experiment.

For the experiments, the animals were randomly divided into 5 groups of 7 rats each. At the second week, rats were fed with a normal diet (standard diet, AIN-93G) that contained 10% corn oil for 1 week, and 4 groups were induced to become diabetic by injection of streptozotocin (STZ). The normal group (NC) was injected with saline instead of STZ and received a basal diet. The diabetic control group, called DMC, was induced with STZ to become diabetic, but did not receive FE supplementation in the feed. The three remaining groups, DM-I, DM-II and DM-III, were induced to become diabetic with STZ and then supplemented with either 1%, 3% or 5% FE, respectively. All treatments continued for 4 weeks. Composition of the experimental diet is presented in Table 1.

Induction of diabetes mellitus in rats

The overnight fasted SD rats were induced to become diabetic by STZ (50 mg/kg, i.p). STZ (Sigma Co., St. Louis, MO, USA) was found to be selectively toxic to the β-cells of pancreatic islets, cells that normally regulate blood glucose levels by producing the hormone insulin. The addition of STZ creates a diabetic state in laboratory animals through the destruction of their pancreatic β-cells (6).

The STZ was freshly dissolved in citrate buffer (0.01 M, pH 4.5) and stored at 4°C prior to use; the injection volume was 1 mL/kg body weight. Animals had free access to food and water after the STZ injection, and diabetes was confirmed in STZ-treated rats by measuring the fasting blood glucose concentration 48 hr post injection. The rats with blood glucose levels above 300 mg/dL, as measured by a glucometer (Active Blood Glucose Meter, ACCU-CHEK®, USA), were considered to be diabetic and were used in this experiment.

Table 1. Composition of experimental diets (%, diet)

Ingredients	Groups[4]				
	NC	DMC	DM-I	DM-II	DM-III
Starch	39.75	39.75	39.75	39.75	39.75
Casein	20	20	20	20	20
Dextrin	13.2	13.2	12.2	10.2	8.2
Cellulose	5	5	5	5	5
Sucrose	10	10	10	10	10
Vitamin mix.[1]	1	1	1	1	1
Mineral mix.[2]	3.5	3.5	3.5	3.5	3.5
DL-methionine	0.3	0.3	0.3	0.3	0.3
Choline bitartrate	0.2	0.2	0.2	0.2	0.2
Corn oil	7	7	7	7	7
Functional extracts[3]	−	−	1	3	5

[1,2]Vitamin mixture and mineral mixture were according to AIN-76™.

[3]Functional extracts (FE) were mixed each 100 g of mistletoe, araliaceous shrub, bush clover, birch, unhulled barley, onion, beet, apple and persimmon in 10 L of water in the depth of sea, thereby fermenting the mixture at dark room temperature for 1 year and then filtered.

[4]NC: not induced diabetes, DMC: diabetic rat, DM-I: fed group 1% of the FE with diabetic rat, DM-II: fed group 3% of the FE with diabetic rat, DM-III: fed group 5% of the FE with diabetic rat.

Measuring of food intake, FER and body weight

Over the 4 week experimental period, food-intake and water-intake were monitored daily at the same time, while body weight and fasting blood glucose (FBG) level were tested every week at the same time. FER (food efficiency ratio) was calculated by total body weight gain (g)/ total food intake amount (g).

Treatment of experimental rats

At the end of the experiment, after overnight fasting, all rats were sacrificed by an overdose of ethyl-ether. Blood samples from the heart were rapidly cooled on ice and 30 min later the serum was separated by centrifuge (Mega 17R, HANILE, Korea) at 3000 rpm for 15 min at 4°C. Liver, heart, kidney, spleen and testis were excised rapidly, chilled and rinsed in ice-cold 0.9% NaCl. The serum and organs of rats were stored under -70°C before analysis, and used for further analysis of antidiabetic activity.

Determination of glucose and glycosylated hemoglobin level in serum

Arterial blood was collected from the heart and the serum was obtained by centrifuging at 3000 rpm for 15

min immediately. Glucose levels in the serum were determined with kit reagents (AM 201-k, Asan, Korea). Glycosylated hemoglobin levels were determined by Hemoglobin A1c kit reagent (Asan, Korea). Whole blood treated by EDTA was used for the analysis.

