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Effects of a Butanol Fraction of *Alisma canaliculatum* and of Selenium on Blood Glucose Levels and Lipid Metabolism in Streptozotocin-Induced Diabetic Rats*

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The purpose of this study was to investigate the effects of a butanol fraction of *Alisma canaliculatum All. Braun et Bouche* (Ac), and of selenium (Se), on plasma glucose and lipid levels in streptozotocin (STZ)-induced diabetic rats. Male Sprague-Dawley rats, fed the AIN-93 recommended diet, were divided into five groups: a non-diabetic control group (no STZ treatment), and four STZ-induced diabetic groups which consisted of a diabetic-control group, an Ac-treated group, an Ac-Se treated group, and a Se-treated group. Diabetes was induced in the rats by an injection of STZ into the tail vein at a dose of 45 mg/kg body weight. The butanol (BuOH) fraction of Ac was orally administered at a rate of 400 mg/kg body weight for 21 days to both the Ac and Ac-Se groups. The supplementation of selenium in the Se and Ac-Se groups was achieved by adding (freshly, every day) 2 mg of Se as Na_2SeO_3 per kg of feed. The rats' body weights and hematocrit (Hct) levels were measured, along with plasma levels of glucose, insulin, cholesterol, HDL-cholesterol, triglyceride (TG), and free fatty acids (FFA). Aminotransferase activities were also analyzed. The non-diabetic rats gained weight, while the diabetic rats lost weight - except in the Ac-Se group, which maintained their initial weight. The blood glucose levels of the Ac group and the Se group were significantly lower than for the diabetic-control group. The plasma triglyceride levels were lowered when both Ac and Se were administered to diabetic rats. The concentrations of plasma FFA in the Ac-Se group were significantly lower compared with the diabetic-control group. Plasma cholesterol levels and alanine aminotransferase activity in the Ac, Ac-Se, and Se groups were significantly lower when compared with the diabetic-control group. Aspartate aminotransferase activity was significantly lower in the Se group compared to the other diabetic groups. These data show that treatment with a butanol fraction of Ac in combination with Se has no synergistic effect. Plasma glucose levels tended to be low when Se was administered to diabetic rats. Supplementation of Se in diabetic rats did not elicit a significant increase in plasma insulin levels or result in hypolipemic effects.

Key words : *Alisma canaliculatum*, selenium, streptozotocin, plasma glucose, lipid metabolites.

INTRODUCTION

Diabetes mellitus has become the most significant chronic disease and cause of death in modern society. In Korea, diabetes was a very rare disease. In 1983 the incidence of diabetes in the population aged between 20 to 29 years and 40 to 49 years was less than 1% and 2%, respectively. However, in 1998 the incidence of diabetes among the population aged 20 to 29 years quadrupled and 40 to 49 years doubled to 2% and 5.1%, respectively[1]. These figures far exceeds the levels among other races and countries such as the Pima Indian and the Nauru tribe[1,2] who are known to have the highest incidence outside of Korea (1.8%). In Korea the death rate from diabetes has increased by 124.5% in the last 10 years[3] and the incidence of diabetes is increasing even in young children due to increases in obesity. Thus, the cause, prevention, and treatment of diabetes has become a very serious public health problem in Korea.

The cause of diabetes is attributed to the westernization of food practices, lack of exercise, and nutritional imbalance of food compositions. Recent westernization of the Korean diet, and the changed food behavior such as fast foods and processed foods prompted the accumulation of reactive oxygen species (ROS) in the body, and the resulting reactive oxygen radicals damage cells and may be directly responsible for increases in diabetes. In diabetes the tissues have more peroxidative damage, and a stimulated free radical production system, compared to non-diabetic tissues.

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In order to resist free radical formation, antioxidative enzymes, as well as nutrients such as vitamin C and selenium which play a role in non-enzymatic protection against lipid peroxidation[4-6], are needed.

Selenium, an antioxidant nutrient, is a mineral which combines with a metalloenzyme, glutathione peroxidase, and controls hydroperoxides and H_2O_2 levels. Selenium is a very important mineral whose characteristics are similar to insulin[7-10]. The addition of Se in diabetes results in lowered blood sugar, and a recovery in the functions and structure of the pancreatic β-cells. Antioxidant supplementation also delays onset of symptoms of diabetes[11-13]. Se is a catalyst in thyroid hormone production, protects inner endothelial cells, facilitates immunological functions, and protects the body against diseases. Selenium prevents apoptosis caused by ROS, and suppresses initial carcinogenic processes[14].

