*Food Sci. Biotechnol. Vol. 17, No. 1, pp. 130 ~ 134 (2008)*

Food Science and Biotechnology



# Hypoglycemic Effect of Onion Skin Extract in Animal Models of Diabetes Mellitus

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**Abstract** Hypoglycemic effect of onion skin extract *in vitro* and *in vivo* was investigated. A methanol extract of onion skin inhibited yeast α-glucosidase with an $IC_{50}$ of 0.159 mg/mL. A single oral administration of the onion skin extract (500 mg/kg) significantly lowered the postprandial area under the glucose response curve to starch (1 g/kg, $p<0.05$). Three-week-old db/db mice were fed an AIN-93G diet or a diet supplemented with a 0.5% onion skin extract for 7 weeks after a 1-week adaptation period. Consumption of onion skin extract significantly reduced the levels of plasma glucose, insulin, and blood glycated hemoglobin as compared with the control group ($p<0.05$). These findings suggest that onion skin is effective in controlling hyperglycemia in animal models of type 2 diabetes mellitus, at least in part by inhibiting α-glucosidase activity.

**Keywords:** onion skin, hypoglycemic effect, α-glucosidase, glucose, db/db mouse

## Introduction

Diabetes mellitus results from defects in insulin secretion, insulin action, or both. Abnormalities in carbohydrate, lipid, and protein metabolism are common in diabetic patients. Cardiovascular disease (CVD) is a major complication and the leading cause of premature death among patients with diabetes (1). It is well known that optimal glycemic control in patients with diabetes mellitus is associated with sustained and decreased rates of diabetes-related cardiovascular complications (2,3).

α-Glucosidase is an enzyme that catalyzes the final step in the carbohydrate digestive process; hence, α-glucosidase inhibitors may delay the use of dietary carbohydrates and minimize increases in postprandial glucose levels. α-Glucosidase inhibitors such as acarbose (4), voglibose (5), and miglitol (6) have been used as oral hypoglycemic agents. However, synthetic α-glucosidase inhibitors have side effects, such as flatulence and abdominal bloating, which may limit their potential as preferred medications (7).

In recent years, numerous studies have been carried out to isolate α-glucosidase inhibitors from natural products, including plant materials, as alternative hypoglycemic agents for diabetes that can be used in addition to conventional treatments (8-11). We screened for α-glucosidase inhibitory activity in onion (*Allium cepa* L.) skin. Onion skin has been found to have strong antioxidant activity and scavenging activity for superoxide radicals (12,13), and quercetin was found to be the major component responsible for this activity (14,15). Both onion skin and peel enhanced antioxidant status in aged rats (16). Since oxidative stress generated by free radical species plays an important role in the progression of diabetes and the development of diabetic complications (17), onion could be beneficial for the prevention and treatment of diabetes via its antioxidative effects. However, the effect of onion skin on glycemic control in diabetes has not been studied previously.

Since intensive glycemic control is a major goal in the treatment of diabetes and essential to reducing the risk of diabetic complications (2,3), it is worth studying the effect of onion skin on glycemic control in diabetes. Thus, we investigated the effect of chronic feeding of onion skin extract on glycemic control in an animal model of diabetes, and measured the α-glucosidase inhibitory activity of onion skin to evaluate its possible use as a hypoglycemic agent and to elucidate the possible underlying mechanism of the glycemic control.

## Materials and Methods

**Reagents** A glucose assay kit from Yeongdong Co. (Seoul, Korea), an insulin assay kit from Linco Co. (St. Charles, MO, USA), and a glycated hemoglobin ($HbA_{1C}$) assay kit from BioSystems (Barcelona, Spain) were used. Cornstarch was obtained from Daesang Co. (Seoul, Korea). Casein, L-cysteine, mineral mixture, and vitamin mixture were purchased from ICN Pharmaceuticals Inc. (Costa Mesa, CA, USA), and *tert*-butyl hydroquinone was purchased from Fluka Co. (Milwaukee, WI, USA). Sucrose and soybean oil were obtained from Cheiljedang Co. (Seoul, Korea). Yeast α-glucosidase, *p*-nitrophenyl-α-D-glucopyranoside, alphacel, choline bitartrate, and all other chemical reagents used in this study were purchased from Sigma Chemical Co. (St. Louis, MO, USA).

