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Effect of *Cornus officinalis Sieb. et Zuccha* Extracts on Physiological and Antioxidative Activities in Streptozotocin Induced Diabetic Rats

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This investigation was performed to study the antioxidant activities of *Cornus officinalis Sieb* extracts and the effect of *Cornus officinalis Sieb* extracts on glucose, lipid metabolism in diabetic rats. DPPH free radical scavanging activitiy and superoxide anion radical scavenging of *Cornus officinalis Sieb* extracts were 94.7% and 92.1%, respectively. Streptozotocin (45 mg/kg body weight, i.p.) induced diabetic rats showed a significant increases of plasma glucose, triglyceride and total cholesterol, concomitantly significant decrease of plasma high density lipoprotein. Glutathione level were decrease in cytosol of liver, lung and brain tissue of rats. Lipid peroxide were increase in microsome of liver cells. Group 1 and 2 were treated with *Cornus officinalis Sieb* extracts 200 mg/kg body weight and 100 mg/kg body weight for 24 days, individually. Group 1 and 2 rats showed decreased plasma glucose, triglyceride, total cholesterol and lipid peroxide in microsome of liver, and increased plasma high density lipoprotein and glutathione in cytosol of liver, lung and brain. The result suggest that *Cornus officinalis Sieb* extracts may normalize the impaired antioxiants status in streptozotocin induced diabetic rats. *Cornus officinalis Sieb* extracts were used to improve the imbalance between free radicals and antioxidant system due to the diabetes.

Key Words: Polyphenol, Diabetes, Antioxidant, Lipid peroxide

INTRODUCTION

Cornus is a traditional medicine used in China, Japan, and Korea (Guilian, 2000). According to ancient records, *Cornus* is recommended to reinforce the muscles and lungs, lower blood pressure, improve the tone of the liver and kidneys (Fikret and Smail, 2003). Recently, *Cornus* that contain the same components as *Cornus* have been the focus of medical research (Lee, 2000). *Cornus* tea, the aqueous extract of *Cornus*, is already known as a functional health food and commonly used in the treatment of allergy (Seo, 2002), plus the extract of *Cornus* has been suggested to have recuperative effects for diabetes and a liver function. (Oberley, 1988; Joo and Jang, 1989) reported that the polyphenol exhibits antioxidant activity toward diabetes models with a good correlation between the polyphenol and the antioxidant activity. Accordingly, the aim of the current study was to investigate the effect of *Cornus* on hyperglycemia and antioxidant in streptozotocin (STZ)-induced diabetic rats.

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MATERIALS AND METHODS

1. Preparation of plant extract

The *Cornus officinalis Sieb. et Zuccha* were first washed well and dried at room temperature and then chopped. The *Cornus* was extracted with 80% methanol by soxhalation and then filtered. Methanol was evaporated in a rotary evaporator at 40~50℃ under reduced pressure. The yield was 3.2 g/100 g.

2. Estimation of the phenolic content

The total concentration of phenols in the extract was determined according to the Folin Ciocalteu method (Kim et al., 2003). The absorbance was read at 750 nm, using a spectrophotometer in a 10 mm cuvette. The total phenol concentration was calculated from the calibration curve, using gallic acid as a standard, and the results were expressed on

a dry weight basis as mg/10 g gallic acid equivalents (GAE).

3. Determination of total flavonoids

Total flavonoids were measured by a colorimetric assay (Kim, 2003). A 1 ml aliquot of appropriately diluted sample or standard solutions of catechin was added to a 10 ml volumetric flask containing 4 ml ddH_2O. At zero time, 0.3 ml 5% $NaNO_2$ was added to the flask. After 5 min, 0.3 ml 10% $AlCl_3$ was added. At 6 min, 2 ml 1 M NaOH was added to the mixture. Immediately, the reaction flask was diluted to volume with the addition of 2.4 ml of ddH_2O and thoroughly mixed. Absorbance of the mixture, pink in colour, was determined at 510 nm versus prepared water blank. Total flavonoids of fruits were expressed on a dry weight basis as mg/10 g catechin equivalents (CE).

4. Evaluation of antioxidant activity using the DPPH method

The antioxidant activity was determined using the DPPH test (Hiroshi and Mitsuaki, 1999). Different dilutions of the phenolic extract were prepared for each variety. An aliquot of 0.3 ml of diluted sample was added to 2.7 ml DPPH solution and vortexed. The decrease in the absorbance was determined at 575 nm when the reaction reached the plateau, using an spectrophotometer in a 10 mm quartz cuvette. Methanol was used to zero the spectrophotometer. The absorbance of the DPPH radical without sample was measured. The ratio [phenolic] (1 g)/[DPPH] (1 g) was plotted against the % of remaining DPPH to obtain the amount of sample necessary to decrease the initial DPPH concentration by 50% (IC_{50}).

