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Inhibitory Effects of *Paeonia suffruticosa* Extracts on Maltase and Sucrase

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Abstract – This study was carried out to investigate inhibitory effect of extracts from root cortex of *Paeonia suffruticosa* on α-glucosidases (EC 3. 2. 1. 20) and postprandial hyperglycemia. Methanol extract and organic solvent (*n*-hexane, ethyl acetate, butanol, aqueous) fractions from the crude drug were determined for the inhibitory activities against maltase, sucrase and α-amylase. The methanol extract from the crude drug strongly inhibited maltase (72%) and sucrase (76%) at the concentration of 100 μg/ml. Among the fractions examined, the ethyl acetate fraction from the natural plant drug showed potent inhibitory effects on maltase (85%) and sucrase (81%) at the concentration of 100 μg/ml. The ethyl acetate fraction from root cortex of *Paeonia suffruticosa* also exhibited significant reductions (21%) of blood glucose elevation in mice loaded with maltose.
Key words – *Paeonia suffruticosa*, postprandial hyperglycemia, α-glucosidase

Introduction

α-Glucosidase are membrane-bound enzymes at the epithelium of the small intestine and 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.

Diabetic and most obese patients suffer from abnormal blood glucose and required control of carbohydrate metabolism. Suppression of intestinal α-glucosidase activity can retard the digestion and absorption of carbohydrates, and therefore, α-glucosidase inhibitors have a possibility to reduce the postprandial elevation of blood glucose levels (Bischo., 1994).

Moutan Cortex Radicis, the root cortex of *Paeonia suffruticosa* Andr. (Ranunculaceae), is an important crude drug used in Chinese traditional medicine as both an analgesic and an anti-inflammatory agent (Lin *et al.*, 1998), and it is prescribed in various Chinese preparations for the treatment of blood stagnation. It has been reported that the methanolic extract of Moutan Cortex Radicis increases antioxidant activity (Lee *et al.*, 2003), prevents the process of herpes simplex virus attachment and penetration (Hsiang *et al.*, 2001) and inhibits enzymes crucial to the life cycle of the human immunodeficiency virus (HIV) (Au *et al.*, 2001).

In the present study, we investigated the inhibitory activity of solvent fractions from the root cortex of *Paeonia suffruticosa* on α-glucosidase of rat intestine and blood glucose elevation in mice.

Experimental

Materials – The root cortex of *Paeonia suffruticosa* was purchased from herb markets in Seoul, Korea. The specimens were authenticated by Dr. S-Y, Hwang, Korea Medical Science Institute Co. Ltd. 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).

Animals – Five to six week old male ICR mice and SD rat 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

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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°C 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°C, 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/ and glucose oxidase 0.384 unit/ml, and incubated for 30 min. 0.5 ml of 1N 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 surcrose, 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°C, 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.

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°C 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°C 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.

Determination of blood glucose – Immediately after oral administration of test samples, 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 methanol extract from the root cortex of *Paeonia suffruticosa* was determined for the inhibitory activities against α-glucosidase isolated from rats. Because it was reported that there was an interesting difference in α-glucosidase activity between yeast and rat origin (Lee and Ji, 2004), α-glucosidase was prepared from the small intestinal brush border of Sprague Dawley male rats instead of yeast α-glucosidase.

The methanol extract from the root cortex of *Paeonia suffruticosa* strongly inhibited maltase and sucrase by more than 70% at the concentration of 100 μg/ml (Fig. 1). The extract also strongly inhibited nonspecific α-

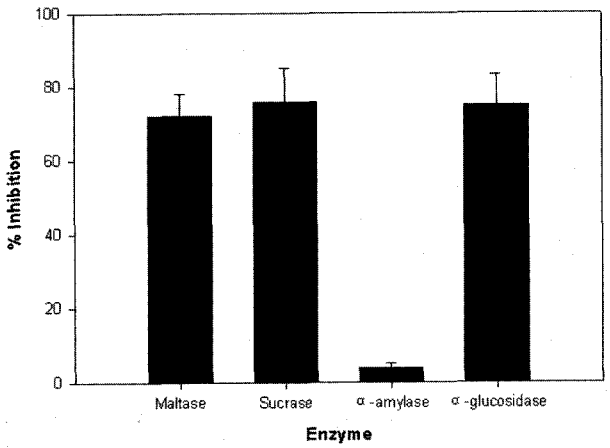

Fig. 1. Inhibitory effects of methanol extract (100 μg/mL) from the root cortex of *Paeonia suffruticosa* on rat intestinal α-glucosidase. All values are expressed as mean ± SD of triplicate tests.

