Improvement of Diabetic Complication by Hydrangea Dulcis Folium in Streptozotocin-Induced Diabetic Rats

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The improvement of diabetic complications such as lipid lowering and anti-oxidative potential of Hydrangea Dulcis Folium (HDF) was studied in streptozotocin-induced diabetic rats. Male Sprague-Dawley rats were divided into 3 groups after induction of streptozotocin (STZ)-diabetes: normal control; diabetic control; diabetic-HDF supplement (hot water extract 40 g/kg diet); and fed experimental diets for 3 weeks. Serum glucose and insulin concentrations, serum lipid profile, intraperitoneal glucose tolerance test, and liver cytosolic antioxidant enzyme levels were measured. The HDF supplement significantly decreased serum glucose concentration, increased insulin level, and improved glucose homeostasis in diabetic control rats. The total cholesterol and triglyceride concentrations in the serum and liver were markedly reduced by HDF treatment in STZ-diabetic rats. Moreover, low density lipoprotein (LDL)-, VLDL-, and high density lipoprotein (HDL)-cholesterol levels were ameliorated in HDF supplemented diabetic rats. Decreased fecal excretions of cholesterol, triglyceride, and bile acid in diabetic rats were significantly increased by HDF consumption. HDF supplement reversed the effects of the oxidative stress system of liver in diabetic rats. Lipid peroxidation of diabetic rats, assessed by thiobarbituric acid reactive substance (TBARS), as well as superoxide dismutase (SOD) activities were significantly increased, and glutathione contents were decreased in diabetic rats. HDF supplement reverted these parameters to near normal value. Our data suggest that HDF supplement could be used to improve the glucose and lipid metabolism as well as to reduce the imbalance between the generation of reactive oxygen species (ROS) and the scavenging enzyme activity in preventing diabetic complications.

Key words  streptozotocin-diabetes; Hydrangea Dulcis Folium; insulin; glucose tolerance; lipid profile; antioxidant enzyme

Diabetes is a major risk factor for premature atherosclerosis, and oxidative stress plays an important role since diabetic monocytes produce increased superoxide anion (O$_2^-$). Hyperglycemia may lead to increased generation of free radicals via multiple mechanisms. The chronic presence of high glucose levels enhances the production of reactive oxygen species (ROS) from protein glycation and glucose autoxidation, which in turn catalyze lipid peroxidation. Accordingly, disturbances of antioxidant defense systems in diabetes have been reported; alteration in antioxidant enzymes, impaired glutathion metabolism, and decreased ascorbic acid levels. Some studies on diabetes reported a significant increase in the products of both plasma and tissue lipid peroxidation compared with control. These findings resulted in new approaches to the treatment of diabetic patients, and the relevant studies focus on the support of antioxidant systems.

Insulin deficiency stimulates lipolysis in the adipose tissue, and gives rise to hyperlipidemia and fatty liver. Accordingly, although diabetes is characterized as a disease of carbohydrate metabolism, abnormalities of lipid and lipoprotein metabolism are commonly observed. Hyperlipidemia, atherosclerosis and gallstones are frequently associated with diabetes, indicating alterations in cholesterol metabolism in this disease. Chylomicron and very-low-density lipoprotein (VLDL) remnants accumulate, leading to high levels of potentially atherogenic particles. In addition, there is triglyceride enrichment of both high-density lipoprotein (HDL), and low-density lipoprotein (LDL). Triglyceride enrichment of these particles leads to decreased levels of HDL and small, dense LDL particles. Therefore, the lipid profile in type 2 diabetic subjects generally consists of elevated triglycerides and LDL cholesterol, and reduced HDL cholesterol.

Recently, herbs have begun to attract attention as functional foods and raw materials for the development of drugs. Herbal medicines derived from plants are being increasingly utilized to treat a wide variety of clinical diseases. Hydrangea Dulcis Folium (HDF, Amacha in Japanese, Kamrocha in Korean), the fermented and dried leaves of Hydrangea macrophylla Serringe var. thunbergii Makino, is a saxifragaceous plant with anticoagulidial, antifungal, anti-ulceric, anti-allergic, anti-hypercholesterolemic, and anti-oxidative activities. Recently Zhang et al. reported that hydrangenol, isolated from H. macrophylla var. thunbergii (Hydrangea Dulcis Folium), lowered blood glucose and free fatty acid levels in KK-A$^+$ mice. In present study, we extended the work to examine the possible anti-diabetic mechanism of HDF in streptozotocin (STZ)-induced diabetic rats as a model system of type-1 diabetes, an example of elevated blood lipids and oxidative stress.