Determination of lipids level in serum

Total lipid levels were determined according to the method of Frings et al. (7). 20 μL of serum was heated with 200 μL concentrated H_2SO_4 in a boiling water bath for 10 min, then 10 mL of phospho-vanillin was added, and the mixture was incubated 37°C for 15 min. The mixture was measured at 540 nm, and olive oil (0～500 mg) was used as standard for calibration curve.

Total cholesterol, triglyceride and high density lipoprotein cholesterol (HDL-C) levels were determined with each kit reagent for total cholesterol (AM 202-k, Asan, Korea), triglyceride (AM 157S-k, Asan, Korea) and HDL-C (AM 203-k, Asan, Korea). The low density lipoprotein cholesterol (LDL-C) level was calculated by Friedewald's equation (8): LDL-C = TC − (HDL-C+TG/5). Atherogenic index (AI) was calculated by the following formula (9): (TC − HDL-C)/HDL-C. Cardiac risk factor (CRF) was calculated by the following formula (10): Total cholesterol/HDL-C.

Determination of GOT and GPT activities

Serum enzyme GOT (glutamic oxaloacetic transaminase) and GPT (glutamic pyruvic transaminase) activities were measured by kit reagent (GOT-PIII, Fuji, Japan) and kit reagent (GPT-PIII, Fuji), respectively.

Determination of lipid peroxidation in serum

100 μL of serum was mixed 1/12 N H_2SO_4 solution and 10% phosphotungstic acid, then centrifuged at 4000 rpm for 10 min. The residue fraction, except the supernatant, was mixed with distilled water and TBA (thiobarbituric acid) reagent. The mixture was incubated in a 95°C water bath for 60 min and then cooled to room temperature in the ice bath. The mixture was added to exactly 4.0 mL n-butanol before being centrifuged at 3000 rpm for 10 min. The absorbance of the butanol fraction was measured at 532 nm. 1,1,3,3-tetraethoxypropan (TEP, Sigma Co., USA) was used as standard for calibration curve, its value was expressed as mmol MDA/dL.

Determination of lipids level in tissues

Lipids from the tissue were extracted according to the method of Folch et al. (11). 0.5 g of tissue was mixed, and extracted well in a C:M solution (chloroform : methanol = 2:1, v/v) and homogenized with the homogenizing stirrer (DAIHAN wise stir® HS-30E, Korea). After homogenization, 300 μL each of homogenate were exhausted by a rotary vacuum evaporator at 30～40°C for determination of total lipids, total cholesterol and triglyceride content in tissues. Determination of lipid levels (total lipid, total cholesterol and triglyceride) in tissues was evaluated using the same methods of lipid determination in serum, described above.

Determination of lipid peroxidation in liver

The methods of Uchiyama and Mihara (12) as well as Lee et al. (13) were used to determine lipid peroxidation in liver tissue. 1.0 g of liver tissue was homogenized in 10 mL of 1.5% KCl solution to obtain a 10% homogenate. 0.5 mL of the homogenate was mixed with 3 mL of 1% H_3PO_4 and 1 mL of 0.67% TBA reagent. The mixture was heated in a 95°C water bath for 45 min and then cooled under running water. 4 mL n-butanol was added to the mixture before being centrifuged at 3000 rpm for 10 min. The butanol fraction was measured at 535 nm and 520 nm. Equal volumes of homogenate without tissue were used as blank. TEP was used as standard for calibration of the curve and its level was expressed as mmol MDA/g wet liver tissue.

Determination of glycogen level in liver

To determine the levels of hepatic glycogen, 0.2 g of liver tissue was dissolved in 1 mL of 30% KOH and after extensive digestion, the glycogen was precipitated by 1.25 mL of 95% ethanol for 5 min. Mixtures were centrifuged at 8000 rpm for 4 min and residues were dissolved in 5 mL of 3rd distilled water. Glycogen was assayed as a reducing sugar by absorbance at 620 nm in 0.2% anthrone-H_2SO_4 solution. D-glucose (Sigma Co., USA) was used as standard for calibration of the curve and the glycogen level was expressed as mg/g wet liver tissue.