Prevention and management of complications caused by diabetes could be achieved by improved food habits, reductions in obesity, and more physical exercise. However, a prolonged regime of drug treatment could raise questions of toxicity, of safety-efficacy, and of cost-effectiveness. In order to prevent such problems and the side effects of chemicals, renewed efforts are being made to use traditional or natural remedies. Recently, research has focused on foods which could increase insulin secretion, on the extraction of naturally occurring antioxidant vitamins, the effects of extraction on antioxidation and antiperoxidation, and on the testing of anticarcinogenic effects[15-18]. In Far Eastern countries such as Korea, research has also focused on pharmaceutical plants which have traditionally been used as folk remedies[19]; research on the mutation-inhibiting effects of plants, and a search for new functional foods, are emerging as important areas of inquiry.

Alisma canaliculatum All. Braun et Bouche (Ac), which is used for the prevention and treatment of diabetes, is a plant belonging to a Family with approximately 13 genuses and 90 species around the world; the Korean Alisma is called *Alisma plantago-aquatica* L. var. *orientale* Samuels. In Korean, this Alisma is called *soguinamul, soitaenamul or mooltaecksa*, and is used, cultured and produced as a drug. The dried subterranean stem of this plant, grown as a perennial in marshy areas around ponds, is called *Alisma canaliculatum All. Braun et Bouche*. The water plantain, Rhizoma Alismatis (Ac's pharmacological Latin name or prescription name) has been used as a food or drug in folk medicine; its roots were used against diarrhea, hypertension, acute intestinal infection, dryness of mucous membranes, dizziness, and jaundice. The Ac, a harmless anti-mutational plant food which suppresses DNA damage, has diuretic properties and cholesterol lowering activities. The Ac consists of 23% starch, 7% protein, triperpene chemicals such as alisol A, alisol B, alisol A monoacetate, alisol B monoacetate, and epialisol A, which belong to the furfurol and triterpenoid branches of refined oil. The Ac contains D-glucose, D-fructose, sucrose, β-sitosterol and choline, with small amounts of alkaloids, aspartic acid, and phytosterols and their derivatives, palmitic acid, stearic acid, oleic acid, and linoleic acid[20,21].

Prior to this study, the effects of Ac were investigated through its supplementation in the diet[22], and through the oral administration of a methanol extract of Ac[23,24]. In the present experiment, Ac, Se, or Ac-Se were given to STZ-induced diabetic rats for 21 days, and blood plasma was collected to study the effects of antioxidants on glucose and lipid metabolism.

MATERIALS AND METHODS

1. Materials

Dry Ac was purchased in the Kyoung-dong market and pulverized. The resulting powder was added to methanol and was then placed in a water bath which was attached to a device for circulating cold air. The extract was filtered in warm temperature conditions. This process was repeated three times. All methanol filtrates were added together, decompressed, and then concentrated. The methanol extracts were fractionated with hexane, chloroform, ethylacetate, butanol, and H_2O. The butanol fraction, which is known to reduce blood glucose, was used for the present experiment. Selenium as the compound Na_2SeO_3 (BDH Laboratory, England) was used in the experiment.

2. Experimental animals, diets and diabetes inducement

Sprague-Dawley rats weighing approximately 230g (Sam-Yook experimental animals) were raised in the laboratory and fed a solid chow (Sam-Yang Animal Feed Co.) for a week prior to the experiment. The rats were then divided into 5 groups. Animals assigned to the 4 diabetic groups were injected with STZ into the tail vein in order to induce diabetes; one group was used as the diabetic control group, with the remaining three groups being designated as the Ac, Ac-Se, and Se groups. STZ was used to induce diabetes in rats, as it is known to specifically affect pancreatic β-cells, induce diabetes, and cause high blood sugar and mild weight reduction. A concentration of 45mg STZ in 0.01M citrate buffer (pH 4.5)/kg body weight[25] was injected into the rats. It is reported that, within 7 hours of STZ injection, insulin secretion increases, but insulin secretion then gradually decreases due to β-cell destruction within 24 hours, and causes high blood sugar levels[26]. Blood was obtained from the eye vein of the animals treated with STZ and centrifuged at 3,000 rpm for 15 minutes (HA300, Hanil

