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Comparison of Glucose Tolerance Effect of Various Commelinaceae Plant Extracts on Hyperglycemic Rats

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Abstract – Blood glucose lowering effects of water extracts from four species of Commelinaceae (*Commelina communis*, *Streptolirion volubile*, *Tradescantia reflexa*, *Aneliema keisak)* were determined on alloxan-induced hyperglycemic rats. In all the experimental groups, the blood glucose level decreased after loading carbohydrates. The blood glucose level in a group treated with *C. communis* extract decreased significantly as compared with the normal group. After loading maltose and sucrose separately in different groups, the blood glucose level decreased in the groups treated with the extracts of *C. communis* and *S. volubile*, and remained approximately unchanged with the extracts of *T. reflexa* and *A. keisak* as compared with the control groups.
Keywords – Alloxan, Blood glucose, Commelinaceae, Hyperglycemic, Rats

Introduction

Rate of diabetes has increased recently due to change in life style, increase in average life span, and bad food habits. Since causes of diabetes are various, it is difficult to diagnose the disease early. Therefore diabetes often progresses to an incurable chronic and wasting condition, often complicated with many diseases like arteriosclerosis, heart disease and brain damage. Diabetes is a serious endocrine disorder affecting carbohydrates, fats, protein metabolism and abnormal excretion of insulin and glucagon and then leads to hyperglycemia, hyperlipidemia, ketosis (Rosetti *et al*., 1990). It can be congenital (Type 1 Insulin dependent) or acquired (Type 2 insulin independent). Type 1 can be diagnosed in childhood and is characterized by abnormally low level of insulin which can be treated by injecting insulin (Atkinson and Maclaren, 1998). Insulin dependent diabetes results from the death of insulin producing β-cell of islets of Langerhans in pancreas (Tisch and McDevitt, 1996). In case of Type 2 diabetes, insulin resistance condition occurs during adulthood, comprising approximately 90% of the total diabetic cases. Obese and persons older than 40 years are more vulnerable to this type of diabetes (Kahn, 1994). Causes of this type of diabetes were deeply studied and are yet to be determined.

Good drugs for diabetes should work fast to prevent excessive increment of blood glucose level after meal. Though insulin and oral blood glucose lowering agents, such as, biguanide and sulfonylurea, and α-glucosidase inhibitors, such as, acarbose, voglibose and miglitoland are being used at present, all of them show undesirable side effects (Pulse *et al*., 1980; Ramaswamy and Flint, 1980). In Korea, 73.9% of patients of insulin independent (Type 2) diabetes over 40 years of age are reported to use folk remedies (Nam, 1994). Many studies to find out new and effective anti-hyperglycemic agents from traditional medicines have been continuously carried out (Kim *et al*., 1993).

Among these, *C. communis* is traditionally known to be good for high blood pressure, diabetes and throat inflammation (Kim *et al*., 1991; Kim *et al.*, 1993; Han and Lim, 1998). Plants of Commelinaceae family in Korea are of six species (Park and Cho, 1994). In the present article, for finding more plants with glucose lowering activity from Commelinaceae family, we screened three more plants, namely *Streptolirion volubile*, *Tradescantia reflexa*, *Aneliema keisak* along with *C. communis* as reference for their glucose lowering as well as glucose tolerance effect.

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Materials and Methods

Plant Materials – All plants and their ethnobotanical information were obtained from "Korean Collection of Herbal Extracts", a biotech company in Korea. A collection of voucher specimen is available with the company (Korean Collection of Herbal Extracts, 2004).

Preparation of plant Extracts of Commelinaceae plants – Whole plant samples dried in shade were soaked in water (10 liter/kg of dry weight) and extracted for 4 hr in hot water. Extracts were filtered through cotton plug and centrifuged at 3000 rpm for 5 min. Upper layer after centrifugation was decanted and concentrated using rotary vacuum evaporator. Dried extracts were stored at –20°C for further studies.

Experimental animals – Male Sprague-Dawley rats (3 weeks old) were bought from Daehan Biolink Co., Ltd. Korea. From the total of 36 rats, 6 groups were made with 6 rats each. They were housed in standard microlon boxes under controlled conditions (temperature 23 ± 1°C, humidity 60 ± 5%, and illumination of 10 hr). Rats were fed freely on food and water.