Statistical analysis

Values were presented as means ± SD with n=7. Statistical difference between the treatments and controls were tested by one-way analysis of variance (ANOVA) followed by the Duncan's multiple range test. A difference in the mean values of $p<0.05$ was considered to be statistically significant. All statistical tests were performed using the computer software program SPSS for Windows (SPSS, version 12.0) for statistics and data analysis.

RESULTS AND DISCUSSION

Food intake and body weight change

Body weight, food intake, increased amount of body weight and food efficiency ratio (FER) in STZ-induced diabetic rats which were fed FE are shown in Table 2. The final body weight in the FE feeding groups was smaller in comparison with the DMC group. After the

Table 2. Changes in body weight, food intake and food efficiency ratio of rats

Group[1)]	Final body weight (g)	Food intake (g/day)	Total body weight gain (g/4 weeks)	FER[2)]
NC	331.67±17.04[d]	20.62±0.33[NS]	136.50±13.75[d]	23.64±2.48[d]
DMC	278.50±15.36[c]	20.83±0.37	71.33±16.48[bc]	12.26±2.93[bc]
DM-I	271.67±38.78[bc]	20.57±0.76	77.67±34.16[c]	13.54±5.98[c]
DM-II	245.50±12.93[ab]	20.63±0.51	47.33±15.88[ab]	8.18±2.67[ab]
DM-III	224.33±19.02[a]	20.87±0.09	29.83±12.83[a]	5.10±2.18[a]

[1)]See the legend of Table 1.
[2)]FER (Food efficiency ratio) = Total body weight gain (g/4 weeks)/ Food intake (g/4 weeks).
[a-d]Values in a column sharing the same superscript letter are not significantly different at $p<0.05$.
NS: not significant.

Table 3. The organ weight of liver, heart, kidney, spleen and testis of rats (g/100 g body weight)

Group[1)]	Liver	Heart	Kidney	Spleen	Testis
NC	4.55±0.45[a]	0.32±0.02[a]	0.71±0.03[a]	0.19±0.01[NS]	1.00±0.05[a]
DMC	6.20±0.60[bc]	0.37±0.04[ab]	0.93±0.13[b]	0.22±0.03	1.23±0.10[b]
DM-I	5.56±0.50[b]	0.35±0.02[ab]	0.98±0.18[b]	0.21±0.04	1.25±0.06[b]
DM-II	6.61±1.10[c]	0.37±0.06[ab]	1.08±0.11[b]	0.23±0.04	1.31±0.18[b]
DM-III	6.13±0.42[bc]	0.38±0.05[b]	1.27±0.15[c]	0.19±0.03	1.35±0.13[b]

[1)]See the legend of Table 1.
[a-c]Values in a column sharing the same superscript letter are not significantly different at $p<0.05$.
NS: not significant.

4-week experiment, the increase in FE feeding groups was 29.83～77.67 g in comparison with initial body weight and the body weight in DM-I group increased in comparison with DM-II and DM-III groups, as well as the DMC group. The FER in the DM-I group was somewhat increased compared to the DMC group, but the supplementation of FE in diet was greater, FER trended downward significantly.

Other studies have shown that in STZ-induced diabetic experimental animals, the glucose utility of cells was decreased and body weight recovery wasn't easily shown as a metabolic characteristic of starvation state (14). Particularly, for STZ-induced diabetes, the hepatic glycogen content was decreased about 4.5 times, so body weight loss in diabetes has been used as an index for indicating the intensity of a diabetic symptom (15). It was reported that, feeding of Saengmaeksan (mixture of ginseng, *Liriope platyphylla* and *Schizandra chinensis*) showed an anti-diabetic effect by recovering body weight loss due to diabetes and increasing the FER (16), which was similar to the result found in this study with the supplementation with 1% FE. In our study, the 1% FE supplementation appeared to be more effective on recovery of body weight loss due to diabetes than did the 3% and 5% supplementation with FE.