Centrifuge Co., Ltd). If the glucose concentration in plasma was higher than 300mg/dL, the rats were considered to be diabetic. The non-diabetic control animals were injected with the same amount of citrate buffer. All animals were given the AIN-93 ration[27] and water ad libitum. The Ac group was given 400mg Ac in 5% Tween 80 solution per kg body weight at a fixed time of the day for 21 days. The Se group was given 2mg Se per kg body weight, mixed into the AIN-93 ration. The Ac-Se group was given Ac and Se together; the non-diabetic control group and the diabetic control group were given 5% Tween 80 solution orally.

Food intakes and body weights were measured daily at a fixed time of the day, and average daily food intakes in each week were calculated. The feed efficiency ratio (FER) was also calculated.

3. Biochemical Analysis

During the experimental period, animals were anaesthesized with ether after food intake, and blood was obtained from the eye vein. The blood was collected in heparinized tubes and was centrifuged at 3,000 rpm for 15 minutes at 4 ℃. Plasma glucose and cholesterol concentrations were determined. After the blood was collected, the liver, heart, kidneys, lungs, and spleen were excised and weighed. The plasma was rapidly frozen at-70 ℃ until further analysis.

Hematocrit levels were determined by using the micro-hematocrit method[28] and the packed red cell volume was measured by using the microcapillary reader. The glucose kit from Young-Dong pharmacy (an adapted the glucose oxidase method[29]) was used to measure the plasma glucose levels by determining the absorbancy at 505 nm. Levels of plasma insulin were determined by using a radioimmunoassay kit (Diagnostic products Co., U.S.A.)[30] in a gamma counter (Peckard, USA). Plasma cholesterol[31,32] was measured using the cholesterol kit (Young-dong pharmaceutical Co.) by measuring absorbancy at 500nm; plasma triglyceride was measured using a triglyceride kit (Young-Dong pharmaceutical Co.) by measuring absorbancy at 545nm, which is an adapted method of Trinder[33]. HDL-cholesterol was measured by the enzymatic method[34]. The activities of aspartate aminotransferase (AST) and alanine aminotransferase(ALT), the activities of AST and ALT were measured by using the Reitman-Frankel method[35].

4. Statistical Analysis

Means and standard deviations were calculated from all data. Significance between experimental groups was determined by the L.S.D. testing method following the application of the ANOVA test using the PC-Stat program.

RESULTS AND DISCUSSION

1. Body weight

Table 1 presents the body weights of the animals according to the experimental treatments. At the end of the experimental period the non-diabetic control group gained weight (+60.4±11.8 g), while the diabetic control group lost weight (-25.6±17.7 g). Among the diabetic groups treated with Ac and Se, the Ac-Se group gained weight (+3.0±29.6 g) compared to the Ac and the Se group. The Ac-Se group appeared to begin gaining weight after 7 days of the treatment, unlike the Ac and Se group.

The rats with STZ-induced diabetes are reported to incur drastic weight reductions and to recover weight only with difficulty, unlike rats with alloxan-induced diabetes[36]. Diabetes is induced by STZ by destroying the β-cells of the pancreas, and the resulting lack of insulin affects the growth and development of the animals due to a reduced energy production from glucose metabolism. Sexton[37] reported that weight reduction occurs in rats treated with STZ because of a reduction in the surface area of blood capillaries where exchange of materials and solutes occurs, and this subsequently results in the shrinkage of skeletal muscles. Se supplementation alone did not affect the weight gain[38]; however the Ac-Se group's weight gain was due to a higher feed efficiency ratio which might be attributed to a slight normalization of glucose metabolism caused by an activation of the Ac aided by Se supplementation.

