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Inhibitory Effects of *Artemisia capillaris* Thumb. on α-Glucosidase and α-Amylase

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ABSTRACT : This study was carried out to investigate inhibitory effect of extracts from *Artemisia capillaris* Thumb. on maltase, sucrase, α-amylase, nonspecific α-glucosidase, and postprandial hyperglycemia. Methanol extract and organic solvent (*n*-hexane, ethyl acetate, butanol, aqueous) fractions from the medicinal herb were determined for the inhibitory activities against maltase, sucrase and α-amylase. The methanol extract from *A. capillaris* strongly inhibited maltase (57%) and α-glucosidase (72%) at the concentration of 100 μg/mℓ. Among the four fractions (*n*-hexane, ethyl acetate, butanol, aqueous) examined, the butanol fraction from *A. capillaris* showed potent inhibitory effects on maltase (73%), sucrase (33%), and α-amylase (75%) at the concentration of 100 μg/mℓ. The butanol fraction from *Artemisia capillaris* also exhibited significant reductions (20%) of blood glucose elevation in mice loaded with maltose. These results suggest that the extract from *Artemisia capillaris* can be used as a new nutraceutical for inhibition on postprandial hyperglycemia

Key words **:** *Artemisia capillaries* Thumb., postprandial hyperglycemia, maltase, α-amylase

INTRODUCTION

Artemisia capillaris Thumb. (Compositae), is an important medicinal herb used in Chinese traditional medicine as antipyretic, anti-inflammatory, diuretic for the treatment of hepatitis and bilious disorder (Chang and But, 1987). Several studies have demonstrated the free radical scavenging, immunosuppressive and vasodilator activities of scoparone (Huang *et al.*, 1991, 1992). Scoparone (6,7-Dimethoxy coumarin) is a major component of the shoot of *Artemisia capillaris*

α-Glucosidases are located in the brush-border surface membrane of intestinal cells, are the key enzymes of carbohydrate digestion (Caspary, 1978). The administration of an α-glucosidase inhibitor would thus retard the digestion and absorption of carbohydrates and, in consequence, the rise in postprandial blood glucose could be suppressed and delayed. The intestinal α-glucosidases are divided into four hydrolase types, namely, maltase (EC 3.2.0.20), glucoamylase (EC 3.2.1.3), sucrase (EC 3.2.1.48), and isomaltase (EC 3.2.1.10). Among them, maltase is the major enzyme which is responsible for the digestion and absorption of dietary starch, whereas sucrase can only hydrolyze sucrose.

Hyperglycaemia, which is the result of decreased insulin sensitivity or decreased insulin secretion from pancreatic β-cells, can further inhibit insulin secretion from pancreas and diminish insulin-mediated glucose uptake in peripheral tissues (Wolffenbuttel and Haeften, 1995). α-Glucosidase inhibitors delay the digestion of oligosaccharide and disaccharide to monosaccharide by inhibiting α-glucosidase on the small intestinal brush-border, and reduce the rate of glucose absorption. Therefore, inhibition of α-glucosidase is considered important in managing noninsulin-dependent diabetes (Bischo, 1994).

In the present study, we investigated the inhibitory activity of solvent fractions from *Artemisia capillaris* on rat intestinal α-glucosidase, pancreatic α-amylase and blood glucose elevation in mice.

MATERIALS AND METHODS

Materials

Artemisia capillaris Thumb. was purchased from herb markets in Seoul, Korea. The medicinal plant was extracted with methanol at room temperature. The methanol extracts were dried under reduced pressure, and then the concentrated methanol extracts were partitioned into n-hexane, ethyl acetate, *n*-butanol, and aqueous fractions. *p*-nitrophenyl-α-D-glucopyranoside as a substrate and all the other reagents were purchased from Sigma (St. Louis, MO).

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Animals

Five to six week old male ICR mice and SD rats were purchased from Daehan Biolink Co. Ltd. (Korea). They were housed in plastic cages with free access to food (until 12 h before use) and water, and were kept in a room at 25 ± 1°C, 55 ± 5% humidity with a 12 h dark-light cycle.

Preparation of crude enzyme solution

A crude α-glucosidase enzyme solution prepared from rat intestinal by careful scraping with a thin spatula, and diluted with cold 0.1 M potassium phosphate buffer (pH 7.0). After breakdown on a sonicator for 15 sec, the suspension was centrifuged at 10,000 rpm, 4℃ for 30 min and the supernatant used as the crude enzyme.