Organ weights

The results of measuring weights of internal organs are shown in Table 3. The weights of organs such as liver, heart, kidney, spleen and testis were measured. The weight of the liver tissue was the highest in the DM-II group and lower in the DM-I group, but there was a little difference between FE feeding groups. The weight of the kidney tissue in the DM- III group was significantly increased in comparison to the NC and the DMC groups, in agreement with the prediction that 5% feeding of FE would cause a heavy burden on the kidney. Moreover, a similar effect was seen with the weight of the liver (3% and 5% feeding groups of FE).

Han et al. (17) reported that when composite plants were administered to diabetic rats, the weights of their internal organs were decreased in comparison to the diabetic control group, but Kim et al. (18) assumed that the increase in weight of the liver tissue that occurred when pine needle extracts were fed to diabetic rats, in comparison with diabetic control group, was the result of simple drug metabolism due to long-time feeding of the test materials, and not from hepatotoxicity due to the test material. In this study, since a 1% feeding of FE did not show a distinct influence on the weights of the internal organs, we suggest that the increase of organ weights in 3% and 5% supplemented rats might be due to overdose of FE.

Blood glucose and glycosylated hemoglobin level

The blood glucose and glycosylated hemoglobin levels of STZ-induced diabetic rats after feeding with FE are shown in Table 4. The blood glucose level was significantly decreased in the DM-I, DM-II and DM-III groups in comparison with DMC group. It has been reported that the decrease in blood glucose level due to the feeding of plants indicated the existence of insulin

Table 4. Glucose and glycosylated hemoglobin levels in serum of diabetic rats fed functional extracts (mg/dL)

Group[1)]	Glucose	Glycosylated hemoglobin
NC	149.75±18.38[a]	2.57±0.58[a]
DMC	224.70±22.23[c]	4.79±0.71[c]
DM-I	164.60±16.51[ab]	3.06±0.48[ab]
DM-II	168.76±27.49[ab]	3.09±0.22[ab]
DM-III	187.18±18.12[b]	3.59±0.76[b]

[1)]See the legend of Table 1.
[a-c]Values in a column sharing the same superscript letter are not significantly different at $p<0.05$.

analogue in plants which contribute to the increase in insulin secretion caused by stimuli to β-cell, the increase in transfer of glucose into cells and consumption thereof (19), and the regeneration of pancreatic cells (20). Because the blood glucose levels in this experiment showed a decrease in the FE-fed rats that was similar to that of the normal group, we suggest that the decrease in blood glucose levels due to FE result from the same mechanism.

The glycosylated hemoglobin level is proportional to the blood glucose level in the case of diabetes, and, regarding that a half life of hemoglobin is 60 days (21), the measurement of glycosylated hemoglobin content is used as an index for indicating blood glucose level for the most recent 60 days. In Table 4, the glycosylated hemoglobin level was decreased about 25.1～36.1% in FE feeding groups, compared with the DMC group. And the glycosylated hemoglobin level in the DM-III group was slightly higher than the DM-I and DM-II groups. Feeding a mixture of 14 types of domestic medicinal herbs known to have physiological functional compounds to STZ-induced diabetic rats decreased glycosylated hemoglobin levels by about 15%, according to published reports (21). In agreement with those results, our studies show that 1% and 3% feeding of FE are effective for decreasing glycosylated hemoglobin levels.

Lipids level in serum

An atherogenic index (AI) and a cardiovascular risk factor (CRF), whose results indicate blood lipid levels after 4-week feeding of FE to diabetic rats are shown in Table 5. Total lipids were higher in all diabetic groups, compared with the NC group. These levels were significantly decreased in FE feeding groups (DM-I, -II and -III groups) in comparison to the DMC group, with the DM-I group showing as much as a 26% decrease in comparison to the DMC group. There was little significant difference in total cholesterol levels between the DMC and FE fed groups, with the total cholesterol level of 1% FE supplement group being the lowest, closest to that of NC group. For triglycerides, there was no significant difference between the DMC and DM-I, -II, and -III groups.