Average daily food intakes in the non-diabetic control and diabetic-control groups were 18.5g and 30.3g, respectively; thus, the diabetic group showed a very high food intake. Food intake was significantly higher in all

Table 1. Changes in body weight of normal and diabetic rats fed on the butanol fraction of *Alisma canaliculatum* with selenium[1]

Group	Initial(0 day)	7 day	14 day	Final(21 day)	Weight gain
Normal	232.7±3.4[NS,2]	253.7± 4.6[a,3]	268.7± 9.6[a]	293.1±15.0[a]	+60.4±11.8[a]
Diabetic-control	231.2±7.5	211.6± 9.6[c]	200.0±13.0[c]	205.6±18.7[c]	-25.6±17.7[c]
Ac	232.1±8.3	217.4± 7.2[bc]	218.7±16.6[bc]	210.1±31.0[bc]	-22.0±32.9[bc]
Ac-Se	230.4±6.3	223.3±11.3[b]	228.1±24.2[b]	233.4±29.0[b]	+3.0±29.6[b]
Se	232.9±6.3	221.6± 8.8[b]	217.7±19.9[bc]	222.3±22.3[bc]	-10.0±22.9[bc]

1) Values are mean ± S.D., n=7.
2) NS : not significant at the $p<0.05$
3) Values with different superscript within the same column are significantly different at the $p<0.05$ by LSD

diabetic rats compared to the non-diabetic rats, and the feed efficiency ratio in the Ac-Se group (+0.004±0.042) was slightly higher than other diabetic groups (Table 2). The continuous loss of body weight in diabetic rats despite the higher food intakes, compared to the non-diabetic control group, might have been due to a regressive change in body metabolism due to diabetes.

2. Organ weight

Table 3 shows the relative weights of the liver, kidneys, lungs, heart, pancreas, and spleen per 100g body weight. The relative weights of the heart, lungs, and pancreas were not significantly different in all groups. However, the relative weight of the liver was significantly higher in all diabetic rats compared to the non-diabetic control group. It was reported that the increased hepatic weight in diabetic rats can be attributed to the lowered function of insulin causing a breakdown of body fat which in turn increases free fatty acids that are utilized for hepatic triglyceride synthesis and are eventually accumulated in the liver[39]. The weights of the kidneys were significantly higher in all diabetic groups compared to the non-diabetic control group. There were no significant differences between the diabetic control group and the experimental diabetic groups in the weight of the kidney. This may be attributed to the increased glomerular filtration of the large quantity of plasma glucose generated after the inducement of diabetes, which would in turn increase the volume and the size of the kidneys; the increased plasma glucose concentration would induce an increased pentose phosphate pathway, causing increased DNA and RNA synthesis, and consequently increased cell division of the kidneys. Thus, the kidneys become large and heavy[40]. Se supplementation did not affect organ weights[41]. Overall nutritional status rather than Se status affects liver diseases, but the Se-supple- mented diet improved hepatic enzyme activities as well as functional improvement of the kidneys and lipid peroxidation. In the present study the liver and the kidneys were the most affected organs; however, 3 weeks of supplementation with the Ac extract and Se did not reduce the size of the enlarged liver and kidneys. The amount of Se supplementation and the length of the supplementation could have made a difference, and also there could have been differences in the biochemical activities of the organs even though the sizes were not changed.

3. Blood glucose and insulin levels

Non-fasting plasma glucose was analyzed every 4 days, and it was significantly higher in diabetic rats compared to the non-diabetic control rats (Table 4). The increase in blood glucose from day 0 to day 21 were: 0.8% in the control group, 68.5% in the diabetic-control group, 50% in the Ac group, 39.7% in the Ac-Se group, and 37.7% in the Se group, respectively. The rate of increase in blood glucose was lower in the supplementation groups compared to the diabetic-control group. After 21 days of supplementation, the blood glucose levels of the Ac and Se groups were significantly lower than the diabetic control group ($p<0.05$). After 14 days and 21 days, the Se supplemented group had a significantly lower blood glucose level compared to the other diabetic groups ($p<0.05$). The supplementation

Table 2. Diet intake and feed efficiency ratio(FER) in normal and diabetic rats fed on butanol fraction of *Alisma canaliculatum* with selenium(g/day)[1)]