Enzyme assay

Maltase activity was measured according to the method of Dahlqvist (Dahlqvist, 1970). The maltase reaction mixture contained 0.1 mℓ of crude enzyme solution, 0.1 mℓ of 2 mM maltose, 0.1 mℓ of sample solution and 0.2 mℓ of 0.1 M phosphate buffer (pH 7.0). After incubation for 40 min at 37℃, the reaction mixture was inactivated on a hot water bath for 2 min, and then centrifuged at 3,000 rpm for 5 min. 0.1 mℓ of supernatant was added to the glucose reagent, consisting of *o*-phenylenediamine 0.005 mg/mℓ, peroxidase 2 unit/mℓ and glucose oxidase 0.384 unit/mℓ, and incubated for 30 min. 0.5 mℓ of 1 N HCl were added to the reaction mixture and the liberated glucose measured colorimetrically at 492 nm (Lee *et al.*, 1983; Tandon *et al.*, 1975).

Sucrase activity was measured according to the method of Dahlqvist (Dahlqvist, 1970). The reaction mixture for the surcrase determination contained 0.1 mℓ of crude enzyme solution, 0.1 mℓ of 10 mM sucrose, 0.1 mℓ of sample solution and 0.2 mℓ of 0.1 M phosphate buffer (pH 7.0). After incubation for 180 min at 37℃, the enzyme inactivated in a hot water bath for 2 min, then centrifuged at 3,000 rpm for 5 min, and 0.1 mℓ of supernatant was then added to 0.1 mℓ of the glucose reagent and incubated for 30 min. 0.5 mℓ of 1 N HCl were added to the reaction mixture and the liberated glucose measured colorimetrically at 492 nm.

Pancreatic α-amylase activity was measured according to the method of Rinderknecht (Rinderknecht *et al.*, 1967). 0.1 mℓ of reaction mixture containing the crude enzyme, 0.2 mℓ of sample and 0.75 mℓ of starch solution (1 unit/20 mM phosphate buffer, pH 7.0) were incubated at 37℃ for 1 h. After addition of 0.5 mℓ of 0.1 N HCl, the reaction mixture was centrifuged at 3,000 rpm for 10 min, and 1.0 mℓ of the supernatant was measured against a reagent blank colormetrically at 620 nm.

Nonspecific α-glucosidase activity was measured according to the method of Dahlqvist (Dahlqvist, 1970). The reaction mixture containing 0.05 mℓ of crude enzyme solution, 0.1 mℓ of sample solution, 0.2 mℓ of 20 mM phosphate buffer (pH 7.0) and 0.25 mℓ of *p*-nitrophenyl-α-D-glucopyranoside (2 mM) was incubated at 37℃ for 30 min and then 0.5 mℓ of 1 M glycine-NaOH (pH 9.0) was added to terminate the reaction. After centrifugation for 10 min, at 3000 rpm, the supernatant was analyzed by measuring the absorbency at 405 nm.

In vivo maltase inhibitory effect

Immediately after oral administration of the *n*-butanol fraction (400 mg/kg) from *Artemisia capillaris* or acarbose (positive control, 80 mg/kg), maltose (2000 mg/kg) was orally given to mice. Sixty minutes later, cardiac blood was collected from the animals with a heparin-treated cylinder. A 50 μℓ of blood was provided for the determination using glucose meter (Super Glucocard™ II, Japan). The purified water was given to the control mice and the data was calculated to determine glucose concentration (mg/dℓ).

Statistical analysis

All values are expressed as mean ± SD. *P* values were calculated from the Student's *t*-test, based on comparisons with appropriate control samples tested at the same time.

RESULTS AND DISCUSSION

The reported spectroscopic method was used to evaluate the *Artemisia capillaris* Thumb. for *in vitro* α-glucosidase inhibition. First, the methanol extract from *Artemisia capillaris* was determined for the inhibitory activities against α-glucosidase and α-amylase isolated the small intestinal brush border of Sprague Dawley male rats.

The methanol extract from *Artemisia capillaris* strongly inhibited maltase and α-amylase by more than 45% at the concentration of 100 μg/mℓ (Fig. 1). The extract also strongly inhibited nonspecific α-glucosidase by 72% using *p*-nitrophenyl-α-D-glucopyranoside as substrate but showed weak inhibitory activity on sucrose.