MATERIALS AND METHODS

Materials and Chemicals Hydrangea Dulcis Folium (HDF) was purchased from Gamrowon (Kangwondo, Korea) in August 2005 and the sample was authenticated by college of Oriental medicine, Semyung University, where the voucher specimen was preserved. The water extract was prepared twice with hot water (100 °C) for 24 h. The extract was filtered through Whatman No. 1 filter paper and evaporated under vacuum at 40 °C. The yield after vacuum evaporation was 9.8%. Most chemicals used in this study were obtained.
from Sigma (St. Louis, MO, U.S.A.) unless otherwise noted. 

**Animals and Diets** Twelve weeks old male Sprague-Dawley rats (Orient, Seoul, Korea) weighing between 200 and 230 g were allowed to acclimatize on a commercial standard rodent pellet food (NIH #31M, Samtako, Korea). Animals were individually housed in cages under 24±2°C, 55±5% humidity with a 12 h light–dark cycle (07:00—19:00). One week later, rats were randomly divided into 3 groups (n=8); normal control (NC), diabetic control (DC), and diabetic-HDF supplements (DH). Insulin deficient type 1 diabetes was induced by single intraperitoneal injection of STZ (50 mg/kg, i.p.) freshly dissolved in citrate buffer (0.01 M, pH 4.5). Control rats received the same volume of vehicle. Tail bleeds were performed 24 h post-injection and animals with a fasting blood glucose concentration above 300 mg/dl were considered diabetic. Normal and diabetic control rats were fed AIN-93 based semi-purified standard diet, and experimental groups were supplemented with 4% HDF extracts (w/w). The composition of the standard diet was as follows (g/100 g): casein, 16.0; DL-methionine, 0.3; corn oil, 10.0; sucrose, 15.0; mineral mix (AIN-93), 3.5; vitamin mix (AIN-93), 1.0; choline bitartarate, 0.2; cellulose, 4.0; and corn starch, 50.0. Body weight and food intake were measured twice a week. Feces were collected for the last 3 d to obtain the cytosol supernatant. The protein amount in the cytosolic fraction was measured by the method of Lowry et al.22) with bovine serum albumin as a standard.

**Intraperitoneal Glucose Tolerance Test (IPTG) and Analytical Procedures** After 3 weeks of experimental periods, rats were fasted overnight and injected with glucose (50 mg/kg, i.p.). Blood samples were collected from the tail vein just prior to and 30, 60, and 90 min after glucose loading, and serum glucose levels were measured using an One touch Basic glucose measurement system (Life Scan Inc., U.S.A.). Rats were killed by decapitation immediately after 90 min blood sample was taken and the blood samples were collected. Serum concentrations of total cholesterol (TC), HDL-cholesterol (HDL-C), and triglyceride (TG) were measured enzymatically using commercially available kits (Youngdong Pharmaceutical Co., Korea). Serum LDL-cholesterol (LDL-C) and VLDL-cholesterol (VLDL-C) levels were measured according to the protocol of Friedwald et al.19) Serum insulin concentrations were determined by using radioimmunoassay kit (Boehringer Mannheim, Germany). The hepatic lipids were extracted using the procedure developed by Folch et al.,20) and the TC and TG concentrations were measured using the same kits as serum. Fecal steroids excretions were assessed by cholesterol and bile acid levels in feces. After extracted with ethyl ether, cholesterol was measured same as liver samples. Bile acid was assayed from the enzymatic method described by Fausa and Skalhegg21) in ethanol extracts of feces.

**Liver Cytosol Preparation** The livers were washed with saline and homogenized in 100 mM K-phosphate buffer, pH 7.4. The homogenate was centrifuged at 600 × g for 10 min, and the supernatant was further centrifuged at 10000 × g for 20 min to remove mitochondria pellet. Finally, the supernatant was further ultracentrifuged at 105000 × g for 60 min to obtain the cytosol supernatant. The protein amount in the cytosolic fraction was measured by the method of Lowry et al.22) with bovine serum albumin as a standard.