Group	1st week	2nd week	3rd week	Mean	FER
Normal	18.5±0.8$^{c,2)}$	17.1±2.9^{b}	20.0±4.7^{b}	18.5±2.4^{b}	+0.158±0.032^{a}
Diabetic-control	25.6±3.8^{b}	33.4±7.6^{a}	32.0±4.7^{a}	30.3±5.2^{a}	-0.044±0.032^{bc}
Ac	27.5±3.6^{ab}	35.9±5.5^{a}	35.4±9.5^{a}	33.0±5.4^{a}	-0.124±0.212^{c}
Ac-Se	25.6±2.0^{b}	36.9±4.2^{a}	38.2±7.2^{a}	33.6±3.5^{a}	+0.004±0.042^{b}
Se	30.3±4.0^{a}	37.7±5.0^{a}	34.9±4.5^{a}	34.3±4.0^{a}	-0.172±0.035^{bc}

1) Values are mean ± S.D., n=7.
2) Values with different superscript within the same column are significantly different at the $p<0.05$ by LSD

Table 3. Organ weight in normal and diabetic rats fed on the butanol fraction of *Alisma canaliculatum* with selenium(100g/body weight)[1)]

Group	Heart	Kidney[3)]	Liver	Lung	Pancreas	Spleen
Normal	0.36±0.07$^{NS,2)}$	0.33±0.02$^{c,4)}$	3.28±0.46^{b}	0.55±0.05^{NS}	0.30±0.11^{NS}	0.24±0.03^{NS}
Diabetic-control	0.34±0.03	0.65±0.04^{ab}	3.93±0.20^{a}	0.66±0.04	0.24±0.05	0.25±0.05
Ac	0.34±0.02	0.69±0.12^{a}	4.20±0.31^{a}	0.68±0.09	0.25±0.07	0.25±0.03
Ac-Se	0.33±0.02	0.64±0.04^{ab}	4.16±0.31^{a}	0.66±0.33	0.26±0.03	0.27±0.02
Se	0.34±0.03	0.58±0.03^{b}	3.99±0.39^{a}	0.66±0.04	0.23±0.05	0.24±3.30

1) Values are mean ± S.D., n=7.
2) NS : not significant at the $p<0.05$
3) Mean of two kidneys
4) Values with different superscript within the same column are significantly different at the $p<0.05$ by LSD

with the butanol extract of Ac in the present experiment showed similar results to the previous study[24] in terms of lowering blood glucose.

According to reports by Reddi and Bollinei[42], diabetic rats induced by STZ show an increased sensitivity to oxygen free radicals and hydrogen peroxide, the breakdown products of the liver, which impose oxidative stress in diabetes and would damage inner endothelial tissue; this would eventually be directly responsible for high blood glucose. The results of the DCCT (diabetes control and complication trial)[43] showed that high blood sugar is directly responsible for diabetic complications as significant hyperglycemia and hypoinsulinemia reduce glucose absorption; the consequent increase in damaged NADH and NAD+ would result in increased peroxidation products, and the resulting increased free radicals and macrophages would destroy the self-immunity of the pancreatic cells[44,45].

According to research on the antioxidant activities of a hot water extract of Chinese medicine[16], Ac was showen to have an scavenging ability and an electron donating activity; thus the Ac was a possible candidate for antioxidant functional foods. The exact mechanism by which the different fractions of Ac extract lower blood glucose is not clarified yet, but some components of the extract have insulin-like activities and act to either delay breakdown of insulin or catalyze the action of insulin[46,47]; however, the effect of Ac on insulin level and on glucose absorption is not clear[48]. The meaning of insulin resistance is when the effect of insulin reduces in the peripheral tissues. Because high blood sugar levels in diabetes cause insulin resistance, glucose control is a very important issue in diabetes[49]. In the treatment of diabetes, the use of a diuretic may increase insulin resistance. The Ac has been prescribed as a diuretic in Chinese medicine. It is possible that the oxidative-damaged β-cells of the Islet of Langerhans in diabetes could be protected by the Ac and Se in the diet.

According to the research by Lizuka et al[50] Se supplementation reduces high blood sugar, achieves a recovery in the function and structure of the pancreatic β-cells; Se acts like insulin[7] in glucose catabolism, gluconeogenesis, fatty acid synthesis, and the pentose phosphate pathway. In our experiment, the Se group had the lowest blood glucose levels compared to other diabetic groups due to selenium's action on steadying blood glucose; although the Ac-Se group did not have the additional blood glucose lowering effect, the Ac would have stabilized actions lowering blood glucose.

