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Antioxidant and antihypertensive activities of acteoside and its analogs

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ABSTRACT. Acteoside (Act), a phenylethanoid glycoside, is an active compound in several plants and traditional herbal medicines. Act along with its structural isomer, isoacteoside (Isoact), and an analog, 6-*O*-acetylacteoside (6-*O*-acetylact), were used in the study to investigate the antioxidant, anti-angiotensin-converting enzyme (ACE), and hemolysis inhibitory activities *in vitro* and antihypertensive activity against spontaneously hypertensive rats (SHR) *in vivo*. We showed that Act, Isoact, and 6-*O*-acetylact effectively scavenged 1,1-diphenyl-2-picryl-hydrazyl radicals (with IC_{50} at 11.4, 9.48, and 9.55 μM, respectively) and superoxide radicals (with IC_{50} at 66.0, 38.5, and 39.1 μM, respectively). As Isoact and 6-*O*-acetylact had similar radical-scavenging activities, only Act and Isoact were used for following studies. Both Act and Isoact inhibited xanthine oxidase activity with IC_{50} at 53.3 and 62.2 μM, respectively. Both Act and Isoact also significantly inhibited ACE activity and the hemolysis induced by 2,2'-azo-bis(2-amidinopropane)dihydrochloride, but the effects of Act were stronger than Isoact. We then orally administered a single dose of Act or Isoact (10 mg/Kg body weight) to SHR and measured the changes of systolic blood pressure (SBP) and diastolic blood pressure (DBP) over 24 h. Act, but not Isoact, showed antihypertensive activity in lowering SBP and DBP. The results suggest the potential usefulness of Act as a health food product for antioxidant protection and blood pressure regulation.

Keywords: Acteoside; Antihypertensive activity; Angiotensin converting enzyme (ACE); Antioxidant; Hemolysis.

INTRODUCTION

Acteoside (Act), a phenylethanoid glycoside containing caffeic acid, 3',4'-dihydroxyphenylethanol, glucose, and rhammose, was first isolated from flowers of *Syringa vulgaris* (Birkofer et al., 1968), and together with the structural isomer of isoacteoside (Isoact) and the derivative of 6-*O*-acetylacteoside (6-*O*-acetylact), has been found in many plants and herbal medicines, such as *Ligstrum purpurascens* (Wong et al., 2001), *Callicarpa dichotoma* (Koo et al., 2006; Lee et al., 2006), *Cistanche deserticola* and *Boschniakia rossica* (Wu et al., 2006), *Scrophularia ningpoenis* (Huang et al., 2008), *Rehmannia glutinosa* (Li et al., 2006). Act was reported to exhibit antimetastatic activity on B16 melanoma cells in C57BL/6 mice models (Ohno et al., 2002). Act, Isoact, and 6-*O*-acetylact were recently shown to inhibit IL-1β-activated expression of intercellular CAM-1 and vascular CAM-1 in human umbilical vein endothelial cells (Chen et al., 2009). Act also protects bovine pulmonary endothelial cells from hydroxyl radical-induced oxidative stress (Chiou et al., 2004) and inhibits nitric oxide and TNF-α production through blocking of AP-1 activation in lipopolysaccharide-stimulated macrophages (Rao et al., 2009). Act and Isoact exhibit neuroprotective activities *in vitro* (Koo et al., 2005). Koo et al. (2006) reported that Act and its aglycones effectively scavenge 1,1-diphenyl-2-picryl-hydrazyl (DPPH) and nitric oxide *in vitro*. However, few studies have compared side-by-side the *in vitro* antioxidant activities of Act and its structurally related compounds such as Isoact and 6-*O*-acetylact.