**Preparation of the methanol extract** Onion skin was

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Received July 11, 2007; accepted October 11, 2007

obtained from a local market in Changnyung, Gyeongnam, Korea. The onion skins were rinsed, freeze-dried, powdered, and extracted 3 times with 10 volumes of methanol for 12 hr at room temperature. The solvent was removed by rotary evaporation at 40°C. The extraction yield was 5.9%. The dry extract was redissolved in dimethyl sulfoxide (DMSO) at a concentration of 5 mg/mL for use as a test material in the *in vitro* study.

**Enzyme inhibition assay** Yeast α-glucosidase inhibitory activity was measured by the method described by Watanabe *et al.* (9) using a microplate reader (model 550; Bio-Rad, Hercules, CA, USA). Yeast α-glucosidase (0.7 U) dissolved in 100 mM phosphate buffer (pH 7.0) containing 2 g/L bovine serum albumin and 0.2 g/L $NaN_3$ was used as the enzyme solution, and 5 mM *p*-nitrophenyl-α-D-glucopyranoside in the same buffer was used as substrate solution. The inhibitory activities of the onion skin extract and acarbose, a positive control, against α-glucosidase were measured at concentrations of 0.50, 0.25, 0.10, and 0.05 mg/mL. The measurements were performed in triplicate, and the 50% inhibitory concentration ($IC_{50}$) was defined as the concentration that inhibited 50% of enzyme activity under the assay conditions.

**Animals and biochemical analyses** The effects of onion skin extract on postprandial blood glucose response were measured in normal rats. Male Sprague-Dawley rats weighing 150-180 g were purchased from Orient Co. (Seoul, Korea). All rats were fed a commercial food *ad libitum* for 2 weeks after arrival. The rats (n=14) were randomly divided into 2 groups. The animals were administered either soluble starch (1 g/kg) alone or starch with the onion skin methanol extract (500 mg/kg) by gastric intubation after an overnight fast. Blood samples were collected from the tail tip at 30, 60, 90, 120, 180, and 240 min after carbohydrate loading. Feed was withheld during the test. Blood samples were centrifuged at 1,000×g for 15 min. Plasma glucose was measured using a commercial glucose assay kit (Yeongdong Co., Seoul, Korea). Plasma glucose levels were expressed as increments from the baseline. Incremental areas under the response curves (AUC) were calculated using the trapezoidal rule, with fasting levels as the baseline.

The hypoglycemic effects of long-term consumption of onion skin extract were determined in db/db mice, an animal model of type 2 diabetes. Three-week-old male db/db (+/+) C57BL/KsL mice (n=14) were purchased from the Korea Research Institute of Bioscience and Biotechnology (Daejeon, Korea). The animals had free access to commercial food during a 1-week adaptation period, and then were randomly divided into control and onion skin treatment groups. The diets for the animals were based on the AIN-93G diet, which contains 39.8% cornstarch, 20% casein, 13.2% dextrinized cornstarch, 10% sucrose, 7% soybean oil, 5% Alphacel, 3.5% mineral mixture, 1% vitamin mixture, 0.3% L-cysteine, 0.25% choline bitartrate, and 0.0014% *tert*-butyl hydroquinone. The control group was offered a standard AIN-93G diet, whereas the treatment group was offered the same diet supplemented with 0.5% (w/w, final concentration) onion skin methanol extract *ad libitum* for 7 weeks. At the end of the experimental period, the mice were sacrificed by heart puncture after an overnight fast. Blood $HbA_{1C}$ levels were measured by the chromatographic method using a commercial assay kit. Plasma glucose and insulin were measured by an enzymatic method and a radioimmunoassay, respectively.

All animals were housed individually in plastic cages and maintained in controlled conditions of 24±5°C and 55 ±5% relative humidity, with a regular 12-hr light/12-hr dark cycle during the experimental period. The experiments were performed according to the guidelines of animal experimentation approved by the Animal Resource Center at Inje University, Korea.

**Statistical analysis** All data are presented as mean± standard deviation (SD). Statistical analyses were performed with Student's *t*-test. *p*-Values less than 0.05 were considered to be significant.

