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Effect of *Paeonia lactiflora* Extracts on α-Glucosidase

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Abstract – This study was carried out to investigate inhibitory effect of extracts from the root of *Paeonia lactiflora* on postprandial hyperglycemia. Organic solvent (hexane, ethyl acetate, butanol, aqueous) extracts from the crude drug were fractionated by high performance liquid chromatography. These fractions were examined to evaluate α-glucosidase (EC 3. 2. 1. 20) inhibition by microplate colorimetric assay. Among the fractions examined, the ethyl acetate fraction from the roots of *Paeonia lactiflora* showed potent inhibitory effects on α-glucosidase. Therefore, further fractionation of the fraction was carried out to isolate the active principles. Finally, we isolated and purified 1,2,3,4,6-penta-*O*-galloyl-beta-D-glucose (PGG) as a active principle by activity-guided fractionation. These results suggest that the extract from the root of *Paeonia lactiflora* can be used as a new nutraceutial for inhibition on postprandial hyperglycemia and PGG might be a candidate for developing an α-glucosidase inhibitor.

Keywords – *Paeonia lactiflora*, postprandial hyperglycemia, α-glucosidase inhibitor, α-glucosidase

Introduction

α-Glucosidase is a membrane-bound enzyme at the epithelium of the small intestine, that hydrolyses the cleavage of glucose from disaccharides and oligosaccharides. Intestinal α-glucosidases are divided into four enzymes: maltase, glucoamylase, sucrase and isomaltase. 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).

The root of *Paeonia lactiflora* Pall. is an important crude drug used in many traditional prescriptions in China and Korea. There are several reports of the various chemical constituents, mainly monoterpenoid glycosides (Murakami *et al.*, 1996; Tanka *et al.*, 2000) and tannins (Nishizawa *et al.*, 1983; Tanka *et al.*, 2000). It is commonly used in nourishing blood, activating circulation, alleviating pain, regulating menstruation, treating liver disease and cancer. The extract from the root of *Paeonia lactiflora* can improve blood flow through its endothelium-dependent vasodilator action on aorta (Goto *et al.*, 1996) and inhibitory effects on thrombosis and platelet aggregation (Wang and Ma, 1990).

In the present study, solvent fractions from the root of *Paeonia lactiflora* were examined the inhibitory effects on α-glucosidase. PGG, 1,2,3,4,6-penta-*O*-galloyl-beta-D-glucose, was isolated from the crude drug by activity-guided fractionation.

Experimental

Materials – The root of *Paeonia lactiflora* and other medicinal plants were 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. α-Glucosidase from bakers yeast (9 U/mg) was purchased from Sigma (St. Louis, MO), and *p*-nitrophenyl glycosides as a substrate was obtained from

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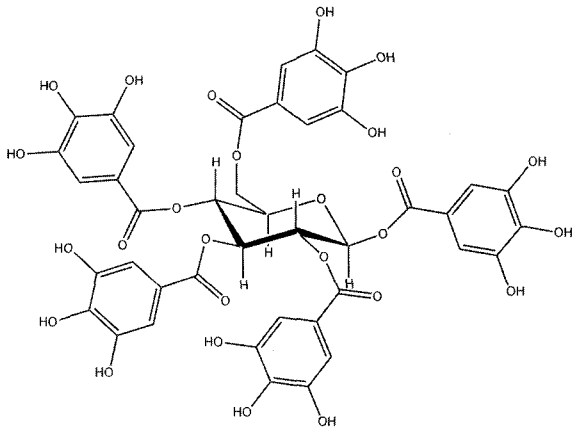
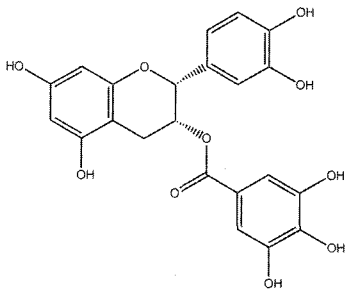
Merck (Darmstadt, Germany). All the other reagents were from Sigma (St. Louis, MO).

