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Inhibitory activity of *Euonymus alatus* against alpha-glucosidase *in vitro* and *in vivo**

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Abstract

The major goal in the treatment of diabetes mellitus is to achieve near-normal glycemic control. To optimize both fasting blood glucose and postprandial glucose levels is important in keeping blood glucose levels as close to normal as possible. α-Glucosidase is the enzyme that digests dietary carbohydrate, and inhibition of this enzyme could suppress postprandial hyperglycemia. The purpose of this study was to test the inhibitory activity of methanol extract of *Euonymus alatus* on α-glucosidase *in vitro* and *in vivo* to evaluate its possible use as an anti-diabetic agent. Yeast α-glucosidase inhibitory activities of methanol extract of *E. alatus* were measured at concentrations of 0.50, 0.25, 0.10, and 0.05 mg/ml. The ability of *E. alatus* to lower postprandial glucose was studied in streptozotocin-induced diabetic rats. A starch solution (1 g/kg) with and without *E. alatus* extract (500 mg/kg) was administered to diabetic rats by gastric intubation after an overnight fast. Plasma glucose levels were measured at 30, 60, 90, 120, 180, and 240 min. Plasma glucose levels were expressed in increments from baseline, and incremental areas under the response curve were calculated. Extract of *E. alatus,* which had an IC_{50} value of 0.272 mg/ml, inhibited yeast α-glucosidase activity in a concentration-dependent manner. A single oral dose of *E. alatus* extract significantly inhibited increases in blood glucose levels at 60 and 90 min ($p < 0.05$) and significantly decreased incremental response areas under the glycemic response curve ($p < 0.05$). These results suggest that *E. alatus* has an antihyperglycemic effect by inhibiting α-glucosidase activity in this animal model of diabetes mellitus.

Key Words: *Euonymus alatus*, α-glucosidase, postprandial glucose, diabetes mellitus

Introduction

Diabetes is the fifth leading cause of death among Koreans (Korea National Statistical Office, 2006). The prevalence of diabetes mellitus is increasing markedly because of an aging population, increased urbanization, and more sedentary lifestyles (King *et al.,* 1998). Keeping blood glucose levels close to normal and preventing diabetic complications are the major goals in the treatment of diabetes mellitus (DCCT Research Group, 1993; UKPDS Group, 1998). Cardiovascular disease (CVD), a major complication of diabetes, is the major cause of morbidity and mortality in patients with diabetes (Centers for Disease Control and Prevention, 1999). Achieving near-normal glycemic control in patients with diabetes mellitus is associated with sustained and decreased rates of diabetes-related cardiovascular complications (DCCT Research Group, 1993; UKPDS Group, 1998). Optimizing both fasting blood glucose and postprandial glucose levels is important in achieving near-normal glucose levels (Abrahamson, 2004). Avignon *et al.* (1997) reported that postprandial glucose levels could be a better marker of glycemic control than fasting blood glucose levels in patients with type 2 diabetes. Microvascular and macrovascular complications are strongly associated with postprandial hyperglycemia (Baron, 1998; Haller, 1998; Jenkins *et al.,* 1988; Mooradian & Thurman, 1998).

At present, α-glucosidase inhibitors are the most common oral agents used to decrease postprandial hyperglycemia, since they can delay the digestion of dietary carbohydrates, resulting in retardation of glucose absorption (Saito *et al.,* 1998; Sels *et al.,* 1999; Stand *et al.*, 1999). In addition, numerous studies have been carried out to isolate effective and safe α-glucosidase inhibitors from natural products, including plant materials, as alternative hypoglycemic agents for diabetes that can be used in addition to conventional treatments (Joo *et al.*, 2006; Li *et al.*, 2005; Shim *et al.*, 2003; Youn *et al.,* 2004).