Several classes of pharmacological agents have been used in the treatment of hypertension (Mark and Davis, 2000). One class of anti-hypertensive drugs, known as angiotensin I converting enzyme (ACE) inhibitors (i.e. peptidase inhibitors), has a low incidence of adverse side-effects and are the preferred class of anti-hypertensive

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agents when treating patients with concurrent secondary diseases (Fotherby and Panayiotou, 1999). ACE (peptidyl-dipeptide hydrolyase EC 3.4.15.1) is a dipeptide-liberating Zn-containing exopeptidase, which removes a dipeptide from the C-terminus of angiotensin I to form angiotensin II, a very hypertensive compound. Several antioxidant peptides (reduced glutathione and carnosine-related peptides) exhibit ACE inhibitory activities (Hou et al., 2003). The first clinically available, orally active ACE inhibitor, captopril, was developed for hypertensive treatments (Ondetti et al., 1977; Borer, 2007). Act has been reported to exert nitric oxide-mediated relaxing effects on the endothelium-intact aortic rings of SD rats (Wong et al., 2001). Ahmad et al. (1995) have reported that Act induces a dose-dependent decrease in systolic blood pressure (SBP) and diastolic blood pressure (DBP) following its intravenous injection into normotensive anaesthetized Wistar rats. However, it is unclear whether orally administered Act and/or its related isomers are antihypertensive *in vivo*. In the present study, we investigated the *in vitro* antioxidant capacity and ACE inhibitory activities as well as the *in vivo* antihypertensive activity of Act, Isoact and/or 6-*O*-acetylact using the spontaneous hypertensive rats (SHRs). These studies are expected to provide useful data for the development of Act as a health food product.

MATERIALS AND METHODS

Materials

2,2'-azo-bis(2-amidinopropane)dihydrochloride (AAPH), ACE (I unit, rabbit lung), butylated hydroxytoluene (BHT), DPPH, N-(3-[2-furyl]acryloyl)-Phe-Gly-Gly (FAPGG), NADH, phenazine methosulfate (PMS), xanthine, and xanthine oxidase were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Captopril was purchased from Calbiochem Co. (CA, USA). Act, Isoact, and 6-*O*-acetylact (Figure 1) were purchased from Equl Corp. (purities > 98%, Shanghai, China). Other chemicals and reagents were from Sigma Chemical Co. (St. Louis, MO, USA).

Scavenging activity of Act, Isoact and 6-*O*-acetylact against DPPH radicals by spectrophotometry

A volume (0.3 mL) of Act, Isoact, and 6-*O*-acetylact (final concentrations were 1.56 to 50 μM), BHT and ascorbic acid (final concentrations were 2.4 to 60 μM) was added to 0.1 mL of 1 M Tris-HCl buffer (pH 7.9) and then mixed with 0.6 mL of 100 μM DPPH in methanol to a final concentration of 60 μM for 20 min under light protection at room temperature (Liu et al., 2004; Lin et al., 2008). The decrease of absorbance at 517 nm was measured and expressed as ΔA517 nm. Deionized water was used as a blank experiment. The scavenging activity of DPPH radical (%) was calculated with the equation: $(\Delta A517_{blank} - \Delta A517_{sample}) \div \Delta A517_{blank} \times 100$. The IC_{50} stands for the concentration of half-inhibition.