Purification of PGG – The roots of *Paeonia lactiflora* (8 kg) was extracted three times with methanol and the methanol extracts (820 g) were partitioned into *n*-hexane, ethyl acetate, *n*-butanol, and water fractions. The ethyl acetate fraction (60 g) was chromatographed over a silica gel column eluting with a gradient of cyclo-hexane/ isoprophyl alcohol mixtures (100 : 0 → 0 : 100) and then PGG (53 mg) was purified by reverse phase C18 column using water/acetonitrile mixtures (100 : 0 → 0 : 100). The structure of PGG was identified by ^{1}H-NMR, ^{13}C-NMR, and electron ionization mass spectrometer spectral data together with published data (Kwon *et al.*, 1999).

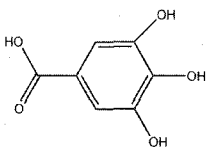
PGG – An amorphous off-white powder; m.p. 185°C; UV λ_{max} (log ε) 286 (4.8) nm, ^{1}H-NMR (400 MHz, DMSO-d_6) δ 6.95, 6.89, 6.83, 6.79, 6.75 (each 2H, s, galloyl-H),6.35 (1H, d, *J*=8 Hz, H of glucose), 5.93 (1H, t, *J*=9 Hz, H-3), 5.42 (1H, t, *J*=9 Hz, H-4), 5.39 (1H, t, *J*=9 Hz, H-2), 4.28-2.58 (3H, m, H-5 and H-6); ^{13}C-NMR (400 MHz, DMSO-d_6) δ 119.02, 118.21, 118.05, 117.95, 117.48 (galloyl C-1), 109.08, 108.94, 108.80, 108.80, 108.80 (galloyl C-2, 6), 145.67, 145.55, 145.49, 145.40, 145.38 (galloyl C-3, 5) 139.59, 139.08, 139.05, 138.90, 138.71 (galloyl C-4), 165.51, 164.89, 164.67, 164.54, 164.02 (galloyl ester), 91.78 (glucose C-1).



PGG



EGCG



Gallic acid

Fig. 1. The structures of PGG, EGCG, and gallic acid.

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.

α-Glucosidase assay – α-Glucosidase activity was measured according to the method of Dahlqvist (Dahlqvist, 1970). α-Glucosidase was assayed using 50 mM phosphate buffer at pH 6.7 and 50 mM sodium citrate buffer at pH 4.5, and the appropriate 1 mM *p*-nitrophenyl glycosides were used as substrates. The enzyme reaction was carried out at 30°C for 30 min, and then 3 volumes of 1 M Na_2CO_3 were added to terminate the reaction. Enzymatic activity was quantified by measuring the absorbency at 405 nm. One unit of α-glucosidase is defined as the amount of enzyme liberating 1.0 μmol of *p*-nitrophenol per min under the assay conditions specified.

Results

Inhibitory effects of fractions of the root extract of *Paeonia lactiflora* on α-glucosidase from rat – The reported spectroscopic method was used to evaluate the various natural products for *in vitro* α-glucosidase inhibition. First, the effect of methanol extracts and four solvent fractions of the root extract of *Paeonia lactiflora* were tested on α-glucosidase from rat small intestinal mucosa. As shown in Fig. 2, the ethyl acetate fraction appeared to be most potent (≈ 93% inhibition) and then methanol extracts (≈ 36% inhibition) at the concentration of 100 μg/ml. The *n*-hexane and aqueous fractions did not affect on α-glucosidase from rat small intestinal mucosa.