## Results and Discussion

**Inhibition of α-glucosidase activity *in vitro* and *in vivo*** The inhibitory activity of the methanol extract of onion skin against yeast α-glucosidase (Fig. 1) were 95.8, 73.3, 35.0, 16.9, and 6.0% at concentrations of 0.50, 0.25, 0.10, 0.05, and 0.025 mg/mL *in vitro*, respectively. Acarbose, an α-glucosidase inhibitor used as an oral hypoglycemic agent, inhibited the enzyme activity by 29.9, 23.5, 12.2, and 4.5% at concentrations of 0.50, 0.25, 0.10, and 0.05 mg/mL, respectively. The onion skin extract had an $IC_{50}$ value of 0.159 mg/mL.

Since α-glucosidase is a key enzyme of carbohydrate metabolism in the small intestine, α-glucosidase inhibitors could delay digestion and reduce postprandial glucose levels (4). In fact, α-glucosidase inhibitors have become the most common oral agents used to improve postprandial hyperglycemia since their introduction in the early 1990s (18). However, because the chronic use of these agents can result in side effects, such as flatulence, abdominal cramping, vomiting, and diarrhea, their use may be limited (19). Therefore, much attention has focused on the search for natural substances that show potent inhibitory activity against α-glucosidase and have fewer side effects (11,20-22). *Rhus chinensis* (20), *Commelina communis* (21), mulberry leaves (22), and *Saururus*

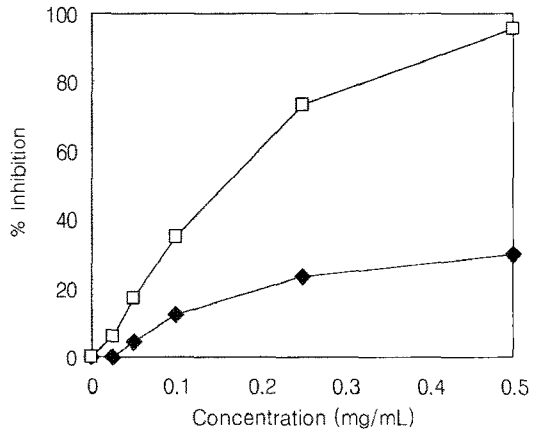


**Fig. 1. Dose-dependent inhibition of yeast α-glucosidase activity of onion skin extract and acarbose.** □, Onion skin extract; ◆, acarbose. Values represent means of triplicate measurements.

*chinensis* Baill leaves (11) have shown potent inhibitory activity against α-glucosidase *in vitro* and *in vivo*. In our study, onion skin extract also showed strong α-glucosidase inhibitory activity *in vitro*. Onion skin is a good source of quercetin (14,15), a noncompetitive α-glucosidase inhibitor (23). The content of quercetin in onion skin is estimated to be between 2 and 10 mg/g dry weight (24-26) Thus, the quercetin in onion skin could be the active compound responsible for the α-glucosidase inhibitory activity.

To investigate the inhibitory effect of onion skin on α-glucosidase activity *in vivo*, we determined the ability of onion skin extract to lower postprandial blood glucose in normal rats. A single oral dose of onion skin extract (500 mg/kg) administered with starch (1 g/kg) inhibited the increase in plasma glucose levels significantly at 30 and 60 min, compared with those of rats administered starch alone ($p<0.05$; Fig. 2). The AUC for the glucose response was significantly lower in the onion skin group (2,353±493 mg · min/dL) than in the control group (3,582±921 mg · min/dL, $p<0.05$; Table 1). These data demonstrate that onion skin extract decreases postprandial glucose levels by inhibiting α-glucosidase activity. Postprandial hyperglycemia is one of the earliest observable abnormalities in diabetes mellitus (27) and has been associated with an increased risk for micro- and macrovascular complications of diabetes (28-32).

**Effect of chronic consumption of onion skin extract on hyperglycemia in db/db mice** The effects of chronic consumption of onion skin extract on glycemic control were determined in db/db mice. The food intake, body weight, and food efficiency ratio of the onion skin group did not significantly differ from those of the control group (Table 2). Chronic feeding of a diet containing 5% *Lonicera japonica* flowers with α-glucosidase inhibitory activity to 8-week-old rats significantly decreased body weight gain, suggesting that α-glucosidase inhibitors may be effective in controlling body weight (33). However, long-term consumption of acarbose does not influence the body weight of db/db mice (34) or diabetic patients (35). In our study, onion skin extract had no significant influence