Euonymus alatus, known as '*gui-jun woo*' in Korea, has been used in Asian folk medicine to treat tumors, regulate blood circulation, and relieve pain in countries including Korea and

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China (Kim *et al.*, 2006; Park *et al.*, 2005). However, there is not enough scientific evidence to support the medical use of *E. alatus*. *Euonymus alatus* has anticancer (Lee *et al.*, 1993) and antioxidative (Oh *et al.*, 2005; Seo *et al.*, 2003) properties and is effective in preventing hyperglycemia and hyperlipidemia in mice fed high-fat diets (Park *et al.*, 2005). Activation of mRNA expression of PPARγ by *E. alatus* extract could improve insulin resistance and hyperlipidemia and thus help to prevent obesity-related type 2 diabetes.

Controlling postprandial glucose levels is an also important strategy in the prevention of type 2 diabetes (Jermendy, 2005). Clinical studies have documented that α-glucosidase inhibitor is effective in controlling both fasting and postprandial hyperglycemia in patients with diabetes (Balflour & McTavish, 1993; Coniff *et al.*, 1995; Holman *et al.*, 1999), and the relative risk of type 2 diabetes could be decreased by α-glucosidase inhibitors in subjects with impaired glucose tolerance and obesity (Jermendy, 2005). Thus, in this study we measured the α-glucosidase inhibitory activity of *E. alatus in vitro* and *in vivo* to evaluate its possible use as an anti-diabetic agent.

Materials and Methods

Reagents

Yeast α-glucosidase, *p*-nitropheny-α-D-glucopyranoside, soluble starch, and streptozotocin (STZ) were purchased from Sigma Chemical Co (St. Louis, MO, USA). A glucose assay kit was obtained from Yeongdong Co (Seoul, South Korea).

Preparation of the methanol extract

Euonymus alatus was obtained from a local market in Busan, Korea. Leaves of *Euonymus alatus* was powdered and extracted with ten volumes of methanol for 12 h three times at room temperature. The solvent was removed by rotary evaporation at 40℃. The extraction yield was 9.5%, and the extract was dissolved in dimethylsulfoxide (DMSO) at a concentration of 5 mg/ml to be used as a test sample.

Measurement of yeast α-glucosidase inhibitory activity in vitro

Yeast α-glucosidase inhibitory activity was determined using the chromogenic method developed by Watanabe *et al.* (1997). 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 an enzyme and a substrate solution, respectively. The enzyme solution (50 μl) and 10 μl of the test sample at various concentrations were mixed, and absorbance at 405 nm was measured 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 zero was measured, and inhibitory activity was calculated as a percentage of the blank control. The inhibitory activities of the *E. alatus* 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 IC_{50} value, i.e., the concentration of the extract that results in 50% inhibition of maximal activity, was determined.

Animals

Male Sprague-Dawley rats weighing between 250 and 280 g were purchased from Bio Genomics, Inc. (Seoul, Korea). The rats were housed individually in stainless steel wire-bottomed cages and located in a room where temperature (23-27℃), humidity (50-60%), and light (0600-1800 hr light and 1800-0600 hr dark cycle) were controlled. The animals were fed a commercial chow (Samyang Co., Seoul, Korea) *ad libitum* for 14 d after arrival. They were rendered diabetic by intravenous injection of STZ (60 mg/kg) in citrate buffer, pH 4.5. Blood samples were taken from the tail tip after 7 d, and blood glucose concentration was measured using a glucometer (Glucotrend Roche Diagnostics, United Kingdom). Animals showing fasting blood glucose levels higher than 200 mg/dl were considered diabetic and used for further study. All animals continued to be fed commercial chow.

Measurement of postprandial blood glucose

The effect of *E. alatus* extract on postprandial glucose was measured in STZ-induced diabetic rats (n=16). The rats were randomly divided into two 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 starch with methanol extract of *E. alatus* (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 at 3,000 rpm for 15 min. Plasma glucose was measured using a commercial glucose oxidase kit (Yeongdong Co., Seoul, Korea). The plasma glucose level was expressed in increments from the baseline. Incremental areas under the response curve (AUC) were calculated using the trapezoidal rule with fasting levels as the baseline. All experiments were performed according to the guidelines of animal experimentation approved by the Animal Resource Center at Inje University.

