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RESEARCH NOTE

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Water Chestnut (*Trapa japonica* Flerov.) Exerts Inhibitory Effect on Postprandial Glycemic Response in Rats and Free Radical Scavenging Activity *in vitro*

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Abstract The α-glucosidase inhibitory and antioxidant effects of water chestnut (*Trapa japonica* Flerov.) were assessed to explore its possible use as an anti-diabetic agent. Methanol extracts of the fruit shell and meat of water chestnut were assayed for inhibitory activity against yeast α-glucosidase and 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity. Effect of fruit shell extract on postprandial glucose response was assessed. Compared with fruit meat, shell extract showed stronger inhibition against α-glucosidase with an IC_{50} of 273 μg/mL. Oral administration of fruit shell extract (500 mg/kg) significantly lowered the postprandial area under the glucose response curve to starch (1 g/kg) in streptozotocin (STZ)-induced diabetic rats ($p<0.01$). Compared with fruit meat, shell extract showed stronger scavenging activity against DPPH, with an IC_{50} of 27.1 μg/mL. The results indicate that the fruit shell of water chestnut was effective in controlling postprandial hyperglycemia and exerted an antioxidant effect. Therefore, water chestnut may be useful in treating diabetes.

Keywords: α-glucosidase, antioxidant effect, postprandial hyperglycemia, *Trapa japonica* Flerov.

Introduction

Diabetes mellitus, a chronic, degenerative disease that is characterized by hyperglycemia, results from defects in insulin secretion, insulin action, or both. Uncontrolled diabetes leads to diabetic complications, including cardiovascular disease, nephropathy, neuropathy, and retinopathy that can decrease quality of life and ultimately be life-threatening (1). Tight glucose control in patients with diabetes is essential to decrease the risk of diabetic complications (2).

α-Glucosidase is an enzyme that catalyzes the digestion of dietary carbohydrates in the small intestine; consequently, α-glucosidase inhibitors may reduce increases in postprandial glucose levels. In fact, α-glucosidase inhibitors (3-5) have been used as oral hypoglycemic agents. Hyperglycemia in diabetes leads to increased formation of reactive oxygen species and free radicals (6); the resulting oxidative stress plays a major role in the progression of diabetes and diabetic complications (7). It has been reported that both antioxidant nutrients and antioxidants derived from plant materials can be useful in attenuating diabetes and diabetic complications, by reducing the detrimental effects of oxidative stress (8,9).

Thus, compounds with both hypoglycemic and antioxidant activities could be lead candidates in the treatment of diabetes. Although there has been enormous progress in the development of medications for diabetes, most current medications show side effects, such as hypoglycemia, weight gain, and abdominal pain (10). As a result, much effort has been directed at the discovery and development of agents that are useful in treating diabetes, with reduced side effects. It has been reported that olive leaf (11,12), *Eucommia ulmoides* oliver (hardy rubber tree) leaf (13,14), *Saururus chinensis* Baill leaf (15,16), and onion skin (17,18) have both hypoglycemic and antioxidant activities.

The water chestnut (*Trapa japonica* Flerov.) is an annual aquatic plant that is found in lakes and ponds in various parts of the world (19), including Korea, Japan, China, India, and North America. The meat of the fruit of water chestnut is consumed primarily in a cooked form and is eaten raw at the tender stage. The fruit meat of water chestnut contains about 80% starch, 5% protein, and significant amounts of vitamins (20). Most studies on water chestnut have focused on its taxonomy and ecology (19,21), but its pharmacological functions have rarely been studied. The fruit shell of water chestnut contains hydrolyzable tannins, such as trapain and eugenin (22), which are known to have antioxidant properties (23).

However, to our knowledge, the antioxidant activity of water chestnut itself has not been determined previously. In this study, the antioxidant activities of extracts of fruit shell and the meat of water chestnut were measured *in vitro* by 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging. Additionally, the α-glucosidase inhibitory activity of water chestnut was measured *in vitro* and *in vivo* to assess its possible value as an anti-diabetic agent, with antioxidant and hypoglycemic effects.

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Materials and Methods

Reagents A glucose assay kit was obtained from Yeongdong Co. (Seoul, Korea) and acarbose from Bayer Korea (Seoul, Korea). Yeast α-glucosidase, *p*-nitrophenyl-α-D-glucopyranoside, soluble starch, streptozotocin (STZ), DPPH, L-ascorbic acid, and all other chemical reagents were purchased from Sigma-Aldrich (St. Louis, MO, USA).

Preparation of the methanol extract Water chestnut (*Trapa japonica* Flerov.) was obtained from a local market in Changnyung-gun, Korea. The fruit was divided into shell and meat, which were freeze-dried, powdered, and extracted with 10 volumes of methanol for 12 hr and then twice with 5 volumes of methanol for 6 hr at room temperature. The solvent was removed by rotary evaporation at 40°C.