Plasma insulin levels were significantly lower in the diabetic control group (0.08 ± 0.00 mIU/ml) compared to the non-diabetic control group (0.64±0.75 mIU/ml). The Ac-Se group had a slightly higher level of insulin (0.15 ± 0.18 mIU/ml), compared to the other diabetic groups; however, there was no significant difference between the diabetic experimental groups and the diabetic control group (Table 5). The study by Lim and Kim[23] showed that a group supplemented with hexane and $CHCl_3$ extracts of Ac had significantly higher levels of insulin and lower blood glucose in rats; however, there was little correlation between insulin level and plasma glucose level. Se supplementation markedly prevented oxidative damage in the blood, liver and muscles in diabetes, as well as in the blood platelets. It may be that the experiment was done in such a short period of time and the mechanism of Ac-Se supplementation is not clear. Although Ac and Se did not recover the pancreatic cells and did not change the level of insulin significantly, it may have worked as defensive functions which helped reduce the level of plasma glucose.

4. Hematocrit levels and aminotransferase activities

Hematocrit levels were slightly lower in the non-diabetic control group compared to the diabetic groups, even though the difference was not significant (Table 5). According to Dai and McNeill[51] the level of hematocrit for control rats and diabetic rats were 47 - 53% and 46 - 55%, respectively. Brooks[36] also reported that the level of hematocrit in control rats and STZ-induced diabetic rats were 40% and 42%, respectively. In the study of diabetic patients[52], high level of hematocrit were associated with increased insulin resistance and increased risk of non-insulin-dependent diabetes. Hu et al[53] reported that Se supplementation did not affect hematocrit levels and other hematologic

Table 4. Plasma glucose levels in normal and diabetic rats fed on the butanol fraction of *Alisma canaliculatum* with selenium(mg/dl)[1]

Group	0 day	3 day	7 day	10 day	14 day	17 day	21 day
Normal	156.9±24.4$^{c,2)}$	123.1± 9.0^{c}	141.0± 12.9^{c}	173.9± 16.9^{b}	142.1± 8.6^{c}	149.1± 24.6^{c}	158.1± 12.5^{c}
Diabetic-control	479.1±44.0^{ab}	574.6±110.3^{a}	547.3± 81.5^{ab}	636.6±105.1^{a}	713.1± 97.2^{a}	698.8± 91.8^{a}	807.2± 74.4^{a}
Ac	445.8±28.2^{b}	498.0± 46.4^{a}	545.3± 71.5^{ab}	631.9± 83.4^{a}	738.8± 52.9^{a}	632.9±104.0^{ab}	668.5±147.3^{b}
Ac-Se	487.5±23.8^{a}	407.4±102.9^{b}	586.1±170.7^{a}	606.8± 87.0^{a}	687.3± 84.4^{a}	638.9±106.2^{ab}	680.8±138.6^{ab}
Se	475.4±52.2^{ab}	379.6± 96.9^{b}	456.6± 60.3^{b}	615.5±144.9^{a}	558.2±193.9^{b}	548.1±138.4^{b}	654.7±154.3^{b}

1) Values are mean ± S.D., n=7.
2) Values with different superscript within the same column are significantly different at the $p<0.05$ by LSD

indices. Thus the results of previous studies support this research that Se supple- mentation did not affect hematocrit levels.

Liver and heart diseases are associated with diabetic complications. Increased activities of aspartate amino-transferase (AST) and alanine aminotransferase (ALT) are used as indices of liver damage, and the results for these enzymes are presented in Table 5.

The ALT activity was significantly lower in the non-diabetic control group compared to the diabetic control group. AST activity was lower in the non-diabetic control group compared to diabetic experimental groups, even though the difference was not significant. Only in the Se supplementation group were both AST and ALT activities significantly lower than in the diabetic control group. The weight of the liver was similar in all groups, but the activities of AST and ALT are normally high in damaged liver cells causing a fatty liver and liver toxicity. The Se-supplemented group, rather than the Ac-Se supplementation group, showed low ALT and AST activities, and AST activity in particular was very similar to the non-diabetic control group. It appears that Ac and Se both improve the liver functions.