**Antioxidant Enzyme Activity and TBARS Concentration of Liver Cytosol** As a marker of the lipid peroxidation product, the hepatic malondialdehyde content was measured using the method of Uchiyama and Mićara23) based on the thiobarbituric acid reaction substance (TBARS). Gluthatione (GSH) content was measured by the enzymatic method of Akerboom and Sies.24) Superoxide dismutase (SOD) activity was determined by the method of Yuan et al.25)

**Statistical Analysis** Data were expressed as mean ± S.E.M. One-way ANOVA was used to determine treatment effects. Differences among means were inspected using Dun-can’s multiple range test and were considered to be significant at p<0.05.

**RESULTS**

**Anti-diabetic Effect** There was a significant increase in food intake by STZ-treatment, and HDF supplement reduced the food intake to near normal control value (Table 1). Daily food intakes of normal, diabetic control rat, and HDF supplemented diabetic rat were 15.4±0.7, 19.7±0.9, and 13.4±1.4 g, respectively. However, induction of diabetes caused significant weight loss resulting in negative body weight gain in diabetic rats. Administration of HDF significantly prevents the weight loss in these rats. Fasting glucose levels were about 4-fold elevated in STZ-diabetic rats, and HDF supplement significantly decreased STZ-induced hyperglycemia by

| Table 1. Effects of Hydrangea Dulcis Folium (HDF) on Body Weight, Food Intake, Serum and Liver Lipids Profile |
|-----------------------------------------------------|-----------------------------------------------------|-----------------------------------------------------|
| **Body weight gain, g/3 week**                       | **Diabetic control**                                 | **HDF** |
| Normal control                                      | −85.1±15.2a                                         | −27.5±18.9a                                         |
| Food intake, kcal/3 week                            | 1338.9±60.8a                                        | 1108.7±115.8a                                       |
| **Serum**                                           |                                                     |                                                     |
| Fasting glucose (mg/dl)                             | 107.3±6.2a                                          | 419.4±32.8b                                         |
| Insulin (µU/ml)                                     | 22.1±1.2a                                           | 10.2±1.0b                                           |
| Total cholesterol, mg/dl                            | 62.8±6.8a                                           | 84.3±1.8b                                           |
| HDL cholesterol, mg/dl                             | 33.3±2.7                                            | 39.8±2.7                                            |
| LDL cholesterol, mg/dl                             | 15.3±3.1a                                           | 26.4±1.3b                                           |
| VLDL cholesterol, mg/dl                            | 10.1±2.1a                                           | 18.1±3.0b                                           |
| Triglyceride, mg/dl                                | 50.5±10.4a                                          | 90.7±14.7a                                          |
| **Liver**                                           |                                                     |                                                     |
| Cholesterol, mg/g                                   | 4.3±0.4a                                            | 7.0±1.3b                                            |
| Triglyceride, mg/g                                  | 16.4±2.0a                                           | 20.7±3.4b                                           |

Values are means ± S.E.M. (n=8); means in same row with different superscripts are significantly different (p<0.05). a: Hydrangea Dulcis Folium.
level in rats with STZ-diabetes. Conversely, there was a significantly increased the level of TBARS and activities of SOD to normal 4 folds above normal levels. Administration of HDF de-
babetic rats, as measured by TBARS, as well as SOD was 2.5—
tive stress system in diabetic rats. Lipid peroxidation of dia-
HDF supplement reversed the effects of the elevated oxida-
centrations and activities of oxidative stress biomarkers.

Serum glucose concentrations after glucose loading in STZ-diabetic rats exhibited significantly higher level during all time points determined compare with normal rats (Fig. 1). The peak increase in serum glucose concentrations in diab-
etic was observed after 60 min of glucose treatment, while that of normal rats observed after 30 min, indicating delayed glucose homeostasis in diabetic rats. HDF supplement showed significant decrease in serum glucose concentration at all time points measured when compared with dia-
etic control rats. Moreover, these groups revealed dramati-
cally improved glucose tolerance showing similar patterns as normal control group.