Statistical analysis

Increment plasma glucose level and AUC of the glucose response curve were expressed as mean ± standard error (SE). All statistical analyses were performed using SAS (version 8.02). Differences in incremental plasma glucose levels and AUC

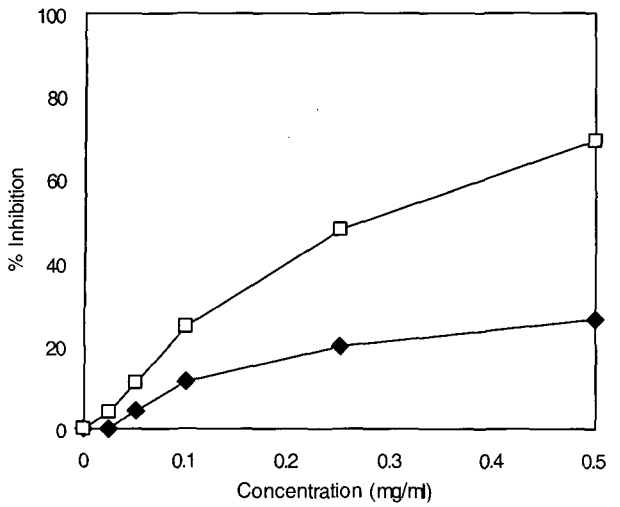

Fig. 1. Dose-dependent inhibition of yeast α-glucosidase activity of *Euonymus alatus* and acarbose. The inhibitory activities of the methanol extract of *Euonymus alatus* or acarbose were measured at concentrations of 0.5, 0.25, 0.1, and 0.05 mg/ml. □, *Euonymus alatus* extract; ◆, acarbose. Values represent means of triplicate measurements.

between the control group and the *Euonymus alatus* group were assessed using Student's *t*-test. Significance was defined as $p < 0.05$.

Results

Inhibition of αglucosidase activity in vitro

The inhibitory activity of the methanol extract of *E. alatus* against yeast α-glucosidase is shown in Fig. 1. The methanol extract of *E. alatus* inhibited yeast αglucosidase activity by 69.4, 48.1, 24.5, 11.3, and 4.2% 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 enzyme activity by 26.5, 20.1, 11.4, and 4.5% at concentrations of 0.50, 0.25, 0.10, and 0.05 mg/ml, respectively. The *E. alatus* extract had an IC_{50} value of 0.272 mg/ml.

Inhibition of αglucosidase activity in vivo

The plasma glucose response to a single oral dose of starch (1 g/kg) alone or starch with *E. alatus* extract (500 mg/kg) is shown in Fig. 2. The incremental plasma glucose levels of the rats that consumed starch were 53.8 ± 8.5, 91.5 ± 5.5, 115.3 ± 10.3, 80.3 ± 8.6, 36.1 ± 5.2, and -0.8 ± 3.2 mg/dl at 30, 60, 90, 120, 180, and 240 min, respectively. The incremental plasma glucose levels of the rats that consumed *E. alatus* extract with starch were 39.4 ± 10.0, 70.8 ± 5.6, 87.8 ± 7.4, 64.8 ± 5.6, 23.1 ± 4.2, -4.6 ± 3.8 mg/dl at 30, 60, 90, 120, 180, and 240 min, respectively. Consumption of *E. alatus* extract significantly decreased incremental plasma glucose levels at 60 and 90 min ($p < 0.05$). The AUC for the glucose response of the *E. alatus* group