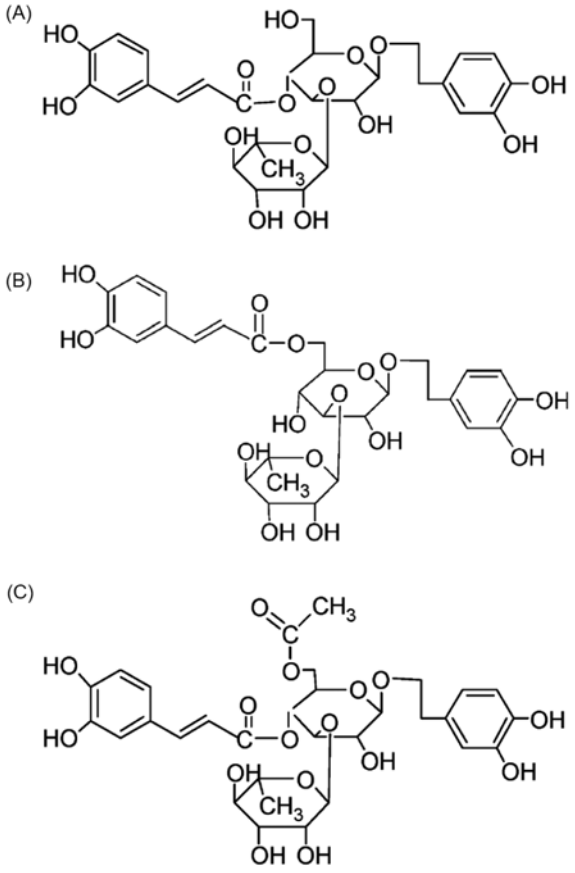

Figure 1. The structure of (A) acteoside, (B) isoacteoside, and (C) 6-*O*-acetylacteoside used in the experiments.

Inhibitory activity of Act and Isoact against xanthine oxidase

The xanthine oxidase activity was measured by determining uric acid formation at 295 nm using xanthine as substrate (Kalckar, 1947). The different amounts of Act and Isoact (the final concentrations were 25, 50, and 75 μM) were pre-mixed with 50 μL of 1 mU/mL xanthine oxidase at 4°C for 1 h, and then the 300 μL of 1 mM xanthine were added. The changes of absorbance at 295 nm were recorded over 2 min and expressed as ΔA295 nm/min. The xanthine oxidase inhibitory activity (%) was calculated as follows: $(\Delta A295\ nm/min_{blank} - \Delta A295\ nm/min_{sample}) \div \Delta A295\ nm/min_{blank} \times 100$. Deionized water was used instead of sample solution as a blank experiment. IC_{50} stands for the concentration of half-inhibition.

Scavenging activity of Act, Isoact, and 6-*O*-acetylact against superoxide radicals by spectrophotometry

The superoxide radical was generated by the PMS-NADH system (Liu et al., 2004; Lin et al., 2008). All 0.2 mL samples, containing different amounts of Act, Isoact, and 6-*O*-acetylact (the final concentration was 15.6, 31.3, 62.5, 125, and 250 μM), were added in sequence to 0.2 mL of

630 μM nitroblue tetrazolium, 0.2 ml of 33 μM PMS, and 0.2 ml of 156 μM NADH in 100 mM phosphate buffer (pH 7.4). Deionized water was used instead of sample solution as a blank experiment. Ascorbic acid (the final concentration was 6, 9, 12, and 24 μM) was used as a positive control. The changes of absorbance at 560 nm were recorded during 2 min and expressed as ΔA560 nm/min. The scavenging activity of superoxide radicals (%) was calculated as follows: (ΔA560 nm/min$_{blank}$ – ΔA560 nm/min$_{sample}$) ÷ ΔA560 nm/min$_{blank}$ × 100. IC_{50} stands for the concentration of half-inhibition.

Determination of ACE inhibitory activity of Act and Isoact by HPLC

Each 50 μL of Act (0.2, 0.4, 0.5, and 2.0 μmole) and Isoact (0.1, 0.2, 0.5, 1.0, and 2.0 μmole) were premixed with 15 μL of 1U/mL ACE for 5 min, and then 200 μL of 0.5 mM FAPGG were added and reacted at room temperature for 10 min (Anzenbacherova et al., 2001). The 800 μL methanol was added to stop the reaction. The blank experiment was FAPGG only. In the control experiment, ACE reacted with FAPGG under the same conditions. Chromatographic separation of FAPGG and FAP was carried out on the Hitachi (Japan) chromatographic system with a 10 μL-loop. The HPLC analysis was performed on a Biosil Aqu-ODS-W 5 μ column (Biotic Chemical Co., Ltd., Taiwan, 250 × 4.6 mm i.d.), particle size 5 μm. The reacted mixture was separated isocratically with a mobile phase consisting of 0.02 M nonylamine (adjusted to pH 2.4 with phosphoric acid) and acetonitrile in a ratio of 67.5:32.5 (V/V) (Anzenbacherova et al., 2001). The flow rate was 1 mL/min; the injection volume was 10 μL; the detector was set at 345 nm. The ACE inhibitions (%) of Act and Isoact were calculated as follows: [(Area of $FAPGG_{blank}$ – Area of $FAPGG_{control}$) – (Area of $FAPGG_{blank}$ – Area of $FAPGG_{sample}$) ÷ (Area of $FAPGG_{blank}$ – Area of $FAPGG_{control}$) × 100.