**Hypolipidemic Effect** The total cholesterol and triglyc-
eride concentrations in the serum and liver were significantly higher in STZ-diabetic rats than normal control animals. The supplementation of HDF suppressed the increase in these parameters in serum and liver of the diabetic rats (Table 1). Similar results were observed for the serum VLDL- and LDL-cholesterol levels. The HDL-cholesterol concentration was not different among groups.

**Fecal Lipid Excretion** Fecal dry weight was signific-
antly higher in diabetic rats than in control due to diabetes-
duced hyperphagia. HDF supplementation weakened its hyperphagia, and the fecal weight was decreased in these rats. Fecal excretions of TG, cholesterol, and bile acid were markedly reduced in STZ-diabetic rats compared with nor-
mal control rats (Table 2). HDF supplement markedly in-
creased fecal TG, cholesterol, and bile acid excretions to near normal values.

**Anti-oxidative Effect** Table 3 shows liver cytosolic concentra-
tions and activities of oxidative stress biomarkers. HDF supplement reversed the effects of the elevated oxida-
tive stress system in diabetic rats. Lipid peroxidation of diab-
etic rats, as measured by TBARS, as well as SOD was 2.5—
4 folds above normal levels. Administration of HDF de-
creased the level of TBARS and activities of SOD to normal level in rats with STZ-diabetes. Conversely, there was a sig-
nificant reduction in the level of GSH by diabetes and it was dramatically increased by HDF supplementation.

### DISCUSSION

STZ-induced diabetes in the rat has been used to study hypercholesterolemia, and has been shown to be sensitive to cholesterol metabolism. We examined the lipid lowering and anti-oxidative potential of HDF in STZ-induced diabetic rats. To our knowledge, this is the first study to show the hypoglycemic, hypolipidemic and anti-oxidative effect of HDF in diabetic rats.

It was evident from the results that HDF reduced the blood glucose and increased insulin level in STZ-induced diabetic rats. The increase in serum insulin level after HDF treatment could be linked to more than one mechanism. The possible mechanism includes the quantitative changes in pancreatic β-
cells by differentiation and proliferation of residual pancre-
atic β-cells after STZ administration. We have observed the increased β-cell mass after HDF treatment in db/db mice (data not presented). It may be also attributable to increased insulin synthesis or secretion by residual β-cells similar to that observed after glibenclamide administration. HDF also acts as a hepatoprotective agent, suggesting enhanced the transport of blood glucose to peripheral tissues and its utilization, which may be another mechanism of action. Nakagiri et al. reported that HDF suppressed α-galactosamine-induced GPT level and the hepatoprotective effect was more potent than milk thistle or tumeric powder. We have also ob-
erved the normalized liver weight by HDF treatment in STZ diabetic rats (data not presented).

The levels of serum lipids are usually elevated in diabetes mellitus, and such an elevation represents the risk of coro-
nary heart disease. In the STZ-induced diabetes, the rise in blood glucose is accompanied by an increase in various blood lipids. Consumption of HDF brought the levels of these blood lipids to near normal values. The effect of HDF

### Table 2. Effects of Hydrangea Dulcis Folium (HDF) on Fecal Steroid Excretion

<table>
<thead>
<tr>
<th></th>
<th>Normal control</th>
<th>Diabetic control</th>
<th>HDFsupplemented group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Feces weight (dry), g/3 d</td>
<td>5.9±0.4a</td>
<td>15.8±1.8b</td>
<td>10.9±0.8a</td>
</tr>
<tr>
<td>Fecal steroid</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Triglyceride, mg/d</td>
<td>70.4±6.1a</td>
<td>24.2±1.5b</td>
<td>54.3±0.4a</td>
</tr>
<tr>
<td>Cholesterol, mg/d</td>
<td>155.9±6.1a</td>
<td>55.7±3.1b</td>
<td>109.4±5.3c</td>
</tr>
<tr>
<td>Bile acid, mg/g feces</td>
<td>69.7±6.2a</td>
<td>39.3±4.4a</td>
<td>59.8±4.3c</td>
</tr>
</tbody>
</table>

Values are means±S.E.M. (n=8); means in same row with different superscripts are significantly different (p<0.05). a) Hydrangea Dulcis Folium. b) TBARS; thiobarbi-
turic acid reactive substance: mg/mg protein. c) µmol/mg protein. d) Superoxide dismutase: units/mg protein/min.