Inhibition assay for α-glucosidase Yeast α-glucosidase inhibitory activity was determined using the chromogenic method of Watanabe *et al*. (24). 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, and 5 mM *p*-nitrophenyl-α-D-glucopyranoside in the same buffer (pH 7.0), were used as the enzyme and substrate solutions. The enzyme solution (50 μL) and 10 μL of the test sample at various concentrations were mixed, and the absorbance was measured at 405 nm using a microplate reader (Model 550; BioRad, Hercules, CA, USA). After incubation for 5 min, 50 μL of the substrate solution were added and incubated for an additional 5 min. The increase in absorbance from time 0 was measured, and inhibitory activity was calculated as a percentage of the blank control. The inhibitory activities of the methanol extract of fruit shell and meat of water chestnut and acarbose, a positive control, against α-glucosidase were measured at concentrations of 25, 50, 100, 250, and 500 μg/mL. The measurements were performed in triplicate, and the 50% inhibitory concentration (IC_{50}) was defined as the concentration that inhibited 50% of the enzyme activity under the assay conditions.

Measurement of postprandial glucose responses Because the fruit shell extract of water chestnut showed much stronger inhibitory activity against yeast α-glucosidase than the meat extract *in vitro,* the inhibitory effect of fruit shell extract was determined *in vivo*. Specifically, the effect of the methanol extract of the shell of water chestnut on postprandial blood glucose response was measured in STZ-induced diabetic rats. Male Sprague-Dawley rats weighing 250-280 g were purchased from Orient Co. (Seoul, Korea). All rats were fed a commercial chow (Samyang Co., Seoul, Korea) *ad libitum* for 2 weeks after arrival. The animals were rendered diabetic by intraperitoneal injection of STZ (65 mg/kg) in citrate buffer, pH 4.5. Blood samples were taken from the tail tip after 1 week and blood glucose concentrations were measured using a glucometer (Glucotrend; Roche Diagnostics, Lewes, UK). Animals showing fasting blood glucose levels higher than 200 mg/dL were used in the study. All animals continued to receive a commercial chow.

STZ-induced diabetic rats (n=16) were randomly divided into 2 groups. After an overnight fast, fasting blood samples were collected from the tail tip. The rats were given soluble starch (1 g/kg) alone or with the extract of the fruit shell of water chestnut (500 mg/kg) by gastric intubation. Blood samples were collected from the tail tip at 30, 60, 90, 120, 180, and 240 min. Food was withheld during the test. Blood samples were centrifuged (1,000×g, 15 min). Plasma glucose was measured using a commercial glucose oxidase kit (Yeongdong Co., Seoul, Korea). Plasma glucose levels were expressed as increments from baseline. Incremental areas under the response curves (AUC) were calculated using the trapezoidal rule, with fasting levels as the baseline.

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 12/12 hr light/dark cycle during the experimental period. All procedures of the animal experiments were approved by the Animal Resource Center of Inje University.

Measurement of DPPH scavenging activity The antioxidant activity was determined by the DPPH assay as described by Blois (25). For radical scavenging measurements, 1 mL of DPPH (0.1 mM) solution prepared in ethanol was mixed with 30 μL of methanol extracts of various concentrations of the fruit shell and meat of water chestnut and L-ascorbic acid, a positive control, in methanol, and the absorbance was measured at 517 nm at room temperature 10 min later. The reduction of the DPPH radical by the fruit shell and meat extract and L-ascorbic acid was determined at concentrations of 12.5, 25, 37.5, 50, 72.5, 100, and 250 μg/mL. All measurements were made in triplicate and IC_{50} was calculated.

Statistical analysis All results are presented as mean± standard deviation (SD). Student's *t*-test was used to evaluate statistical significance between the mean values of the control and experimental group in the *in vivo* study. A p values less than 0.05 were considered significant.

Results and Discussion

Inhibition of α-glucosidase activity *in vitro* The inhibitory activities of methanol extracts of the fruit shell and meat of water chestnut and acarbose against yeast α-glucosidase are shown in Fig. 1. The inhibitory activities of fruit shell and meat extract of water chestnut were 59.8 and 13.8%, respectively, at a concentration of 500 μg/mL. Acarbose, an α-glucosidase inhibitor used as an oral hypoglycemic agent, inhibited the enzyme activity by 29.4% at the same concentration. The shell extract of water chestnut dose-dependently inhibited the enzyme activity, with an IC_{50} value of 273 μg/mL.