An HDL-cholesterol (HDL-C) level was significantly increased after feeding of FE, with the 1% and 3% FE-fed groups showing levels similar to that of the NC group. The LDL-cholesterol (LDL-C) level was effectively decreased in the 1% and 3% feeding groups in comparison with the DMC group. AI was the highest in the DMC group, and it could be assumed that, in the 1% feeding group it was recovered up to the level of NC group and so the attack rate of arteriosclerosis, one of diabetes' complications, could be decreased due to FE feeding. For the CRF, the 1% and 3% FE-supplemented groups showed a lower value compared with the DMC group, like the AI, it is predicted that feeding of FE at 3% or less could cause a decrease of risks resulting from complications of diabetes.

Metabolic disorders of lipids, mainly observed in diabetes, indicate the increase of triglycerides and cholesterol and the decrease of HDL-C in blood (22,23). It is also reported that for diabetes caused by disorder of circulatory system, in which blood glucose isn't controlled, activation of intestinal HMG-CoA reductase is increased, and then transfer of cholesterol into circulatory blood is elevated (24). It is thought that the decrease of the blood cholesterol level in the 1% FE feeding group, has high correlation with the decrease in blood glucose (Table 4), and such results are similar to those of reports of Han et al. (17). It was reported that mistletoe (25),

Table 5. Lipid profiles in serum of diabetic rats fed functional extracts

Group[1)]	Total lipids	Total cholesterol	Triglyceride	HDL-C	LDL-C	AI	CRF
	(mg/dL)						
NC*	205.78±7.73[a]	60.29±1.67[a]	50.57±4.08[a]	35.96±1.30[c]	14.21±0.77[a]	0.68±0.02[a]	1.68±0.02[a]
DMC	321.85±9.66[c]	66.35±5.90[bc]	59.85±4.50[b]	31.09±1.85[a]	23.29±7.41[bc]	1.14±0.27[cd]	2.14±0.27[cd]
DM-I	238.10±21.74[b]	62.60±3.04[ab]	57.65±3.59[b]	34.96±2.12[bc]	16.11±2.19[ab]	0.79±0.06[ab]	1.79±0.06[ab]
DM-II	250.85±11.66[b]	68.80±4.19[cd]	60.24±3.83[b]	34.67±2.64[bc]	22.08±5.15[bc]	1.00±0.19[bc]	2.00±0.19[bc]
DM-III	309.95±10.70[c]	72.55±2.96[d]	62.23±4.47[b]	31.73±2.81[ab]	28.37±5.91[c]	1.30±0.26[d]	2.30±0.26[d]

[1)]See the legend of Table 1.
[a-d]Values in a column sharing the same superscript letter are not significantly different at $p<0.05$.
HDL-C: high density lipoprotein cholesterol, LDL-C: low density lipoprotein cholesterol, AI: atherogenic index, CRF: cardiac risk factor.

Table 6. Total protein, albumin and globulin in serum of diabetic rats fed functional extracts (mg/dL)

Group[1)]	Total protein	Albumin	Globulin
NC	6.41±0.39[NS]	4.45±0.28[NS]	1.95±0.19[NS]
DMC	6.21±0.16	4.35±0.08	1.87±0.22
DM-I	6.51±0.39	4.50±0.15	2.02±0.43
DM-II	6.29±0.12	4.35±0.59	1.94±0.64
DM-III	6.09±0.12	4.26±0.20	1.84±0.30

[1)]See the legend of Table 1.
NS: Not significant.

Table 7. GOT and GPT activities in serum of diabetic rats fed functional extracts (μ/dL)

Group[1)]	GOT	GPT
NC	53.50±3.32[ab]	2.75±0.96[a]
DMC	62.25±7.72[c]	4.50±1.00[b]
DM-I	52.75±5.91[a]	5.00±1.15[b]
DM-II	57.50±2.52[abc]	6.75±0.50[c]
DM-III	61.25±3.77[bc]	8.75±1.50[d]

[1)]See the legend of Table 1.
[a-d]Values in a column sharing the same superscript letter are not significantly different at $p<0.05$.