5. Effect on lipid concentrations

Table 6 shows the results for plasma cholesterol levels. Plasma cholesterol levels fluctuated during the experimental period. In the beginning of the experiment plasma cholesterol levels were similar between the different groups. On the fourth day, plasma cholesterol significantly increased in all diabetes-induced rats compared to the non-diabetic control. On the seventh day the Ac-Se group and Ac group showed significantly lower plasma cholesterol levels compared to the diabetic-control group. When diabetes is not under control, there is a hepatic hydroxymethyl glutaryl-CoA (HMG-CoA) reductase and an increase in intestinal HMG-CoA reductase activities which enhances the mobilization of intestinal cholesterol, resulting in hypercholesterolemia and hyperlipidemia[54]. Hsu et al[55] found out that isoflavone reduced total cholesterol. As previous mentioned by study of Nam and Kang[16], Ac is one of antioxidative foods. This present experiment showed that the butanol fraction of Ac, and Se, which is antioxidative could be used effectively to reduce plasma cholesterol.

Table 7 shows that plasma triglyceride levels were lower in the Ac and Se supplemented groups compared to the diabetic control group. The reason why plasma triglyceride increases in diabetes is because blood free fatty acid is converted to triglyceride more rapidly than normal[56]. The results of the present experiment agree with previous research[57] where plasma cholesterol levels increased significantly in the diabetic-control group compared to the non-diabetic control group; this may be attributed to the abnormal glucose metabolism which induced impairment in lipid metabolism.

Plasma HDL-cholesterol levels were significantly

Table 5. Insulin and hematocrit levels, ALT and AST activities in normal and diabetic rats fed on the butanol fraction of *Alisma canaliculatum* with selenium[1)]

Group	Insulin(mIU/ml)	Ht(%)	ALT(KA unit/L)	AST(KA unit/L)
Normal	0.64 ± 0.75^{a}	$44.9\pm1.9^{b,2)}$	35.5 ± 5.1^{c}	90.6 ± 20.4^{bc}
Diabetic-control	0.08 ± 0.00^{b}	46.3 ± 2.0^{ab}	131.4 ± 75.3^{a}	124.7 ± 37.6^{ab}
Ac	0.08 ± 0.00^{b}	47.6 ± 2.6^{a}	83.6 ± 9.9^{b}	103.8 ± 12.5^{bc}
Ac-Se	0.15 ± 0.18^{b}	47.3 ± 1.6^{a}	91.4 ± 27.2^{b}	144.6 ± 58.3^{a}
Se	0.11 ± 0.06^{b}	47.6 ± 2.4^{a}	77.3 ± 10.7^{b}	83.5 ± 24.1^{b}

1) Values are mean ± S.D., n=7.
2) Values with different superscript within the same column are significantly different at the $p<0.05$ by LSD

Table 6. Plasma cholesterol levels in normal and diabetic rats fed on the butanol fraction of *Alisma canaliculatum* with selenium(mg/g)[1)]

Group	0 day	3 day	7 day	10 day	14 day	17 day	21 day
Normal	$67.0\pm12.4^{NS,2)}$	$80.3\pm11.9^{b,3)}$	77.7 ± 16.5^{c}	75.5 ± 16.0^{b}	85.1 ± 15.9^{b}	72.8 ± 10.7^{b}	62.7 ± 6.7^{c}
Diabetic-control	71.4 ± 4.3	111.0 ± 11.0^{a}	121.6 ± 19.9^{a}	115.0 ± 21.5^{a}	119.6 ± 14.5^{a}	115.4 ± 11.8^{a}	122.3 ± 39.4^{a}
Ac	67.6 ± 8.2	121.8 ± 43.0^{a}	98.6 ± 13.9^{b}	100.8 ± 12.0^{a}	124.0 ± 30.2^{a}	102.9 ± 15.6^{a}	90.5 ± 8.1^{b}
Ac-Se	94.3 ± 45.6	101.7 ± 15.5^{ab}	100.1 ± 12.3^{b}	104.1 ± 13.8^{a}	129.1 ± 33.1^{a}	114.2 ± 17.6^{a}	91.6 ± 29.0^{b}
Se	76.0 ± 31.3	105.9 ± 18.4^{a}	116.1 ± 26.8^{ab}	101.2 ± 12.4^{a}	120.3 ± 28.6^{a}	103.0 ± 6.9^{a}	88.6 ± 14.5^{b}

1) Values are mean ± S.D., n=7.
2) NS : not significant at the $p<0.05$
3) Values with different superscript within the same column are significantly different at the $p<0.05$ by LSD

higher in the diabetic-control group, and this difference was not observed between the diabetic-control group and other diabetic groups (Table 7). The Ac group showed somewhat reduced plasma HDL-cholesterol levels compared to the diabetic-control group and other groups. Although the reduction in blood glucose could have led to a reduction in triglycerides, this did not affect the levels of HDL-cholesterol. The presence of higher levels of HDL-cholesterol is associated with a lower incidence of atherosclerosis[58].