Inhibitory effects of the fractions of the root of *Paeonia lactiflora* on α-glucosidase from yeast – In order to compare our results obtained by screening natural products for α-glucosidase inhibitory activity with those of α-glucosidase from bakers yeast, α-glucosidase from yeast was therefore used. As shown in Fig. 3, there are some differences in α-glucosidase activities of the fractions

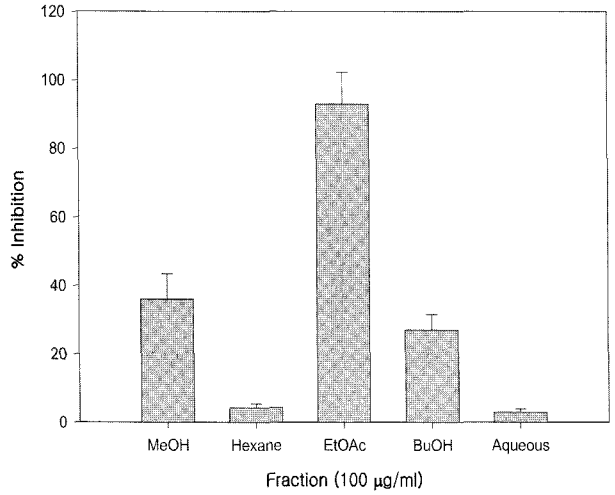


Fig. 2. Effects of fractions (100 μg/mL) from the root of *Paeonia lactiflora* on rat α-glucosidase. All values are expressed as mean ± SD of triplicate tests.

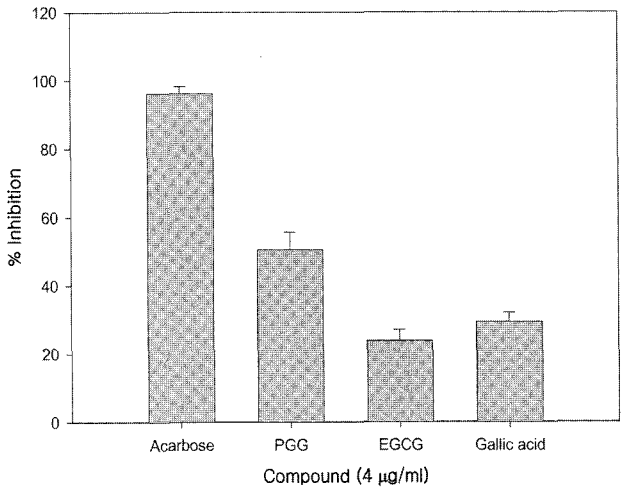


Fig. 4. Inhibitory effects of PGG, EGCG, and gallic acid on rat intestinal α-glucosidase. All values are expressed as mean ± SD of triplicate tests.

of the root extract of Paeonia lactiflora between yeast and rat origin. The ethyl acetate fraction appeared to be most potent (≈ 65% inhibition), and then butanol fraction (≈ 39% inhibition) at the concentration of 100 μg/ml. The hexane fraction also affected on yeast α-glucosidase activity (≈ 38% inhibition).

Isolation and purification of PGG – Since the ethyl acetate fraction of the methanol extract of the root of *Paeonia lactiflora* showed potent inhibitory activity, further fractionation of the fraction was carried out to isolate the active principles. Finally, we isolated and purified of PGG as a active principle by activity-guided fractionation.

Inhibitory effects of PGG and phenolic compounds on α-glucosidase from rat – To investigate the structure-activity relationships of gallate and gallic acid moieties for the inhibition of α-glucosidase activity, we also examined (−)-epigallocatechin gallate (EGCG), gallic acid, and gallacetophenone. As shown in Fig. 4, gallic acid, and EGCG also inhibited the α-glucosidase activity. At the concentration of 4 μg/ml, PGG inhibited by 50% ($IC_{50} \approx 4$ μg/ml), gallic acid by 29% ($IC_{50} \approx 8$ μg/ml), EGCG by 23% ($IC_{50} \approx 10$ μg/ml). At the same con centration, a synthetic inhibitor, acarbose, inhibited by 94% ($IC_{50} \approx 0.5$ μg/ml) of α-glucosidase activity.