### Table 3. Liver Cytosolic Contents of Thiobarbituric Acid Reactive Substance (TBARS), Gluthathione, and Activities of Superoxide Dismutase

<table>
<thead>
<tr>
<th></th>
<th>Normal control</th>
<th>Diabetic control</th>
<th>HDFsupplemented group</th>
</tr>
</thead>
<tbody>
<tr>
<td>TBARSb</td>
<td>9.2±0.8a</td>
<td>23.0±3.5b</td>
<td>9.2±0.6a</td>
</tr>
<tr>
<td>Gluthathionec</td>
<td>3.5±0.2a</td>
<td>1.0±0.1b</td>
<td>4.9±0.9b</td>
</tr>
<tr>
<td>SODd</td>
<td>52.4±5.1a</td>
<td>214.5±8.7b</td>
<td>51.8±6.1a</td>
</tr>
</tbody>
</table>

Values are means±S.E.M. (n=8); means in same row with different superscript are significantly different (p<0.05). a) Hydrangea Dulcis Folium. b) TBARS; thiobarbi-
turic acid reactive substance: mg/mg protein. c) µmol/mg protein. d) Superoxide dismutase: units/mg protein/min.
on diabetic hyperlipidemia could be through its control of hyperglycemia. Laakso suggested that the level of glycemic control is the major determinant of total and VLDL-TG concentrations. Moreover, improved glycemic control following sulfonylurea therapy decreased the serum VLDL and TG concentrations.

Several hypotheses of the mechanism(s) causing hypocholesterolemic effects have been proposed. The most frequently suggested mechanism is interference with intestinal cholesterol and bile acid absorption, leading to an increase in fecal neutral sterol and bile acid excretion. In the present study, excretion of fecal cholesterol and bile acid was significantly greater in HDF group than in the diabetic control group, suggesting lower intestinal absorption of cholesterol following HDF consumption. These results coincide with the serum and hepatic effect in these groups.

The increase of free radical mediated-toxicity is well documented in clinical diabetes and STZ-diabetic rats. The elevated levels of toxic oxidants in diabetic animals are due to the processes such as glucose oxidation and lipid peroxidation. STZ-diabetic rats, a model of type-1 diabetes mellitus, are characterized by several irregularities in endogenous antioxidant enzymes. In the present study, we have observed significant decrease in blood glucose and elevated insulin level when STZ-diabetic rats were administered HDF. The capacity of HDF to improve hyperglycemia is an essential trigger for the liver to revert to its normal homeostasis during experimental diabetes. The concentration of liver cytosolic TBARS was significantly increased after the induction of diabetes, whereas administration of HDF decreased the level of TBARS. An increase in lipid peroxide concentration in the liver of diabetic animal has been observed. GSH is the most important biomolecule against chemically induced toxicity and can participate in the elimination of reactive intermediates by reducing hydroperoxides in the presence of GSH-Px. GSH also functions as free radical scavenger and in the repair of radical-induced biological damage. The decrease in GSH level represents increased oxidative stress in these animals.

ROS scavenging enzymes can respond to conditions of increased oxidative stress with a compensatory mechanism that increases the enzyme activity in diabetic rats. In the current study, the hepatic SOD activity was significantly increased in diabetic rats compared with normal control rats probably to increase dismutation of superoxide anions. Insulin deficiency promotes β-oxidation of fatty acids with resulting increase in H₂O₂. H₂O₂ has been reported to be an inducer of SOD in tissues and hence an increase in SOD activity is observed in diabetic states, which correlate with our results. Also, increase in SOD activity could be due to its induction by increased production of superoxide (·O₂⁻) radical. The current findings suggest that diabetic rats were exposed to oxidative stress, but HDF supplementation can, in part at least, reduce the imbalance between the generation of ROS and the scavenging enzyme activity.

Present study suggest that HDF supplement could be used to improve the glucose and lipid metabolism as well as to reduce the imbalance between the generation of ROS and the scavenging enzyme activity in diabetic conditions. The mechanism underlying the protective action of HDF remains to be defined.

REFERENCES