Inhibitory activity of Act and Isoact against AAPH-mediated hemolysis

The free-radical chain oxidation of rat red blood cells (RBC) through AAPH-mediated hemolysis (Miki et al., 1987). Rat blood was placed into heparinized tubes and centrifuged at 1000 ×*g* for 10 min. After being washed with 0.15 M NaCl thrice, the packed RBC was obtained by a centrifugation at 1000 ×*g* for 10 min. The different amounts of Act and Isoact (the final concentrations were 2, 5, and 10 μM) were mixed 25 μL of 20% RBC suspension (V/V, in 10 mM PBS) and 25 μL of 500 mM AAPH solution at 37°C for 0, 1, 1.5, 2, 2.5, 3, and 3.5 h with gentle shaking. Each mixture was centrifuged at 1000 ×*g* for 10 min, and the supernatant was measured at 536 nm. Deionized water was used instead of AAPH solution or sample solution, respectively, as a blank group or as a control group. The hemolysis inhibition (%) of Act and Isoact at 3 h or at 3.5 h was calculated as follows: (ΔA536 nm$_{control}$ – ΔA536 nm$_{sample}$) ÷ ΔA536 nm$_{control}$ × 100.

Antihypertensive effects of Act and Isoact on SHR

The effects of orally administered Act, Isoact, and captopril by feeding tube (2.0 × 80 mm) on the reduced SBP and the reduced DBP were determined according to the method of previous reports (Lin et al., 2006; Lin et al., 2008; Han et al., 2011). All animal experimental procedures followed published guidelines (National Science Council, 1994). The male SHRs (8 weeks of age, National Laboratory Animal Center, Taipei) were housed individually in steel cages kept at 24°C with a 12-h light-dark cycle and had free accessee to a standard mouse/rat chow (Prolab® RMH2500, 5P14 Diet, PMI Nutrition International, Brentwood, MO) and water. SHRs were randomly divided into control and sample treatments for SBP and DBP determinations (six rats per group). For a short-term antihypertensive experiment, 0.5 mL of 10 mg Act or Isoact/Kg of SHR or 5 mg captopril/Kg of SHR were orally administered once, and tail blood pressure was measured at 2, 4, 6 and 24 h after a single oral administration. The 0.5 mL distilled water was used for a blank experiment. An indirect blood pressure meter (BP-98A, Softron Co. Ltd. Tokyo, Japan) was used to measure SBP and DBP four times at each determination for each treatment.

Data analysis

Values are presented as means ± SD and analyzed using one-way ANOVA, followed by the post hoc Tukey's test for multiple mean comparisons. Student's *t*-test was performed, when only two groups of data were compared (such as between Act and Isoact at the same concentration). A p value < 0.05 is considered statistically significant. The statistical analysis was performed using SPSS for Windows, version 10 (SPSS, Inc., Chicago, IL, USA).

RESULTS

Scavenging activity of DPPH and superoxide radicals

Act, Isoact, and 6-*O*-acetylact exhibited dose-dependent DPPH scavenging activities at pH 7.9 (Figure 2). The IC_{50} values were 11.4, 9.48, and 9.55 μM, respectively, for Act, Isoact, and 6-*O*-acetylact. The IC_{50} values of positive controls of ascorbic acid and BHT were 13.1 and 18.5 μM, respectively.

Inhibition of xanthine oxidase activity

Act and Isoact were showed to exhibit dose-dependent inhibition of xanthine oxidase (Figure 3). The IC_{50} was calculated to be 53.3 and 62.2 μM, respectively, for Act and Isoact.