Induction of hyperglycemia in rats – Thirty rats in 5 groups which were starved for 14 hr before peritoneally injecting 180 mg/kg body weight (BW) of alloxan (Sigma, St. Louis, USA, dissolved in 0.5 mL physiological saline) to induce hyperglycemia (Lee *et al.*, 1998). After 48 hr blood samples were obtained from rats and blood glucose level was determined using one touch blood glucose meter (Lifescan kit, Johnson & Johnson Co, USA). Rats with blood glucose levels over 250 mg/mL were selected as hyperglycemic rat for later experiments.

Injection of plant extracts – In experiments for blood glucose lowering effect, plant extracts dissolved in 0.5 mL of physiological saline solution were injected to 100 mg/kg BW twice a day at interval of 9 hr, for ten days. For disaccharide load experiments, disaccharide and extract were combined in 2 mL, which was orally administered to give concentration of 500 mg extract/kg BW. The rats in group 1 (control) were administered distilled water instead of extract.

Determination of blood glucose level – Plants extracts were peritoneally injected to alloxan-induced hyperglycemic rats. Blood samples were collected from tail vein and blood glucose levels were determined using one touch blood glucose meter.

Glucose load experiment – To determine the degree of diabetes, 5 weeks old Sprague-Dawley rats with induced hyperglycemia were injected with 100mg of extract/kg BW for ten days (0.5 mL solution twice a day) and starved

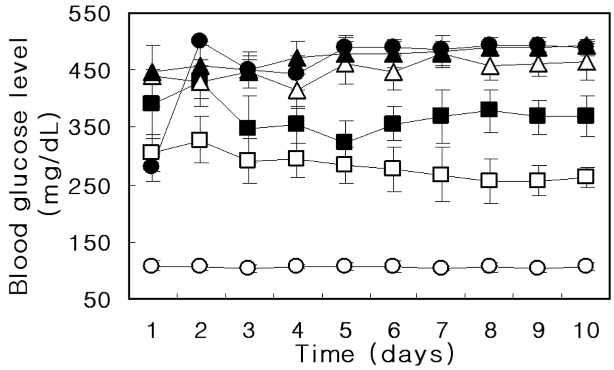

Fig. 1. Effect of extracts of Commelinaceae plants on blood glucose level in alloxan-induced hyperglycemic rats. Blood glucose levels were determined after treatment. Normal diet without any treatment (○); Alloxan (180 mg/kg BW) was injected to induce hyperglycemia (●); Alloxan induced hyperglycemic rats were peritoneally injected with water extracts of Commelinaceae (100 mg/kg BW) for tens days. *C. communis* (□) *S.volubile* (■), *T. reflxa* (△), and *A. keisak* (▲).

for 14 hr before orally administering glucose (0.1 g glucose / 100 g BW). Blood samples were collected before glucose load and 30, 60, 90, and 120 min after the load and the glucose levels were determined.

Disaccharide load experiment – To determine the glucose tolerance effect of plant extracts after disaccharide load, maltose and sucrose were injected to 14 hr starved normal rat to 4 g disaccharide/kg BW and blood samples were collected 35 min after the load, at which blood glucose level was the maximum.

Statistical Analysis of data – Results were expressed as mean ± Standard deviation (S.D.). The data were analyzed using ANOVA to confirm the test of statistic significance. The P values less than 0.05 were considered as statistically significant.

Results

Blood glucose lowering effect of extract of Commelinaceae plants in hyperglycemic rats – Glucose lowering effect of plant extracts is shown in Fig. 1. Highest detection range was 500 mg/mL, values over this range were reported as 500 mg/mL. It was observed that blood glucose levels in case of groups treated with extracts of *C. communis* and of *S. volubile*, decreased to significantly lower levels as compared to the other two plants. The glucose lowering effect is considered to be due to the increase in insulin excretion or/and inhibition of α-glucosidase (Sook *et al.*, 1992).