The effects of the four solvent (*n*-hexane, ethyl acetate, *n*-butanol, aqueous) fractions from the medicinal herb were tested on maltase. As shown in Fig. 2, *n*-butanol fraction appeared to be most potent (73% inhibition) and then ethyl acetate fraction (71% inhibition) at the concentration of 100 μg/mℓ. However the *n*-hexane and aqueous fractions did not affect on maltase.

We next investigated the effects of the four fractions from the medicinal herb on sucrase. As shown in Fig. 3, the butanol

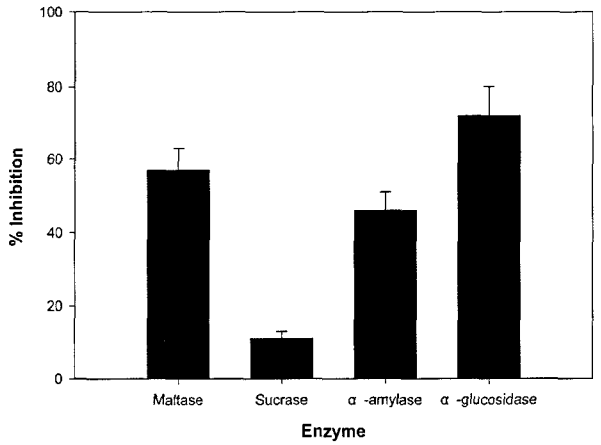

Fig. 1. Inhibitory effects of methanol extract (100 μg/mL) from *Artemisia capillaris* on rat intestinal α-glucosidase. All values are expressed as mean ± SD of triplicate tests.

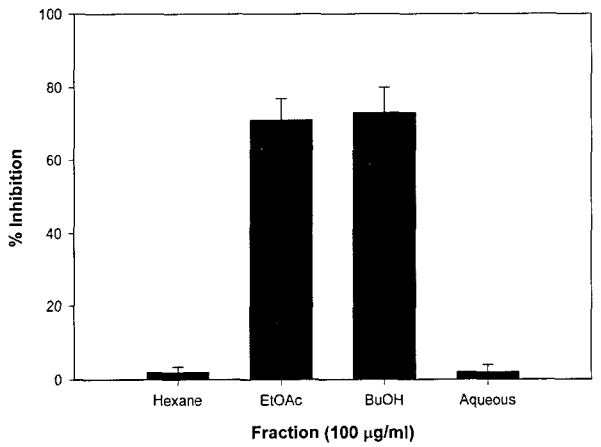

Fig. 2. Inhibitory effects of fractions (100 μg/mL) from *Artemisia capillaris* on maltase. All values are expressed as mean ± SD of triplicate tests.

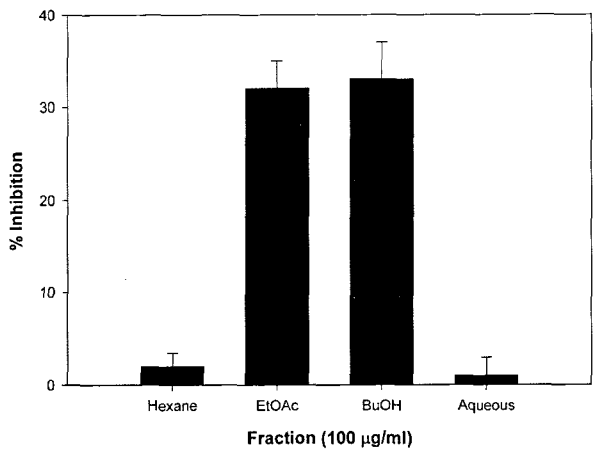

Fig. 3. Inhibitory effects of fractions (100 μg/mL) from *Artemisia capillaris* on sucrase. All values are expressed as mean ± SD of triplicate tests.