Inhibition of superoxide dismutase activity

Because both Act and Isoact inhibited xanthine oxidase activity, we used the PMS-NADH system to generate superoxide radicals (Liu et al., 2004; Lin et al., 2008). Act,

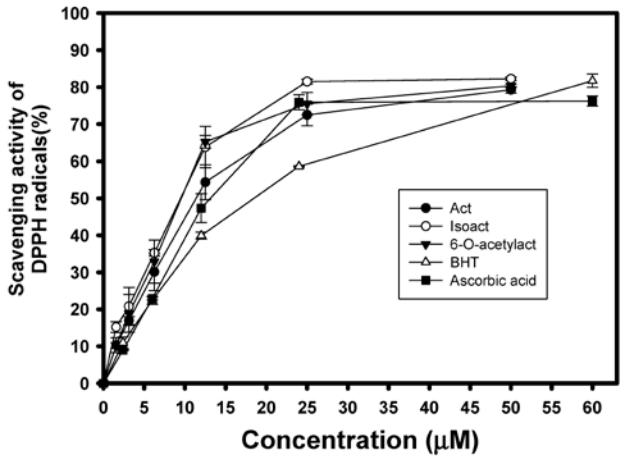

Figure 2. Scavenging activities of different concentrations (1.56~50 μM) of acteoside (Act), isoacteoside (Isoact) and 6-*O*-acetylacteoside (6-*O*-acetylact) on 1,1-diphenyl-2-picryl-hydrazyl (DPPH) radical. The scavenging activity of DPPH radical (%) was calculated with the equation: $(\Delta A517_{blank} - \Delta A517_{sample}) \div \Delta A517_{blank} \times 100$.

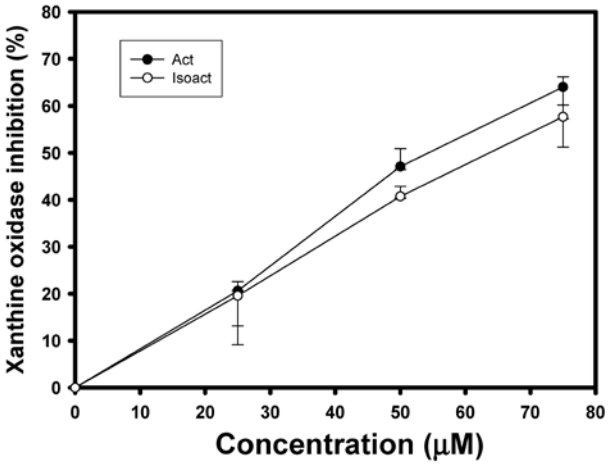

Figure 3. Inhibitory effects of different concentrations (25, 50, and 75 μM) of acteoside (Act) and isoacteoside (Isoact) on xanthine oxidase activities. The inhibition of xanthine oxidase (%) was calculated as following: $(\Delta A295\ nm/min_{blank} - \Delta A295\ nm/min_{sample}) \div \Delta A295\ nm/min_{blank} \times 100$.

Isoact, and 6-*O*-acetylact exhibited dose-dependent superoxide radical scavenging activities (Figure 4). The IC_{50} values were 66.0, 38.5, and 39.1 μM, respectively, for Act, Isoact, and 6-*O*-acetylact. The IC_{50} values of the positive control of ascorbic acid were 9.0 μM. From the results of Figures 2 and 4, it were clear that Isoact and 6-*O*-acetylact had similar radical-scavenging activities against DPPH and superoxide radicals, and thus only Act and Isoact were selected for further biological activity screenings.

ACE inhibitory activities of Act and Isoact

In the preliminary test, we found that Act and Isoact interfered with the absorbance of the ACE substrate FAPGG