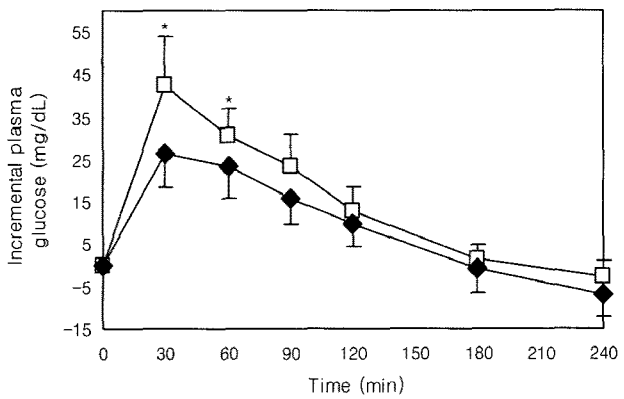


**Fig. 2. Increase in plasma glucose after administration of onion skin extract to rats.** In the control group (□), starch (1 g/kg) was administered orally to rats after an overnight fast. In the onion skin group (◆), starch (1 g/kg) plus onion skin methanol extract (500 mg/kg) was administered orally to rats after an overnight fast. Values represent mean±SD. *Significantly different at $p<0.05$.

**Table 1. Area under the glucose response curve for rats**

| Group[1)] | AUC (mg · min/dL) |
|---|---|
| Control | 3,582±921 |
| Onion skin | 2,353±493*[2)] |

[1)]Control group, starch (1 g/kg) was administered orally to the rats after an overnight fast; onion skin group, starch (1 g/kg) mixed with the onion skin methanol extract (500 mg/kg) was administered orally to the rats after an overnight fast. Values represent mean±SD (n=7).
[2)]*Significantly different at $p<0.05$.

**Table 2. Body weight, food intake, and feed efficiency ratio in db/db mice fed a basal diet or a diet supplemented with onion skin extract[1)]**

| Group | Body weight (g) | Food intake (g/day) | Feed efficiency ratio (%)[2)] |
|---|---|---|---|
| Control | 40.0±2.0 | 4.2±0.5 | 9.5±1.2 |
| Onion skin | 38.6±2.5 | 4.1±0.4 | 8.6±1.1 |

[1)]Values represent mean±SD.
[2)]Feed efficiency ratio (%)=[body weight gain (g)/food intake (g)]×100.

on the body weight of db/db mice, an animal model of type 2 diabetes and obesity.

Consumption of onion skin extract significantly decreased the fasting plasma glucose (371±39 mg/dL) and insulin (4.0±0.6 ng/mL) levels as compared to the control group (432±48 mg/dL and 4.7±0.5 ng/mL, respectively, $p<0.05$; Fig. 3). Blood $HbA_{1C}$ (6.1±0.5) was significantly reduced in the onion skin group as compared to the control group (6.9±0.7%, $p<0.05$).

Maintaining blood glucose at levels close to normal and preventing diabetic complications are major goals in the treatment of diabetes mellitus (2,3). Intensive glycemic control is also essential for reducing the risk of diabetes-related complications, including cardiovascular complications (3). In this study, consumption of onion skin extract for 7 weeks was effective in reducing plasma glucose and insulin levels in db/db mice. It has also been reported that chronic feeding of *touchi* extract containing α-glucosidase inhibitory activity reduced both fasting glucose and insulin levels in KKY mice (36) and in patients with type 2 diabetes (37). A number of studies have reported that acarbose monotherapy reduces fasting blood glucose significantly in patients with type 2 diabetes, and this effect is secondary to the lowering of postprandial hyperglycemia (35,38-41). It was suggested that reducing glucose toxicity through decreasing postprandial glucose responses with a α-glucosidase inhibitor such as acarbose leads to improved overall glycemic control (42). It has also been suggested that acarbose induces a prolonged increase in the intestinal hormone glucagon-like peptide-1 (GLP-1), which can effectively reduce hyperglycemia in diabetic patients (40, 43-45). It is possible that onion skin extract could reduce glucose toxicity by decreasing postprandial blood glucose and increasing GLP-1 through its α-glucosidase inhibitory action, resulting in reduced fasting blood glucose levels.