Glucose load experiment – For the determination of insulin excretion capacity of pancreas, glucose tolerance was determined by checking the glucose level of blood

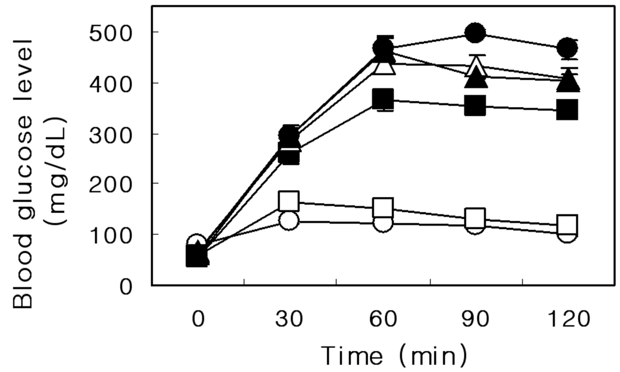

Fig. 2. Effect of extracts of Commelinaceae plants on glucose tolerance in alloxan-induced hyperglycemic rats. Untreated group (○); Hyperglycemic rats were peritonially injected with extracts (100 mg/kg B.W.) for 10 days. Starved rats (14 hr) were peritoneally injected with 50% glucose (0.1 g glucose/100g BW). Blood glucose level were determined at 30, 60, 90, 120 min. Hyperglycemic rats treated with none (●), extracts of *C. communis* (□), *S. volubile*(■), *T. reflexa* (△), and *A. keisak* (▲).

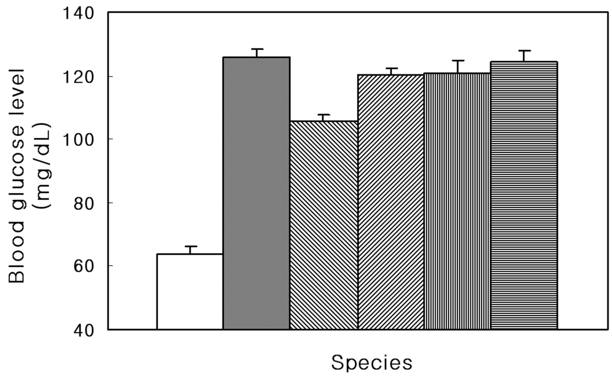

Fig. 3. Effect of extracts of Commelinaceae on blood glucose level after oral load of maltose. Untreated group (□); Hyperglycemic rats were orally loaded with maltose (4 g/kg BW) and extracts (500 mg/kg BW). Maltose only (■), Maltose+*C. communis* extract (▧), Maltose + *S. volubile* extract (▨), Maltose +*T. reflexa* extract (▥), and Maltose+*A. keisak* extract (▤).

collected at different time after the injection of certain amount of glucose (Fig. 2). It was observed that the blood glucose level in control group was 80.67 ± 3.56 mg/mL before load and 102 ± 4.07 mg/mL at 120 min after the load while in hyperglycemic group it was 56 ± 3.90 mg/mL before load and 464 ± 17.44 mg/mL at 120 min after load. In the group treated with extracts of *C. communis*, *S. volubile, T. reflxa and A. keisak,* the glucose level was 60.5 ± 5.47 mg/mL, 55.67 ± 4.97 mg/mL, 65.5 ± 5.09 mg/mL and 64.67 ±.4.32 mg/mL before and 117.3 ± 7.63 mg/mL, 345 ± 4.56 mg/mL, 406.3 ± 21.54 mg/mL and 403.5 ± 12.10 mg/mL at 120 min after load, respectively. Interestingly, at 120 min the blood glucose level of extract treated group was significantly lower than the hyperglycemic control group ($p < 0.05$). The decrease in blood glucose level of group treated with *C. communis* extract was similar to untreated normal group ($p < 0.01$).

In the above experiment, extracts of *C. communis* and *S. volubile* decreased the glucose level in hyperglycemic rats and after glucose load. These results observed may be due to decrease in the breakdown rate of pancreas and increase in insulin excretion. Further, the glucose tolerance effect of *T. reflxa* and *A. keisak* were lower as compared to the other two but were still evident as shown in figure. Since glucose level sharply increased in these groups up to 60 min, it is difficult to decisively conclude that glucose tolerance is improved in these cases. The above results indicate that the inhibition of glucose absorption is considered to be inhibited in these cases but still the exact mechanism is not yet known at present and further study is required for clarification.