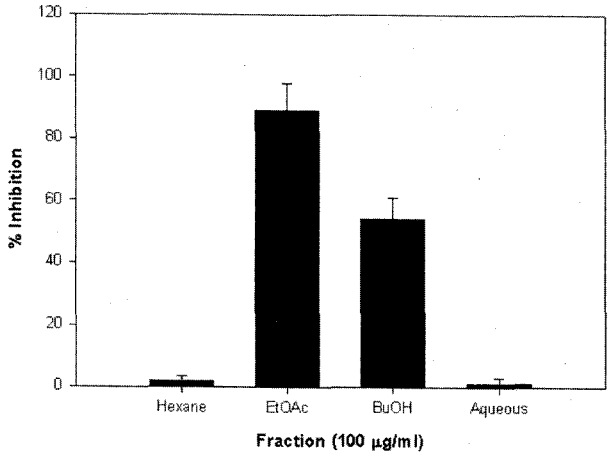

Fig. 2. Inhibitory effects of fractions (100 μg/mL) from the root cortex of *Paeonia suffruticosa* on maltase. All values are expressed as mean ± SD of triplicate tests.

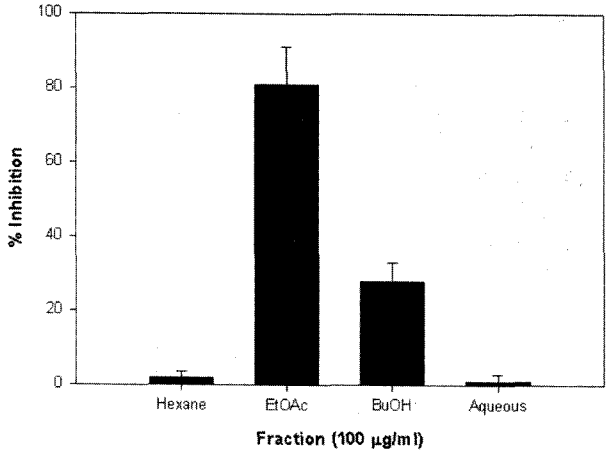

Fig. 3. Inhibitory effects of fractions (100 μg/mL) from the root cortex of *Paeonia suffruticosa* on sucrase. All values are expressed as mean ± SD of triplicate tests.

glucosidase (≈ 75% inhibition) using *p*-nitrophenyl-α-D-glucopyranoside as substrate but showed weak inhibitory activity on α-amylase.

The effects of the four solvent (*n*-hexane, ethyl acetate, butanol, aqueous) fractions from the crude drug were tested on maltase. As shown in Fig. 2, the ethyl acetate fraction appeared to be most potent (≈ 89% inhibition) and then butanol fraction (≈ 54% 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 crude drug on sucrase. As shown in Fig. 3, the ethyl acetate fraction exhibited highly effective inhibition (≈ 81% inhibition) and then butanol fraction (≈ 28% inhibition) at the concentration of 100 μg/ml. However

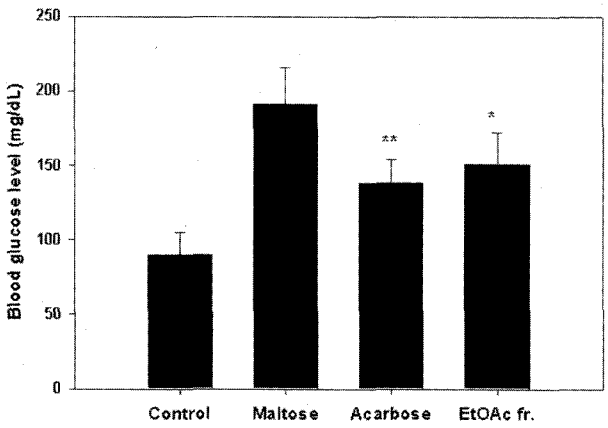

Fig. 4. Inhibitory effects of acarbose (80 mg/kg) and the ethyl acetate fraction (400 mg/kg) from root cortex of *Paeonia suffruticosa* on blood glucose elevation in mice loaded maltose (2 g/kg). *Statistically significant compared with the control data (*, $p < 0.05$; **, $p < 0.01$).

the *n*-hexane and aqueous fractions also showed no inhibitory activity against sucrase.