The consumption of onion skin extract was also effective in lowering blood glycated hemoglobin. Several clinical trials have demonstrated that acarbose reduces $HbA_{1c}$ (19,46,47). $HbA_{1c}$ is a marker that represents

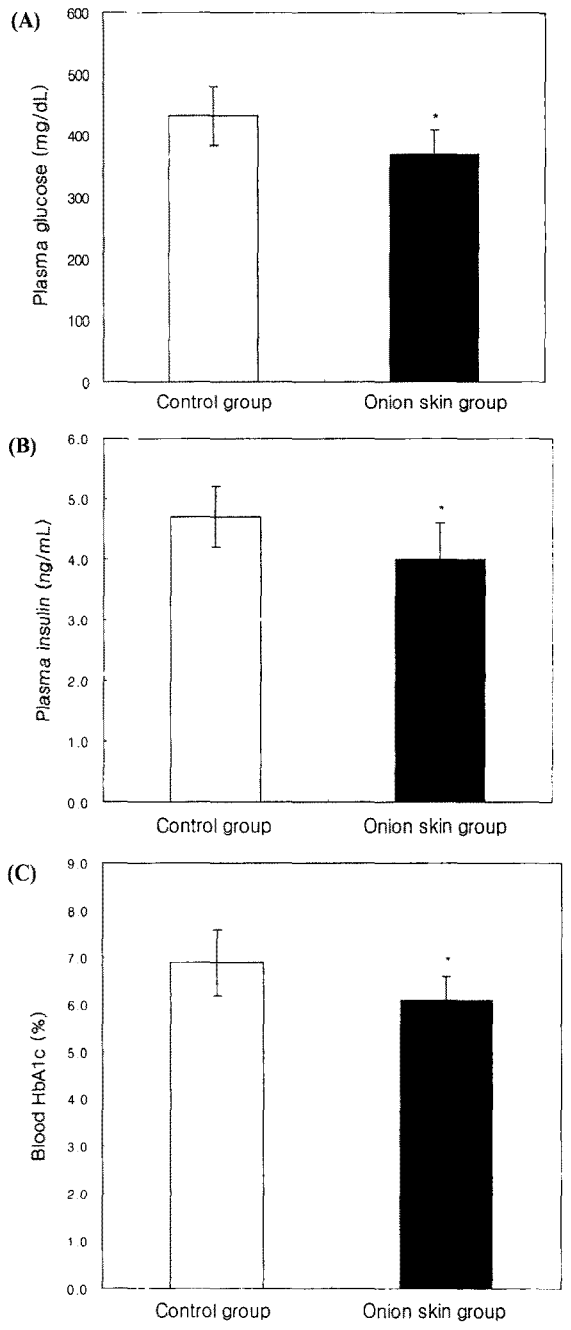


**Fig. 3. Hypoglycemic effects of onion skin on db/db mice.** A, Fasting plasma glucose; B, plasma insulin; C, blood glycated hemoglobin ($HbA_{1C}$). Values represent mean ± SD. *Significantly different at $p<0.05$.

chronic blood glucose control and reflects both fasting plasma glucose and postprandial glucose. It is well known that reduced levels of $HbA_{1c}$ are associated with decreased complications of diabetes, such as cardiovascular complications, neuropathy, and retinopathy (3,48,49). Thus, onion skin extract could be useful in the control of hyperglycemia and the reduction of cardiovascular and other complications of diabetes mellitus.

At present most onion skin is discarded as food processing waste. The data from this study suggest that onion skin could have potential for conversion into useful hypoglycemic agents rather than being discharged as a byproduct. It was reported that onion flesh has antithrombotic effect *in vitro* and *in vivo* and there was no correlation between the antithrombotic effect and quercetin content (50). Since CVD is a major complication and the leading cause of premature death among people with diabetes (1), onion flesh could be beneficial for improvement of cardiovascular complications. Onion skin and peel showed antioxidative effects in aged rats (16). Onion skin contains high amount of quercetin which has strong antioxidant activity and scavenging activity for superoxide radicals (24-26), Thus consumption of onion skin and flesh together could beneficial in prevention diabetes and reducing diabetic complications.

In conclusion, onion skin extract was effective in controlling hyperglycemia in an animal model of diabetes, at least in part by inhibiting α-glucosidase activity. Thus, onion skin extract may be useful for improving overall glycemic control and reducing the risk of diabetic complications.

## Acknowledgments

This research was supported by the Program for the Training of Graduate Students in Regional Innovation of the Ministry of Commerce, Industry, and Energy of the Korean Government.

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