Disaccharide load experiment – To test effect of extracts on digestion of disaccharide by α-glucosidase activity, the combined solution of hot water extracts and disaccharide were given orally to the 14 hr starved rats. The blood glucose level at 35 min was used for the determination of α-glucosidase inhibition.

Maltose – The blood glucose level at 35 min after load of maltose is shown (Fig. 3). The glucose level was 125 ± 2.79 mg/mL in hyperglycemic control group loaded with maltose only. The glucose level was 105.7 ± 1.97 mg/mL, 120.3 ± 2.16 mg/mL, 120.8 ± 4.26 mg/mL), and 124.3 ± 3.33 mg/mL in group treated with both maltose and the extracts of C. *communis*, *S. volubile, T. reflexa* and *A. keisak* respectively. Comparing with hyperglycemic control group, glucose level was significantly lower in groups treated with extracts of C. *communis* ($p < 0.01$), *S. volubile* and T. *reflexa* ($p < 0.05$). Thus, from the result these extracts can be considered to inhibit the intestinal absorption effectively.

Sucrose – Blood glucose levels at 35 min after load of sucrose is shown in Fig. 4. The glucose level in hyperglycemic control group loaded with only sucrose was 113.3±2.16 mg/mL. The glucose levels was 66.17±2.14 mg/mL, 105.3±2.50 mg/mL, 110.8 ± 2.86 mg/mL and 113.7±3.20 mg/mL in the groups treated with sucrose and extract of C. *communis*, *S. volubile, T. reflxa* and *A. keisak* respectively. Comparing with hyperglycemic contral group, extracts of *C. communis* and *S. volubile* seems to prevent sharp increase in blood glucose level by inhibiting activity of α-glucosidase.

Discussion

Carbohydrates are the major constituents of human

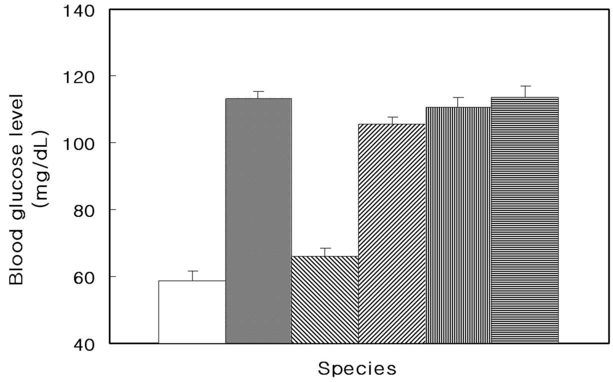

Fig. 4. Effect of extracts of *Commelinaceae Family* on blood glucose level after oral load of sucrose. Untreated group (□); Hyperglycemic rats were orally loaded with sucrose (4 g/kg BW) and extracts (500 mg/kg BW). Sucrose only (■), Sucrose+*C. communis* extract (▧), Sucrose+*S. volubile* extract (▨), Sucrose+*T. reflexa* extract (|||), and Sucrose+*A. keisak* extract (≡).

diet. They are broken down to disaccharides which are further digested to monosaccharides by intestinal α-glucosidase. There is a sharp increase in the blood glucose level after the meals due to rapid absorption of glucose in the blood. In normal human intestinal α-glucosidase, such as maltase and sucrase are properly inhibited to prevent sharp increase in blood glucose level after food consumption (Chung *et al.*, 1996). Hence, inhibition of α-glucosidase will prove useful in treating Diabetes mellitus. A number of α-glucosidase inhibitors from natural sources have beer identified (Yoshikuni, 1988; Choi, 1996).

In the present research, experiment was carried out to determine the effect of Commelinaceae plant extracts on breakdown of maltose and sucrose by intestinal α-glucosidase. In case of maltose, the blood glucose level decreased on treating with extracts of C. *communis*, *S. volubile,* and *T. reflxa* while in case of sucrose it decreased to lower level on administration of *C. communis*, and *S. volubile* extracts. From these data, extracts of *C. communis* and *S. volubile* seems to prevent sharp increase in blood glucose level by inhibiting activity of α-glucosidase.

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