Since, the methanol extract from the root cortex of *Paeonia suffruticosa* and the ethyl acetate fraction from the methanol extracts were effective on maltase, sucrase, and nonspecific α-glucosidase activities, the effect of the methanol extract seems to be ascribed to the effect of the 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 mg/kg). As shown Fig. 4, the ethyl acetate fraction (400 mg/kg) from root cortex of *Paeonia suffruticosa* and acarbose significantly reduced the blood glucose elevation in comparison with positive control in mice loaded with maltose (2 g/kg) by 21% and 28% respectively.

α-Glucosidase inhibitors are currently the most commonly used oral agents for improving postprandial hyperglycemia and the prospect of blood glucose control without hyperinsulinemia and body weight gain (Mooradianand Thurman, 1999; Lee and Kim, 2000). Inhibition of α-glucosidase and amylase should result in delayed carbohydrate digestion and glucose absorption with attenuation of postprandial hyperglycemic excursions. α-Glucosidase inhibitors including acarbose, miglitol, and voglibose are at present available for the treatment of patients with type II diabetes mellitus. In this experiment, the ethyl acetate fraction from root cortex of *Paeonia suffruticosa* was shown to have an inhibitory effect on α-glucosidase *in vitro* as well as on blood glucose elevation in mice loaded with maltose *in vivo*. Therefore, the crude

drug offer the possibility of being developed as a α-glucosidase inhibitor for controlling postprandial blood glucose elevation in patients with fewer side effects, because they have been clinically used as natural plant drug for a long time without serious problems.

All the results so far obtained suggest that the ethyl acetate extracts from the root cortex of *Paeonia suffruticosa* contain α-glucosidase inhibitory substances. However, the mechanism of action and the nature of the active molecules remain to be elucidated. So, further analysis of the crude drug extracts, including additional purification and chemical characterization, should permit the identification of these interesting principles possessing α-glucosidase inhibitory activity.

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References

Au, T.K., Lam, T.L., Ng, T.B., Fong, W.P., and Wan, D.C., A Comparison of HIV-1 integrase inhibition by aqueous and methanol extracts of Chinese medicinal herbs. *Life Sci.* **68**, 1687-1694 (2001).

Bischo., H. Pharmacology of α-glucosidase inhibition. *Eur. J. Clin. Invest.* 24, 3-10 (1994).

Caspary, W.F., Sucrose malabsorption in man after ingestion of α-glucosidehydrolase inhibitor. *Lancet* **1**, 1231-1233 (1978).

Dahlqvist, A., Assay of intestinal disaccharidases. *Enzymol. Biol. Clin.* **11**, 52-56 (1970).

Hsiang, C.Y., Hsieh, C.L., Wu, S.L., Lai, I.L., and Ho, T.Y., Inhibitory effect of anti-pyretic and anti-inflammatory herbs on herpes simplex virus replication. *Am. J. Chin. Med.* **29**, 459-467 (2001).

Lee, S.E., Hwang, H.J., Ha, J.S., Jeong, H.S., and Kim, J.H., Screening of medicinal plant extracts for antioxidant activity. *Life Sci.* **73**, 167-79 (2003).

Lee, S.J. and Ji, S.T., Effect of *Paeonia lactiflora* extracts on α-glucosidase. *Nat. Prod. Sci.* **10**: 223-227 (2004).

Lee, S.M., Bustamante, S.A., and Koldovsk, O., The effect of alpha-glucosidase inhibiton on intestinal disaccharidase activity in normal and diabetic mice. *Metabolism* **32**, 793-799 (1983).

Lee, Y.G. and Kim, S.D., Effect of palmiwon on diabetesprone BB rats. *Food Sci. Biotechnol.* **9**, 157-162 (2000).

Lin, H.C., Ding, H.Y., and Wu, Y.C., Two novel compounds from *Paeonia suffructicosa*. *J. Nat. Prod.* **61**, 343-346 (1998).

Mooradian, A.D. and Thurman, J.E., Drug therapy of postprandial hyperglycemia. *Drugs* **57**, 19-29 (1999).

Rinderknecht, H., Wilding, P., and Haverback, B.J., A new method for the determination of α-amylase. *Experientia* (Basel), **23**, 805 (1967).

Tandon, R.K., Srivastava, L.M., and Pandey, S.C., Increased disaccharidase activity in human diabetics. *Am. J. Clin. Nutr.* **28**, 621-625 (1975).

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