5. Animals and experimental design

Rats aged 4~5 weeks and weighed 150~200 g, maintained in metabolic cages in a temperature-controlled (25℃) room were submitted to a 12-h dark/light cycle (artificial lights, 7A.M.-P.M.). The rats were divided into six groups comprising of six animals in each group as follows: N: Normal control rats receiving 0.1 M citrate buffer (pH 4.5), D: Diabetic controls, DV: Diabetic rats given α-tocopherol acetate (200 mg/kg b.w/day) in corn oil orally for 33 days, DC 100 and DC 200: Diabetic rats given *Cornus* extracts (100 and 200 mg/kg b.w/day) in aqueous solution orally for 33 days, DA: Diabetic rats given Acarbose (BAYER) (20 mg/kg b.w/day) in aqueous solution orally for 33 days.

6. Diabetes model

The animals received a dose of 50 mg/kg of streptozotocin (Sigma), dissolved in citrate buffer 0.1 mol/l, pH 4.4, injected intraperitoneally. After 3 days, the diabetes developed with 300 mg/dl of glycemia determined by Glucocard II (ARKRAY).

7. Biochemical parameters

All animals were monitored weekly throughout the experiment. Blood was collected by tail vein and was placed in tubes for centrifugation at 3,000 rpm for 10 min in order to obtain the serum. The total cholesterol and lipoproteins fraction, HDL-cholesterol and triglycerides, were measured by an enzymatic assay (ASAN). At the end of the experiment, liver was collected by perfusion method and obtain microsome from liver and brain by centrifugation method.

8. Determination of Malondialdehyde (MDA)

Malondialdehyde (MDA) levels were estimated by the heating method (Buege, 1978). The principle of the method is the spectrophotometric measurement of the color generated by the reaction of thiobarbituric acid (TBA) with MDA. For this purpose, 15% trichloroacetic acid solution was added to 0.3 ml supernatant in each centrifuge tube and the tubes were placed in a boiling water bath for 15 min. After cooling in tap water, the tubes were centrifuged at 1,000 g for 10 min and 2 ml of the supernatant was added to 0.375% TBA solution in a test tube. The tube was then placed in a boiling water bath for 15 min. The solution was then cooled in tap water and its absorbance was measured using a spectrophotometer at 535 nm. The concentration of MDA was calculated by the absorbance coefficient of the MDA-TBA complex and is expressed as nM/ mg protein.

9. Determination of Reduced Glutathione (GSH)

Reduced glutathione was estimated by the method (Ellman, 1959). To cytosol, added 2.0 ml of 0.6 mM DTNB reagent and 0.2 M phosphate buffer (pH 8.6) to a final volume of 4.0 ml. The absorbance was read at 412 nm against a blank containing buffer instead of sample. A series of standards treated in a similar way also run to determine the glutathione content. The amount of glutathione is expressed as nM/mg protein.

10. Statistical analysis

Results are presented as mean value standard deviation. Statistical analysis between experimental results was based on AVOVA. Significant difference was statistically considered at the level of $P<0.001$ or $P<0.05$.

RESULTS

1. Polyphenol and flavonoid contents

The total phenolic contents of the dry *Cornus* per 10 g was at the level of 11.7±0.9 mg GAE. The content of total flavonoids in 10 g dry *Cornus* was at the level of 2.9±0.2 mg CE.

Table 1. Antioxidative activities of *Cornus officinalis* extracts by DPPH free radical scavenging activity and Superoxide anion radical scavenging activity

Sample	IC_{50} (mg/ml)
	DPPH
Total pholyphenol of *Cornus*	0.057±0.002
Flavonoid of *Cornus*	0.018±0.001
α-tocopherol	0.073±0.001
BHT	1.038±0.001

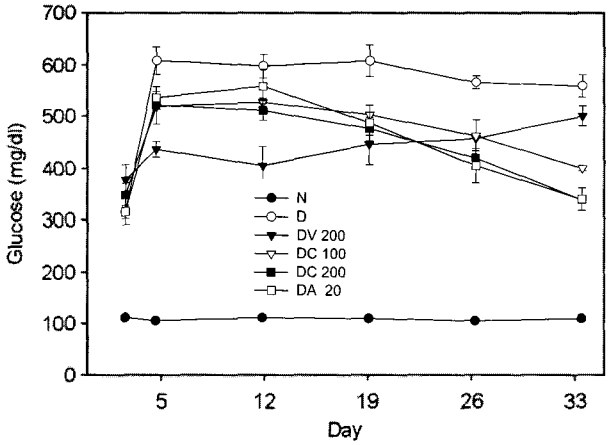

Fig. 1. Changes in blood glucose level by the oral administration of *Cornus officinalis Sieb. et Zuccha* extracts in streptozotocin-induced diabetic rats.
*Group N-normal control 0.9% NaCl oral administration, D-diabetes control: diabetic rats induced by streptozotocin 0.9% NaCl oral administration, DV-diabetic rats in streptozotocin-induced α-tocopherol acetate 200 mg/kg body weight oral administration, DC 100 and DC 200-diabetic rats induced by streptozotocin *Cornus* extracts 100 mg/kg body weight and 200 mg/kg body weight oral administration, DA-diabetic rats induced by streptozotocin Acarbose 20 mg/kg body weight oral administration.