Because α-glucosidase is a key enzyme involved in the last step of the digestion of dietary carbohydrates, α-glucosidase inhibitors such as acarbose (3), voglibose (4), and miglitol (5) have been used as oral agents to control postprandial hyperglycemia (26). 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 (10). In recent years, many studies have been conducted to identify natural substances that show potent inhibitory activity against α-glucosidase with

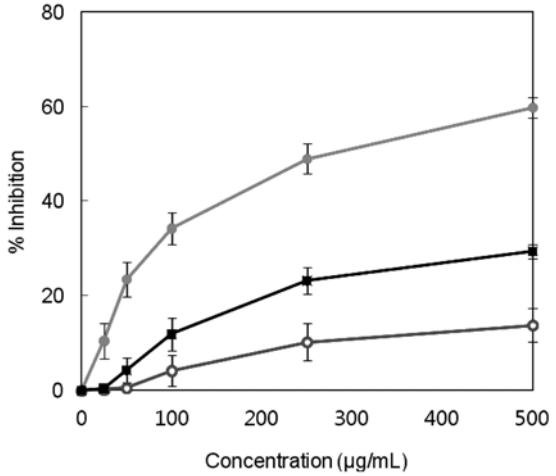

Fig. 1. Dose-dependent inhibition of yeast α-glucosidase activity of extracts of the fruit shell and meat of water chestnut. ● Fruit shell extract of water chestnut; ○ fruit meat extract of water chestnut; ■ acarbose. Values represent means of triplicate measurements.

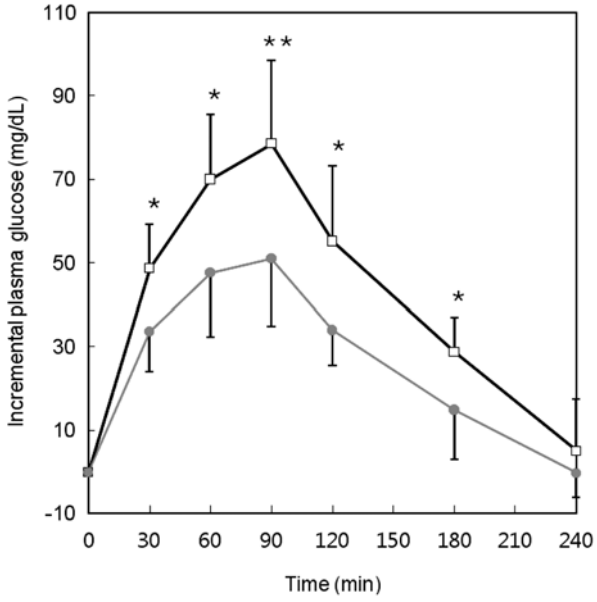

Fig. 2. Increase in plasma glucose after administration of fruit shell extract of water chestnut to STZ-induced diabetic rats. Control group (□), starch (1 g/kg) was administered orally to rats after an overnight fast. water chestnut group (●), starch (1 g/kg) plus methanol extract of fruit shell of water chestnut (500 mg/kg) was administered orally to STZ-induced diabetic rats after an overnight fast. Values represent mean±SD. Significantly different at $*p<0.05$ and $**p<0.01$.

fewer side effects (23,27,28). Touchi extract (27), *Commelina communis* (28), and *S. chinensis* Baill leaves (16) have shown potent inhibitory activity against α-glucosidase *in vitro* and *in vivo*, and touchi extract has been approved as a health/functional food that can help to control postprandial blood glucose by the Korean Food & Drug Administration (KFDA) (29). In our study, the α-glucosidase inhibitory activity of the fruit shell extract of water chestnut was twice as strong as that of acarbose and four times stronger than the meat extract *in vitro*, at a concentration of 500 μg/mL.

Inhibition of postprandial hyperglycemia in STZ-induced diabetic rats The ability of the fruit shell extract of water chestnut to lower postprandial blood glucose in STZ-induced diabetic rats after consumption of starch was assessed. Incremental plasma glucose levels of rats that consumed starch alone reached a peak of 78.5±20.0 mg/dL at 90 min (Fig. 2). A single oral dose of fruit shell extract of water chestnut (500 mg/kg) administered with starch (1 g/kg) inhibited the increase in plasma glucose levels significantly at 30, 60, 90, 120, and 180 min, compared to those of rats given starch alone ($p<0.05$ at 30, 60, 120, and 180 min; $p<0.01$ at 90 min). The AUC for the glucose response was significantly lower in the fruit shell extract of water chestnut group (6,443±1,858 mg·min/dL) than in the control group (10,607±2,634 mg·min/dL, $p<0.01$; Table 1). These data demonstrate that fruit shell extract of water chestnut decreased postprandial glucose levels by inhibiting α-glucosidase activity. Inoue *et al.* (30) reported that an α-glucosidase inhibitor that reduced incremental blood glucose at the peak time point could reduce the AUC of the blood glucose response curve. In our study, fruit shell extract of water chestnut both flattened the peak postprandial blood glucose levels and decreased the AUC of the blood glucose response curve.