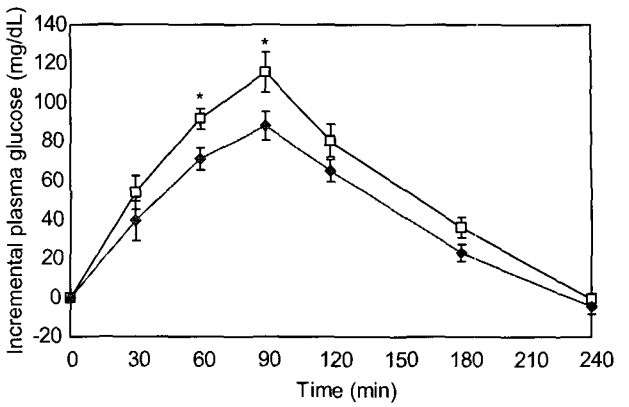

Fig. 2. Postprandial glucose response to *Euonymus alatus* extract in STZ-induced diabetic rats. In the control group (□), starch (1 g/kg) was administered orally to rats after an overnight fast. In the *Euonymus alatus* group (◆), starch (1 g/kg) plus *Euonymus alatus* methanol extract (500 mg/kg) was administered orally to rats after an overnight fast. Values represent mean ± SE (n = 8). * Significantly different at $p < 0.05$.

Table 1. Area under the curve (AUC) of postprandial glucose responses of STZ-induced diabetic rats

Group	AUC (mg · iin/dl)
Control group	12,541 ± 951
Euonymus alatus group	9,350 ± 668*

Control group: Starch (1 g/kg) was administered orally to a rat after an overnight-fast. *Euonymus alatus* group: starch (1 g/kg) with the methanol extract of *Euonymus alatus* (500 mg/kg) was administered orally to a rat after an overnight-fast. Values represent mean ± SE (n=8).
*Significantly different at $p < 0.05$.

(9,350 ± 668 mg·min/dl) was significantly lower than that of the control group (12,541 ± 951 mg·min/dl, $p < 0.05$, Table 1).

Discussion

α-Glucosidase is a key enzyme in carbohydrate digestion in the small intestine (Li *et al.*, 2005). Therefore, α-glucosidase inhibitors could delay digestion of dietary carbohydrates to reduce postprandial glucose. In fact, α-glucosidase inhibitors have become the most common oral agents used to improve postprandial hyperglycemia since their introduction in the early 1990s (Mooradian & Thurman, 1999). However, because the chronic use of synthetic α-glucosidase inhibitors can have undesirable side effects, such as flatulence, diarrhea, and abdominal cramping, their use may be limited (Mooradian & Thurman, 1999). Therefore, attention has focused on natural substances that show potent inhibitory activity against α -glucosidase and have fewer side effects (Joo *et al.*, 2006; Li *et al.*, 2005; Shim *et al.*, 2003; Youn *et al.*, 2004). Galls of *Rhus chinensis* (Shim *et al.*, 2003), *Commelina communis* L. (Youn *et al.*, 2004), flowers of *Punica granatum* (Li *et al.*, 2005), and *Saururus chinensis* Baill (Joo *et al.*, 2006) have shown potent inhibitory activity against α-glucosidase.

In this study, we investigated the inhibitory effect of *E. alatus*

against α-glucosidase to elucidate the possible use of *E. alatus* as an antihyperglycemic agent. Inhibition activity of the methanol extract of *E. alatus* against yeast α-glucosidase was about 2.6 times that of acarbose *in vitro* at a concentration of 0.5 mg/ml (Fig. 1). *Euonymus alatus* extract showed inhibitory activity against yeast α-glucosidase in a dose-dependent manner and had an IC_{50} value of 0.272 mg/ml.

We determined the effect of *E. alatus* on postprandial hyperglycemia in STZ-induced diabetic rats after consumption of starch. Postprandial plasma glucose peaked 90 min after consumption of starch in the control group. The *E. alatus* extract significantly suppressed incremental plasma glucose at 60 and 90 min (Fig. 2) and significantly decreased the AUC for the glucose response curve. These data demonstrate that *E. alatus* exerts α-glucosidase inhibitory activity *in vivo* to decrease postprandial glucose levels. Inoue *et al.* (1997) reported that an α-glucosidase inhibitor that flattens the peak postprandial blood glucose levels reduces the AUC of the blood glucose response curve. In our study, *E. alatus* extract decreased both incremental blood glucose at the peak time point and the AUC.