2. DPPH radical scavenging activity

The concentration of an antioxidant needed to decrease the initial DPPH concentration by 50% (IC_{50}) is a parameter widely used to measure antioxidant activity (Concepcion et al., 1998). The lower the IC_{50}, the higher is the antioxidant activity (Brand et al., 1995). The scavenging activity of the *Cornus* extracts is shown in Table 1. Flavonoid of *Cornus* had the highest hydrogen-donating capacity, closely followed by total pholyphenol of *Cornus*, while BHT was the weakest of all. The antioxidant activity of the extracts was expressed in concentration of inhibition 50%.

3. Contents of blood, triglyceride, cholesterol and HDL in serum

The grucose content decreased significantly in the DC 200 group compared with the DA group (Fig. 1). The lipid

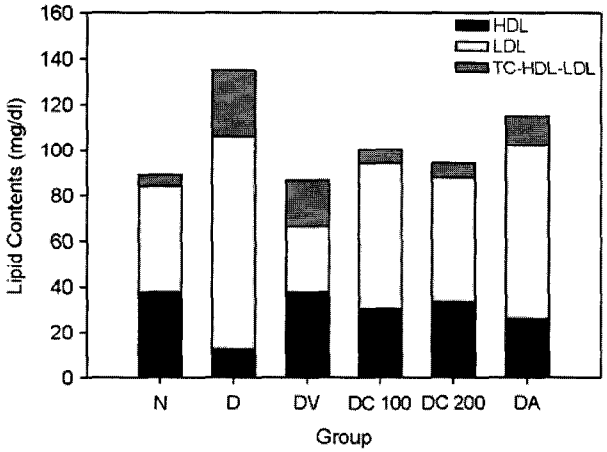

Fig. 2. Changes in cholesterol and HDL by the oral administration of extracts from *Cornus officinalis* in streptozotocin-induced diabetic rats.

Table 2. GSH and MDA concentration in cytosol of tissue of 6 group

Group	Liver		Brain	
	MDA[1)]	GSH[2)]	MDA	GSH
N	3.73±0.67	10.33±1.69	3.08±0.16	8.84±0.27
D	8.71±0.31	4.96±1.25	8.66±1.03	4.62±0.32
DV	7.68±0.25	7.88±0.59	4.04±0.18	8.93±0.28
DC 100	6.24±0.15	5.34±0.24	7.32±0.17	8.78±0.21
DC 200	5.13±0.31	6.58±0.23	6.51±0.97	8.85±0.52
DA	7.23±0.10	5.55±0.17	8.66±1.07	6.61±0.54

1) MDA: molonaldehyd (nM/ mg protein)
2) GSH : glutathion (nM/ mg protein)

composition in serum (Fig. 2) showed that the cholesterol content in the serum also decreased in the diabetic groups compared with the normal group, except for the DA group, which maintained the normal level. The HDL-cholesterol content increased significantly in the DC 200 and DV group compared with the diabetic groups, yet the DA group exhibited a less significant increase than the DC 100 group.

4. Determination of Malondialdehyde and Reduced Glutathione

As can be seen from Table 2, the level and brain of MDA in the liver was increased in untreated diabetic rats compared with the rats in the normal group and the DC 200 group. Interestingly, *Cornus* extracts treatment significantly reduced the MDA level to the control level in liver and brain tissue, compared with the untreated diabetic group. In the untreated diabetic group, the GSH contents were significantly lower than the normal group. GSH was increased in *Cornus* extracts treated diabetic rats compared with Acarbose treated diabetic rats and untreated diabetic rats.

DISCUSSION

As previously demonstrated, radical generated during metabolism can enter into reactions that, when uncontrolled, can affect certain processes leading to clinical manifestations (Kim, 1995). Radicals are key participants in damage caused by diabetic complications. Oxidative stress, superoxide production and an imbalance in antioxidant enzymes have been related to diabetic complications. Diabetes is one of the pathological processes known to be related with an unbalanced production of reactive oxygen species, such as hydroxyl radicals (HO), superoxide anions (O_2) and hydrogen peroxide (H_2O_2). Therefore, cells must be protected from this oxidative injury by antioxidant enzymes. Determination of malondialdehyde by thiobarbituric acid is used as an index of the extent of lipid peroxidation. As previously demonstrated, malondialdehyde used as the best available measure of global radical was substantially elevated in diabetes (Shaban and Helmy, 2003). In the present study, we found significantly decreased glutathione level and increased malondialdehyde concentrations in diabetic rats as compared with control subjects. Our results confirm previous data of an enhanced reactive radical level in diabetes mellitus (Miroslava et al., 2000). Higher amounts of radical play a role in the diabetic complications as well as in a number of disease states. As a safeguard against the accumulation of radical, enzymatic antioxidant activities exist. Our results demonstrate that serum glutathione level correlates positively with malondialdehyde level in diabetes. Our findings of the positive relationship of glutathione level and serum malondialdehyde concentrations may support an idea of an imbalance between the antioxidant enzymes system and radical level in diabetes. An overproduction of radical, especially in diabetes, cannot be properly balanced by the antioxidant enzymes.

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