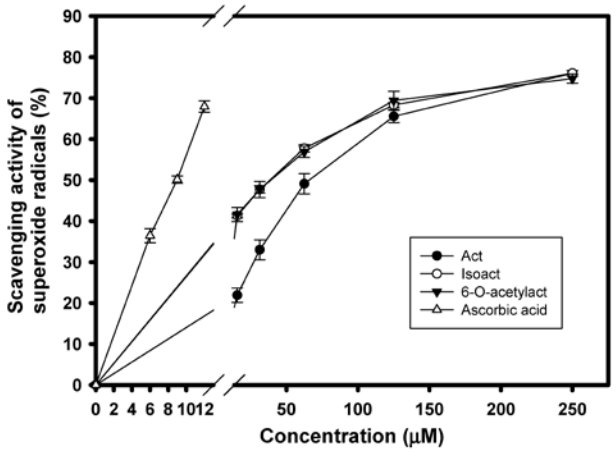

Figure 4. Scavenging effects of different concentrations (15.6~250 μM) of acteoside (Act), isoacteoside (Isoact) and 6-*O*-acetylacteoside (6-*O*-acetylact) on superoxide radical. The changes of absorbance at 560 nm were recorded during 2 min and expressed as ΔA560 nm/min. The scavenging activity of superoxide radicals (%) was calculated as follows: $(\Delta A560\ nm/min_{blank} - \Delta A560\ nm/min_{sample}) \div \Delta A560\ nm/min_{blank} \times 100$.

and its hydrolyzed product FAP at 345 nm as used for continuous spectrophotometric assay (Holmquist et al., 1979). Therefore, the separation coupled with detection of Act or Isoact and FAPGG by HPLC was used to monitor ACE inhibitory activities. Figure 5A to Figure 5D are typical HPLC chromatograms from 10 μl reaction mixtures of a blank test (FAPGG only, Figure 5A); control test (ACE reacted with FAPGG to produce FAP, Figure 5B); 0.5 μmole of Act (312.5 μg), ACE and FAPGG (Figure 5C); and 0.5 μmole of Isoact (312.5 μg), ACE and FAPGG (Figure 5D). The area of FAPGG in Figure 5B was the lowest and that in Figure 5A the highest of four typical HPLC chromatograms. The areas of FAPGG in Figures 5C and 5D are dependent on the ACE inhibitory activities of different concentrations of Act and Isoact. The calculated ACE inhibitions of Act and Isoact are shown in Figure 5E. Act showed higher ACE inhibitory activities than did Isoact, and the IC_{50} of the former was calculated to be 472 μM by area in the HPLC chromatograms.

Inhibitory activity of Act and Isoact against AAPH-mediated hemolysis

The inhibitory activities of Act and Isoact against AAPH-mediated hemolysis at concentrations of 2, 5 and 10 μM were evaluated over 3.5 h (Miki et al., 1987). The results of Figure 6A demonstrate that the hemolysis in rat RBC dramatically increased (expresses as ΔA536 nm) after 3-h or 3.5-h reactions in the presence of AAPH radicals (as control groups, white cycle symbol). Little or no hemolysis was observed in the absence of AAPH radicals during the 3.5-h reaction (as blank groups, black cycle symbol). Therefore, the hemolysis inhibition of Act and Isoact at 3 h or at 3.5 h was calculated as follows: $(\Delta A536\ nm_{control} - \Delta A536\ nm_{sample}) \div \Delta A536\ nm_{control} \times 100$. Figure 6B