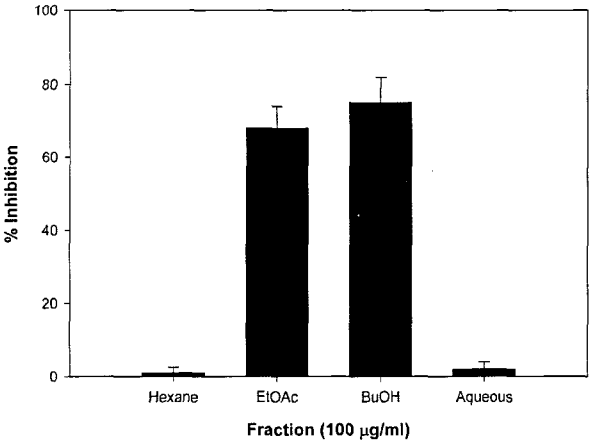

Fig. 4. Inhibitory effects of fractions (100 μg/mL) from *Artemisia capillaris* on α-amylase. All values are expressed as mean ± SD of triplicate tests.

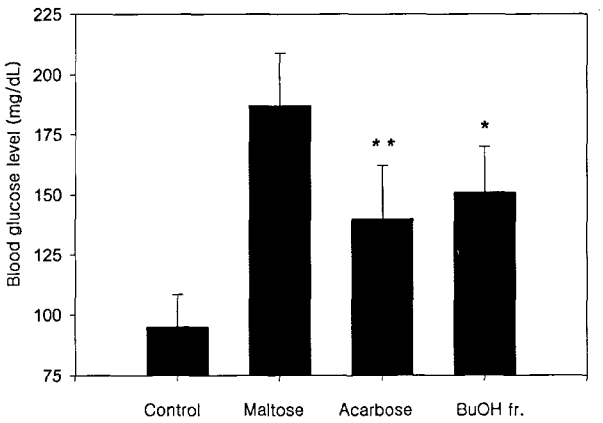

Fig. 5. Inhibitory effects of acarbose (80 ㎎/㎏) and the butanol fraction (400 ㎎/㎏) from *Artemisia capillaris* on blood glucose elevation in mice loaded maltose (2 g/㎏). *Statistically significant compared with the control data (*, $p < 0.05$; **, $p < 0.01$).

fraction exhibited highly effective inhibition (33% inhibition) and then ethyl acetate fraction (32% inhibition) at the concentration of 100 μg/㎖. However the *n*-hexane and aqueous fractions also showed no inhibitory activity against sucrase.

And then, we investigated the effects of the four fractions from the *Artemisa* on α-amylase. As shown in Fig. 4, the *n*-butanol fraction exhibited highly effective inhibition (75% inhibition) and then ethyl acetate fraction (68% inhibition) at the concentration of 100 μg/㎖. However the *n*-hexane and aqueous fractions also showed no inhibitory activity against α-amylase.

Since, the methanol extract from *Artemisia capillaris* and the *n*-butanol fraction from the methanol extracts were effective on maltase, sucrase, and nonspecific α-glucosidase activi-

ties, the effect of the methanol extract on α-glucosidase seems to be ascribed to the effect of the butanol and ethyl acetate fraction.

A further investigation was performed on blood glucose elevation in mice loaded maltose. Acarbose, a synthetic α-glucosidase inhibitor, was employed as a positive control (80 ㎎/㎏). As shown Fig. 5, the butanol fraction (400 ㎎/㎏) from *Artemisia capillaris* and acarbose significantly reduced the blood glucose elevation in comparison with positive control in mice loaded with maltose (2 g/㎏) by 20% and 25% respectively.

α-Glucosidase catalyzes the final step in the digestive process of carbohydrates, and hence α-glucosidase inhibitors could retard the digestion of dietary carbohydrates to suppress postprandial hyperglycemia (Watanabe *et al.*, 1997). Inhibition of membrane-bound intestinal α-glucosidase and pancreatic α-amylase should result in delayed carbohydrate digestion and glucose absorption with attenuation of postprandial hyperglycemic excursions. α-Glucosidase inhibitor such as acarbose, miglitol, and voglibose are known to block the enzymatic degradation of complex carbohydrates in the small intestine. Thus, less carbohydrate is absorbed and the carbohydrate that is absorbed is delayed (Mooradian and Thurman, 1999).

In this experiment, the *n*-butanol and ethyl acetate fractions from *Artemisia capillaris* were shown to have inhibitory effects on membrane-bound intestinal α-glucosidase and pancreatic α-amylase enzymes *in vitro* as well as on blood glucose elevation in mice loaded with maltose *in vivo*.

Therefore, these results suggest that the extract from *Artemisia capillaris* can be used as a new nutraceutical for inhibition on postprandial hyperglycemia.

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