Postprandial hyperglycemia is one of the earliest observable abnormalities in diabetes mellitus. Postprandial hyperglycemia is highly correlated with glycated hemoglobin levels and is a better predictor of glycated hemoglobin levels than fasting glucose (Soonthornpun *et al.*, 1999). Glycated hemoglobin is highly associated with a higher risk of cardiovascular disease and coronary heart disease mortality (Campbell *et al.*, 2001). Postprandial hyperglycemia is strongly correlated with risks for micro- and macrovascular complications of diabetes (Campbell *et al.*, 2001). Bastyr *et al.* (2000) demonstrated that diabetes therapy focused on lowering postprandial glucose rather than fasting glucose could be a better treatment.

In conclusion, *E. alatus* showed strong inhibitory activity against α-glucosidase *in vitro* and *in vivo*. Thus, chronic consumption of *E. alatus* could be helpful in improving hyperglycemia and preventing diabetic complications. Further study to identify the active component responsible for the inhibition of α-glucosidase is strongly recommended.

Literature Cited

Abrahaamson MJ (2004). Optimal glycemic control in type 2 diabetes mellitus: fasting and postprandial glucose in context. *Arch Intern Med* 164:486-491.

Avignon A, Radauceanu A & Monnier L (1997). Nonfasting plasma glucose is a better marker of diabetic control than fasting plasma glucose in type 2 diabetes. *Diabetes Care* 20:1822-1826.

Balflour JA & McTavish D (1993). Acarbose. An update of its pharmacology and therapeutic use in diabetes mellitus. *Drugs* 46: 1025-1054.

Baron AD (1998). Postprandial hyperglycaemia and α-glucosidase inhibitors. *Diabetes Res Clin Pract* 40:S51-55.

Bastyr EJ, Stuart CA, Brodows RG, Schwartz S, Graf CJ, Zagar A & Robertson KE (IOEZ Study Group) (2000). Therapy focused on lowering postprandial glucose, not fasting glucose, may be superior for lowering HbA1c. *Diabetes Care* 23:1236-1241.

Campbell RK, White JR & Nomura D (2001). The clinical importance of postprandial hyperglycemia. *Diabetes Educ* 27:624-637.

Centers for Disease Control and Prevention (1999). *Diabetes Surveillance Report*, Atlanta, GA: US Department of Health and Human Services.

Coniff RF, Shapiro JA, Robbins D, Kleinfield R, Seaton TB, Beisswenger P & McGill JB (1995). Reduction of glycosylated hemoglobin and postprandial hyperglycemia by acarbose in patients with NIDDM. *Diabetes Care* 18:817-824.

Haller H (1998). The clinical importance of postprandial glucose. *Diabetes Res Clin Pract* 40:S43-49.

Holman RR, Cull CA & Turner RC (1999). A randomized double-blind trial of acarbose in type 2 diabetes shows improved glycemic control over 3 years (U.K. Prospective Diabetes Study 44). *Diabetes Care* 22:960-964.

Inoue I, Takahashi K, Noji S, Awata T, Negishi K & Katayama S (1997). Acarbose controls postprandial hyper-proinsulinemia in non-insulin-dependent diabetes mellitus. *Diabetes Res Clin Pract* 36:143-151.

Jenkins DJ, Wolever TM & Jenkins AL (1988). Starchy foods and glycemic index. *Diabetes Care* 11:149-159.

Jermendy G (2005). Can type 2 diabetes mellitus be considered preventable? *Diabetes Res Clin Pract* 68:S73-S81.

Joo HJ, Kang MJ, Seo TJ, Kim HA, Yoo SJ, Lee SK, Lim HJ, Byun BH & Kim JI (2006). The hypoglycemic effect of *Saururus chinensis* Baill in animal models of diabetes mellitus. *Food Science and Biotechnology* 15:413-417.

Kim CH, Kim DI, Kwon CN, Kang SK, Jin UH, Suh SJ, Lee TK & Lee IS (2006). *Euonymus alatus* (Thunb.) Sieb induces apoptosis via mitochondrial pathway as prooxidant in human uterine leiomyomal smooth muscle cells. *Int J Gynecol Cancer* 16: 843-848.