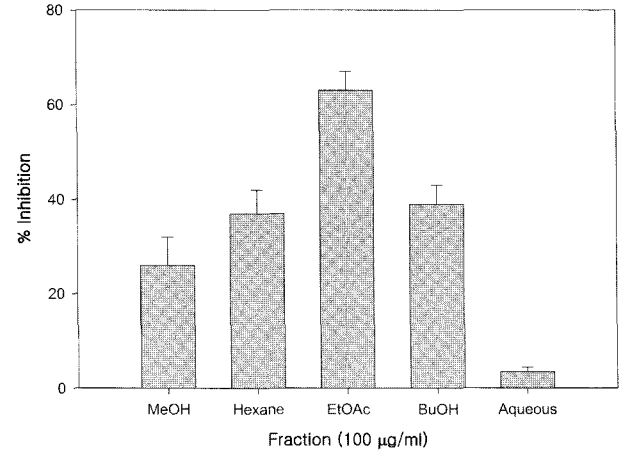


Fig. 3. Effect of fractions (100 μg/mL) from root of Paeonia lactiflora on α-glucosidase from yeast. All values are expressed as mean ± SD of triplicate tests.

Discussion

Intestinal α-glucosidases play a physiologically important role in the digestive process of dietary carbohydrates. 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. In order to evaluate the *in vitro* α-glucosidase inhibitory activity of a compound, α-glucosidase from the small intestinal mucosa should be used in the assay. However, most of in vitro studies on natural (Sawada *et al.*, 1993) or synthetic compounds (Horii *et al.*, 1986) have used α-glucosidase from bakers yeast. In our results also, there is an interesting difference in α-glucosidase activity between yeast and rat origin (Fig. 2 and 3). Voglibose and acarbose, which are known to be excellent *in vivo* α-glucosidase inhibitors (Bischoff, 1994; Odaka *et al.*, 1992), had 10^{-4} to 10^{-2} times lower or no ability to inhibit α-glucosidase from baker's yeast relative to that from small intestines (Oki *et al.*, 1999). A similar result was obtained for glucono-1,5-lactone that was

identified as a inhibitor against rabbit α-glucosidase by Cogoli and Semenza (1975), in which it inhibited only mammalian α-glucosidases. The poor inhibitory effect against baker's yeast α-glucosidase compared to mammalian α-glucosidases agreed with the results of Kameda *et al.* (1984), who had reported that aminocyclitol from Streptomyces hygroscopicus fermen-tation broth and its derivatives showed more potent inhibitory activity against pig α-glucosidase than against baker's yeast. These findings, together with the fact that the oral administration of acarbose in noninsulin-dependent diabetes subjects was allowed to moderate the postprandial blood glucose level (Toeller, 1994), suggested that the conventional α-glucosidase inhibitory study against baker's yeast may not give us any practical information concerning prevention of glucose absorption.

Since, the methanol extracts of the root of *Paeonia lactiflora* and the ethyl acetate fraction of the methanol extracts were effective on α-glucosidase activity, the effect of the methanol extracts seems to be ascribed to the effects of the ethyl acetate fraction. The ethyl acetate fraction of the root of *Paeonia lactiflora* also was determined *in vivo* inhibition effect on blood glucose elevation in mice. Therefore, these results suggest that the extract of *Paeonia lactiflora* can be used as a new nutraceutial for inhibition on postprandial hyperglycemia. In this study, molecules which block α-glucosidase were screened from natural products and PGG was isolated from the ethyl acetate fraction from the roots of *Paeonia lactiflora* as an active compound by bioassay-guided fractionation. Therefore, it was revealed that PGG also inhibits the α-glucosidase activity and is expected to improve postprandial hyperglycemia in diabetes

It is noteworthy that PGG, composed of glucose and five gallic acids, and gallic acid show more potent α-glucosidase inhibitory activity than EGCG.. These observations suggest that gallic acid moieties or hydroxyl groups may be essential for α-glucosidase inhibitory activity of gallotannins.

It is reported that green tea inhibited both yeast and rat α-glucosidases (Oki *et al.*, 1999). Matsumoto and Hara (1992) have demonstrated that the increase in blood glucose level after a meal was significantly suppressed by administering green tea extract (60 mg) to rat. EGCG is the most abundant components of green tea catechins.

In summary, the extract from the root of *Paeonia lactiflora* showed potent inhibitory activity on α-glucosidase. PGG was isolated and purified as a active principle by activity-guided fractionation. Although further studies are needed to elucidate the molecular mechanisms, these results suggest that PGG could be a candidate for developing an α-glucosidase inhibitory agent.