Araliaceous shrub (26), onion (27), and other plants contained in FE, have an effect on improvement of the internal lipids. The results of this experiment suggest that a 1% FE supplementation is effective for the decrease of diabetes complications such as hyperlipidemia and arteriosclerosis. However, the increased lipid components observed with the 3% and 5% feeding groups may stimulate a reverse action (28), resulting from an overdose of the test material.

Total protein, albumin and globulin level in serum

Total protein, albumin and globulin levels in the serum are shown in Table 6. In 4-week feeding of FE to diabetic rats, there were no significant differences of total protein, albumin and globulin levels in the serum. Although serum protein levels aren't usually influenced by insulin (29), when a person has diabetes, protein catabolism is increased and used as source of calorie, which results in decreased protein levels and loss of body weight (30).

There were no significant differences between the DMC group and the FE-supplemented groups in terms of in serum protein level, but the final weight was decreased as the concentration of FE was increased (Table 2).

GOT and GPT activities in serum

Serum GOT and GTP activities in diabetes were significantly higher in the DMC group than the NC group (Table 7). STZ leads to liver damage by causing light degeneration of lipids in the liver, and GOT and GPT activities are used as an index for liver damage (31). The GOT activity in the DM-I group was significantly decreased in comparison with the DMC group, suggesting that 1% feeding of FE can prevent liver damage from diabetes. The GPT activity in the DM-III group was about 1.9 times higher than the DMC group, and the weight of the kidneys in the DM-III group was significantly higher than the DMC group (Table 2). The kidney is an organ that is more sensitive to oxidative stress than other internal organs, and has higher possibilities of being damaged; thus, it is likely that the induction of diabetes results in a greater likelihood for development of kidney diseases (32).

Lipid levels in liver and kidney

Lipid levels in liver and kidney tissues after 4-week feeding of FE to STZ induced diabetic rats are shown in Table 8. Total lipid levels in the liver were decreased in FE-supplemented groups compared to the DMC group, but there was little significant difference between them; however, the differences were greater in the liver compared with kidney tissue. The total cholesterol level was higher in kidney than in liver tissue, and was significantly decreased in kidney tissue for FE-supplemented groups in comparison with the DMC group. Triglyceride levels in liver and kidney tissues for FE fed groups was significantly lower, compared with the DMC group. There was no significant difference in the lipid profiles in liver and kidney tissues between the three FE-supplemented groups.

Table 8. Total lipid, total cholesterol and triglyceride in liver and kidney of diabetic rats fed functional extracts (mg/g wet tissue)

Group[1)]	Liver			Kidney		
	Total lipid	Total cholesterol	Triglyceride	Total lipid	Total cholesterol	Triglyceride
NC	10.41±0.93[a]	1.77±0.26[NS]	8.03±0.56[b]	7.95±1.08[NS]	3.61±0.16[a]	5.50±1.22[a]
DMC	12.13±0.57[b]	1.87±0.17	10.22±0.82[c]	8.98±1.94	4.32±0.12[b]	11.95±1.86[b]
DM-I	11.07±0.59[ab]	1.44±0.33	6.36±0.97[a]	7.34±0.75	3.30±0.80[a]	7.38±2.78[a]
DM-II	11.40±1.45[ab]	1.53±0.07	4.90±1.67[a]	7.42±0.80	3.24±0.48[a]	7.10±0.94[a]
DM-III	11.58±0.31[ab]	1.75±0.36	5.05±1.07[a]	7.47±1.36	3.08±0.38[a]	6.81±1.83[a]

[1)]See the legend of Table 1.
[a-c]Values in a column sharing the same superscript letter are not significantly different at $p<0.05$.
NS: Not significant.