King H, Aubert RE & Herman WH (1998). Global burden of diabetes, 1995-2025: prevalence, numerical estimates, and projections. *Diabetes Care* 21:1414-1431.

Korea National Statistical Office (2006). The cause of death Statistics 2005. *Annual Report of on the Cause of Death Statistics*. Korea National Statistical Office, Seoul. Republic of Korea

Lee H, Kim HK & Ha TY (1993). Antitumor effect of winged *Euonymus* against chemically induced and malignant cell implanted-tumors in mice. *Korean Journal of Immunology* 15:243-253.

Li Y, Wen S, Kota BP, Peng G, Li GQ, Yamahara J & Roufogalis BD (2005). *Punica granatum* flower extract, a potent α-glucosidase inhibitor, improves postprandial hyperglycemia in Zucker diabetic fatty rats. *J Ethnopharmacol* 99:239-244.

Mooradian AD & Thurman JE (1999). Drug therapy of postprandial hyperglycemia. *Drugs* 57:19-29.

Oh BY, Hwang SK, Cheong MY, Sin HS, Park BH, Lee JH & Kim S (2005). Components and biological activity of aqueous extract isolated from winged stem of *Euonymus alatus*. *Korean Journal of Food Science and Technology* 37:898-904.

Park SH, Ko SK & Chung SH (2005). *Euonymus alatus* prevents the hyperglycemia and hyperlipidemia induced by high-fat diet in ICR mice. *J Ethnopharmacol* 102:326-335.

Saito N, Sakai H, Sekihara H & Yajima Y (1998). Effect of an α -glucosidase inhibitor (voglibose), in combination with sulphonilureas, on glycaemic control in type 2 diabetes subjects. *J Int Med*

Res 26:219-232.

Sels JP, Huijberts MS & Wolffenbuttel BH (1999). Miglitol, a new alpha-glucosidase inhibitor. *Expert Opin Pharmacother* 1:149-156.

Seo KS, Lim JK, Park JH, Kim CH, Chung GY & Jeong HJ (2003). Antioxidative activity and biological properties in extracts of *Euonymus alatus* (Thnub.) Sieb. *Korean Journal of Life Science* 13:1-8

Shim YJ, Doo HK, Ahn SY, Kim YS, Seong JK, Park IS & Min BH (2003). Inhibitory effect of aqueous extract from the gall of *Rhus Chinensis* on alpha-glucosidase activity and postprandial blood glucose. *J Ethnopharmacol* 85:283-287.

Soonthornpun S, Rattarasarn C, Leelawattana R & Setasuban W (1999). Postprandial plasma glucose: a good index of glycemic control in type 2 diabetic patients having near-normal fasting glucose levels. *Diabetes Res Clin Pract* 46:23-27.

Stand E, Baumgartl HJ, Fuchtenbusch M & Stemplinger J (1999). Effect of acarbose on additional insulin therapy in type 2 diabetic patients with late failure of sulphonylurea therapy. *Diabetes Obes Metab* 1:215-220.

The Diabetes Control and Complications Trial (DCCT) Research Group (1993). The effect of intensive treatment of diabetes on the development and progression of long-term complications in the diabetes control in insulin-dependent diabetes mellitus. *N Engl J Med* 329:977-986.

UK Prospective Diabetes Study (UKPDS) Group (1998). Tight blood pressure control and risk of macrovascular and microvascular complications in type 2 diabetes: UKPDS 38. *BMJ* 317:703-713.

Watanabe J, Kawabata J, Kurihara H & Niki R (1997). Isolation and identification of α-glucosidase inhibitors from Tochu-cha (Encommia ulmoides). *Biosci Biotechnol Biochem* 61:177-178.

Youn JY, Park HY & Cho KH (2004). Anti-hyperglycemic activity of *Commelina communis* L.: inhibition of α-glucosidase. *Diabetes Res Clin Pract* 66S:S149-155.