The level of free fatty acids was significantly higher in the diabetic-control group, and significantly lower in the Ac-Se group, compared to the diabetic-control group; levels of free fatty acids in the Ac-Se group were at the level of the non-diabetic control group. According to a previous report[59], increased free fatty acid levels increase resistance to insulin, and the accumulation of triglycerides in β-cells inhibits insulin production by increased fat oxidation and increased fatty acyl-CoA (even though the mechanism is not clear). Free fatty acids and triglycerides stimulate ROS production in white blood cells and increase oxidation stress. But Se and the butanol fraction of Ac supplementation may carry phytochemicals which could have inhibited the production of active oxygen, and resulted in the changed level of lipid metabolism.

CONCLUSION

This experiment is a study related to the prevention and treatment of diabetes. The effects of the butanol fraction of *Alisma canaliculam* (AC), a plant which has been used as a folk remedy to reduce plasma glucose level, and selenium (Se, Na_2SeO_3) which functions as an antioxidant in diabetes, were studied. Ac and Se were supplemented to the diet of rats for 3 weeks and the effects on plasma glucose and lipids were determined.

The experimental animals were Sprague-Dawely rats weighing approximately 230 g. In some rats, streptozotocin (45mg/kg body weight) was injected into the tail vein in order to induce diabetes. The rats treated with streptozotocin were divided into 4 groups: the diabetic-contol group, the Ac group, the Ac-Se group and the Se group. These four groups were compared with the streptozotocin-untreated group (non-diabetic control). Animals were given the AIN-93 diet and water ad *libitum*. The Ac groups were orally given 400mg of a butanol fraction of Ac/kg body weight, once a day. The groups treated with Se were given 2mg Se/kg of feed. The AC-Se group was given both Ac and Se. Food intakes and body weight were measured at a fixed time every day. After sacrifice, hematocrit levels, and plasma levels of glucose, insulin, cholesterol, HDL-cholesterol, triglycerides and free fatty acids were determined; AST and ALT activities were also measured.

During the 3 weeks experimental period, weight gain was significantly higher in the Ac-Se group compared to the diabetic control group. and plasma glucose levels were shown to be significantly lower in the Ac and Se group compared to the diabetic control group. Plasma cholesterol levels were significantly lower in the Ac, Se, and Ac-Se groups compared to the diabetic control group. Plasma triglyceride levels were not shown to be different between the diabetic-control group and other treated groups, but tended to be lower in the Ac group and the Se group compared to Ac-Se group. Plasma free fatty acid levels were significantly lower in the Ac-Se group. AST activity was the lowest in the Se group

The results have shown that the butanol fraction of Ac, and Se, could improve plasma lipid metabolism.

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Table 7. HDL-cholesterol, free fatty acid(FFA) and triglyceride(TG) levels in normal and diabetic rats fed on the butanol fraction of *Alisma canaliculatum* with selenium[1]

Group	HDL-cholesterol (mg/dl)	FFA (μEq/L)	TG (mg/dl)
Normal	44.0± 7.2[b,2]	695.7±136.6[a]	52.9±25.0[b]
Diabetic-control	65.3±14.4[a]	954.6±240.9[b]	80.2±23.5[a]
Ac	57.8± 9.2[a]	776.6±195.4[ab]	62.9±19.2[ab]
Ac-Se	64.0± 8.9[a]	688.8±147.5[a]	83.2±29.4[a]
Se	61.7± 7.1[a]	787.7±103.6[ab]	74.7±14.0[ab]

1) Values are mean ± S.D., n=7.
2) Values with different superscript within the same column are significantly different at the $p<0.05$ by LSD

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