An antidiabetic effect in diabetes depends on obstruct of insulinase activity in liver and kidney or increase of sensitivity of insulin receptor by supplemented functional materials (33). According to the results of this experiment, the total cholesterol and triglyceride levels in liver tissue were lower than those in kidney tissue, which were similar to the results reported by Kim (34), indicating that many lipid peroxides are generated because lipid accumulation is high in kidney tissue and resistance to lipid peroxidation was low. Shin (35) reported that the decrease in lipid level in liver tissue of diabetic rats depends on functional materials supplemented in the diet. The lipid level in liver and kidney tissues for the 1% FE feeding group was lower than that for 3% and 5% feeding groups. These results suggest that a concentration of 1% FE in the diet is in the optimum level for controlling body lipids level in diabetes.

Lipid peroxidation in serum and liver

Lipid peroxidation in serum and liver was significantly higher in the DMC group compared with the NC group, as shown in Table 9. There was no significant difference between the FE fed groups and the DMC group for the level of lipid peroxidation in the serum, but peroxidation levels were significantly decreased in liver tissue such that the levels in the DM-II and DM-III groups was lower compared with NC group.

Lipid peroxidation causes cancer, arteriosclerosis and aging as a result of increased oxidative stress of cells in tissue and decreased *in vivo* antioxidant defense power; therefore, lipid peroxidation is used as an index for indicating the extent of cell damage (36). It has been reported that, due to increase of oxidative stress in case of diabetes, the level of lipid peroxidation is increased in liver and kidney tissues as well as in the vascular system (37,38), and the generation of lipid peroxidation is different depending on the sensitivity of each organ (39). Accordingly, the decrease of lipid peroxides due to FE supplementation was significant in liver tissue rather than serum, and it is assumed that FE can protect tissue by obstructing oxidation of lipids accumulated in liver tissue as a result of diabetes.

Moreover, it is known that phenolic compounds among natural plants have antioxidant activity (40) by obstructing generation of malondialdehyde (MDA) (41). Therefore, we believe the results of this experiment involved interaction of the natural materials extracts from the plants to create the FE.

Glycogen contents in liver

The hepatic glycogen content of diabetes rats fed FE is shown in Table 10. The contents in the DMC group were significantly lower than NC group, but were increased in rats fed FE.

Table 9. Lipid peroxidation contents in serum and liver of diabetic rats fed functional extracts

Group[1)]	Serum (mmol/dL)	Liver (mmol/g)
NC	41.44±4.58[a]	139.56±11.09[b]
DMC	60.33±5.94[b]	155.85±9.29[c]
DM-I	57.19±3.37[b]	137.71±12.53[b]
DM-II	67.19±14.69[b]	106.59±2.23[a]
DM-III	90.89±10.06[c]	105.48±10.61[a]

[1)]See the legend of Table 1.
[a-c]Values in a column sharing the same superscript letter are not significantly different at $p<0.05$.

Table 10. Glycogen content in liver of diabetic rats fed functional extracts

Group[1)]	Glycogen content (mg/g wet tissue)
NC	19.85±4.40[c]
DMC	12.46±0.99[a]
DM-I	14.96±2.90[b]
DM-II	14.00±1.37[b]
DM-III	15.05±1.42[b]

[1)]See the legend of Table 1.
[a-c]Values in a column sharing the same superscript letter are not significantly different at $p<0.05$.

For STZ-induced diabetes, insulin becomes deficient due to the destruction of pancreatic β-cells, then glycogenase is activated and body glycogen content is decreased. Moreover, in the liver, insulin resistance is generated within 3 days after STZ administration and a considerable decrease in the glycogen content is generated, rather than in the muscles (32). In this experiment, supplementation with FE caused an increase of the hepatic glycogen content, it is considered as the same tendency that FE feeding groups show significant decrease in serum glucose in comparison with the DMC group (42).

We demonstrated throughout this study that the administration of 1% FE in diet has created remarkable effects on blood glucose and total cholesterol levels in STZ-induced diabetic rats. Also, FE has shown significantly more anti-lipoperoxidation activity in liver tissue. Therefore our results indicate that the 1% feeding of FE exerts hypoglycemic and hypolipidemic effects in STZ-induced diabetic rats.

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