Postprandial hyperglycemia is one of the earliest observable abnormalities in diabetes mellitus and is a better predictor of glycated hemoglobin levels than fasting glucose (31). Glycated hemoglobin levels are highly associated with an increased risk of micro- and macrovascular complications (32). One of the main benefits of oral hypoglycemic agents with α-glucosidase inhibitory activity is the control of postprandial hyperglycemia, which can reduce micro- and macrovascular complications (33). It was suggested that diabetes therapy focused on lowering postprandial glucose, versus fasting glucose, could be a better treatment (34). Thus, the fruit shell extract of water chestnut could be useful in reducing the risk of cardiovascular complications, by controlling postprandial hyperglycemia in diabetics.

Table 1. Area under the glucose response curve in STZ-induced diabetic rats

Group[1)]	AUC (mg·min/dL)[2)]
Control	10,607±2,634
Water chestnut	6,443±1,858**

[1)]Control group, soluble starch (1 g/kg) was administered orally to STZ-induced diabetic rats after an overnight fast; water chestnut group, starch (1 g/kg) mixed with the methanol extract of the fruit shell of water chestnut (500 mg/kg) was given orally to the rats after an overnight fast.
[2)]Area under response curve; Values represent mean±SD (n=8); **Significantly different at $p<0.01$.

Free radical scavenging activity against DPPH radicals As shown in Fig. 3, the fruit shell extract of water chestnut exhibited high free radical scavenging activity (94.3%), equivalent to that of the standard L-ascorbic acid (96.5%) at a concentration of 250 μg/mL, and the fruit meat extract showed lower activity (25.2%). The shell extract showed

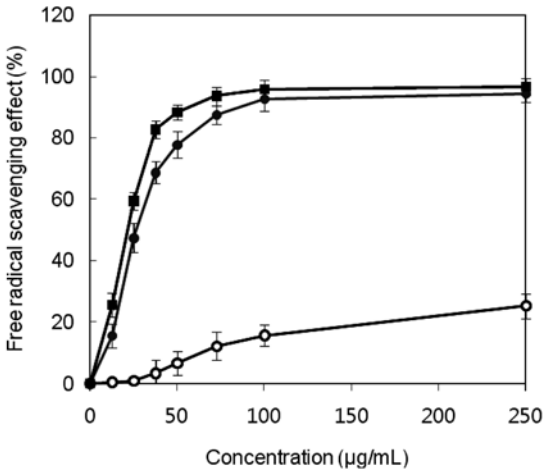

Fig. 3. Free radical scavenging activity of extracts of fruit shell and meat of water chestnut. The scavenging activities of the methanol extract of fruit shell and meat of water chestnut and L-ascorbic acid against the radical DPPH were measured at concentrations of 12.5, 25, 37.5, 50, 72.5, 100, and 250 μg/mL. ● Fruit shell extract of water chestnut; ○ fruit meat extract of water chestnut; ■ L-ascorbic acid. Values represent means of triplicate measurements.

dose-dependent scavenging activity, with an IC_{50} value of 27.1 μg/mL, similar to that of L-ascorbic acid (21.6 μg/mL).

Diabetes mellitus increases oxidative stress, which contributes to the progression of diabetes and the development of diabetic complications (7). Hyperglycemia can overdrive the electron transport chain in cells, resulting in overproduction of superoxide anions (35). Auto-oxidation of glucose and protein glycation can further increase production of oxygen free radicals. Thus, antioxidant treatments have been proposed to decrease oxidative damage and to be beneficial in the treatment of diabetes and to improve complications resulting from diabetes. It has been demonstrated that antioxidant vitamins, such as vitamins C and E, can reduce oxidative stress and lipid peroxidation in animals and patients with diabetes (10,11,36). It has been suggested that antioxidants may inhibit the formation of reactive oxygen species, scavenge free radicals, or increase activity of the antioxidant defense enzyme systems (37). Antioxidants present in plants have also shown protective effects against oxidative damage resulting from diabetes (9).

In this study, a methanol extract of the fruit shell of water chestnut showed strong antioxidant activity, comparable to that of L-ascorbic acid. Soluble tannins contained in the fruit shell of water chestnut (22) may be the active compounds responsible for this high antioxidant activity. Further studies to isolate and identify the antioxidant compound(s) from the shell part would be valuable. Additionally, the extract of the fruit shell of water chestnut controlled postprandial blood glucose in diabetic animals by inhibiting α-glucosidase. Typically, the fruit shell of water chestnut is discarded as food waste, and only the fruit meat is eaten. The results of our study suggest that the fruit shell of water chestnut could be usefully mined for hypoglycemic and antioxidant agent(s), rather than being discarded. Further study is necessary to examine the hypoglycemic and antioxidant effects of chronic consumption of water chestnut and their beneficial effects in improving diabetic complications in animals and patients with diabetes.

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