References

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

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

Cogoli, A. and Semenza, G., A probable oxocarbonium ion in the reaction mechanism of small intestinal sucrase and isomaltase. *J. Biol. Chem.* **250**, 7802-7809 (1975).

Dahlqvist, A., Assay of intestinal disaccharidases. *Enzymologica Biologica et Clinica.* **11**, 52-56 (1970).

Goto, H., Shimada, Y., Akechi, Y., Kohta, K., Hattori, M., and Terasawa, K., Endothelium-dependent vasodilator effect of extract prepared from the roots of Paeonia lactiflora on isolated rat aorta. *Planta Medica.* **62**, 436-439 (1996).

Horii, S., Fukas,e H., Matsuo, T., Kameda, Y., Asano, N., and Matsui, K., Synthesis and alpha-D-glucosidase inhibitory activity of N-substituted valiolamine derivatives as potential oral antidiabetic agents. *J. Med. Chem.* **29**, 38-46 (1986).

Kameda, Y., Asano, N., Yoshikawa, M., and Takeuchi, M., Valiolamine, a new α-glucosidase inhibiting aminocyclitol produced by Streptomyces hygroscopicus. *J. Antibiot.* **37**, 1301-1307 (1984).

Kwon, O. G., Kim, S. H., Chun, B. Y., Park, C K., and Son, K. H., Isolation of antimicrobial components from Moutan Cortex. *Kor. J. Pharmacogn.* **20**, 340-344 (1999).

Matsumoto, N. and Hara, Y., Prophylaxis of obesity and effect of suppressing digestion by tea catechins. *Jpn. Food Ind.* **34**, 26-31 (1992).

Murakami, N., Saka, M., Shimada, H., Matsuda, H., Yamahara, J., and Yoshikawa, M., New bioactive monoterpene glycosides from Paeoniae Radix. *Chem. Pharm. Bull.* **44**, 1279-1281 (1996).

Nishizawa, M., Yamagishi, T., Nonaka, G., Nishioka, I., Nagasawa, T., and Oura, H., Tannins and related compounds. XII. Isolation and characterization of galloylglucoses from Paeoniae Radix and their effects on urea-nitrogen concentration in rat serum. *Chem. Pharm. Bull.* **31**, 2593-2600 (1983).

Odaka, H., Miki, N., Ikeda, H., and Matsuo, T., Effect of a disaccharidase inhibitor, AO-128, on postprandial hyperglycemia in rats. *J. Jpn. Soc. Nutr. Food Sci.* **45**, 27-31 (1992).

Oki, T., Matsui, T., and Osajima, Y., Inhibitory effect of α-glucosidase inhibitors varies according to its origin. *J. Agric. Food Chem.* **47**, 550-553 (1999).

Sawada, Y., Tsuno, T., Ueki, T., Yamamoto, H., Fukagawa, Y., and Oki, T., Pradimicin Q, a new pradimicin aglycone, with alpha-glucosidase inhibitory activity. *J. Antibiot.* **46**, 507-10 (1993).

Tanaka, T., Kataoka, M., Tsuboi, N., and Kouno, I., New monoterpene glycoside esters and phenolic constituents of Paeoniae radix and increase of water solubility of proantho cyanidins in the presence of paeoniflorin. *Chem. Pharm.*

Bull. **48**, 201-207 (2000).

Toeller, M., R-Glucosidase Inhibitors in Diabetes: Efficacy in NIDDM Subjects. *Eur. J. Clin. Invest.* **24**, 31-35 (1994).

Wang, Y. and Ma, R., Effect of an extract of Paeonia lactiflora on the blood coagulative and fibrinolytic enzymes. *Zhong Xi Yi Jie He Za Zhi.* **10**, 101-102 (1990).

Wolffenbuttel, H.R. and Hoeften, W.V., Prevention of complications in non-insulin-dependent diabetes mellitus (NIDDM). *Drugs* **50**, 263-288 (1995).

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