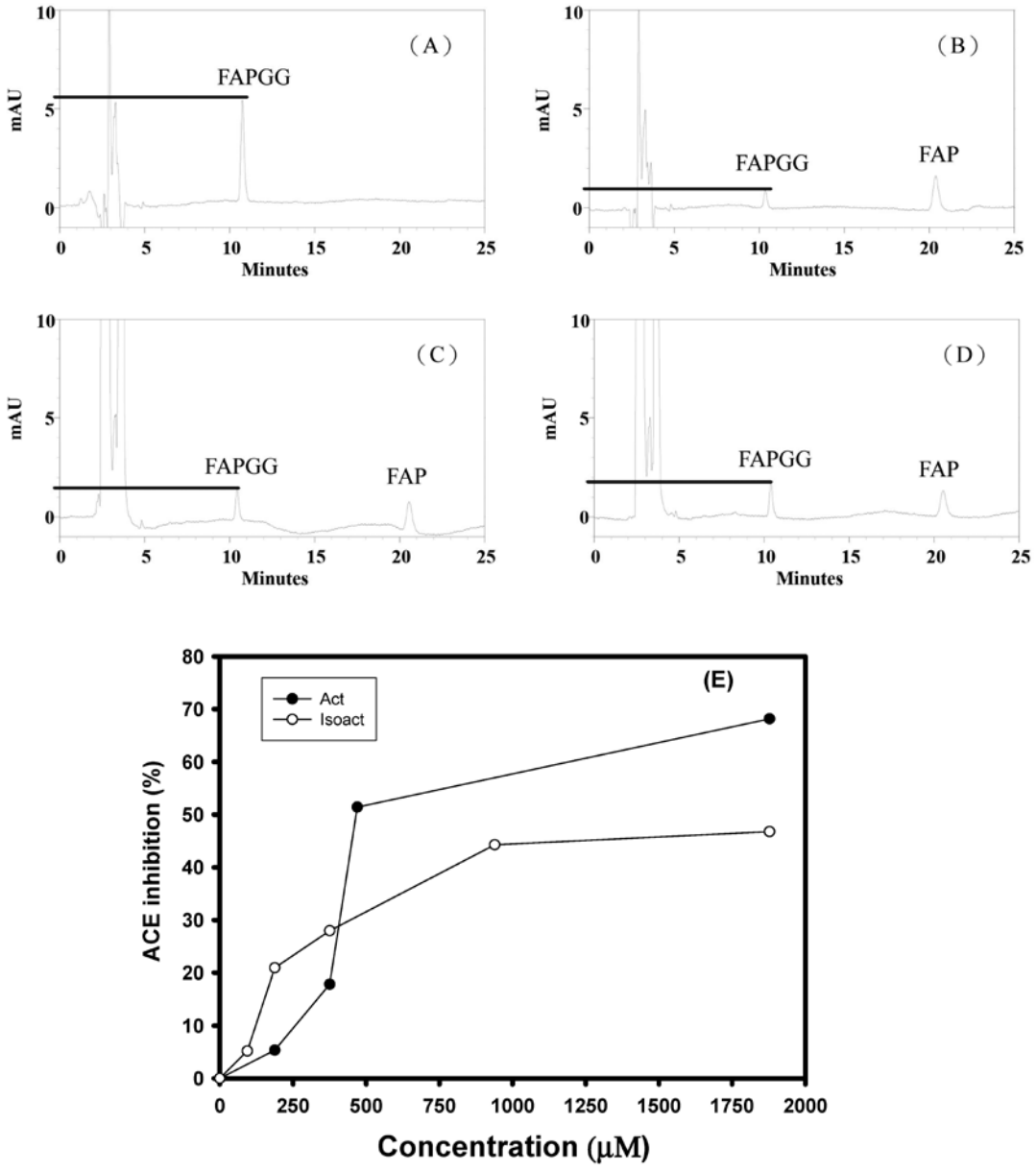

Figure 5. HPLC chromatograms of Lichrospher 100 RP-18 endcapped column for the effects of acteoside (Act) and isoacteoside (Isoact) on ACE activity. (A) blank test (FAPGG only); (B) control test (15 μU ACE reacted with FAPGG to produce FAP); (C) 0.5 μmole of Act (312.5 μg), 15 μU ACE and FAPGG; (D) 0.5 μmole of Isoact (312.5 μg), 15 μU ACE and FAPGG. The mobile phase consisting of a mixture of 0.02 M nonylamine (adjusted to pH 2.4 with phosphoric acid): acetonitrile, 67.5:32.5 (V/V). The flow rate was 1 mL/min; the injection volume was 10 μL; the eluted analytes were detected at 345nm. (E) The ACE inhibitions (%) of different concentrations of Act (188, 376, 470 and 1878 μM) and Isoact (93.9, 188, 376, 939, and 1878 μM) were calculated as follows: [(Area of FAPGG$_{blank}$ – Area of FAPGG$_{control}$) – (Area of FAPGG$_{blank}$ – Area of FAPGG$_{sample}$)÷ (Area of FAPGG$_{blank}$ – Area of FAPGG$_{control}$) × 100.

shows that both Act and Isoact at 2, 5 and 10 μM exhibited concentration-dependent inhibition on AAPH-induced hemolysis, with the inhibitory effects of Act significantly stronger than those of Isoact at each concentration used.

Antihypertensive effects of Act and Isoact on SHR

SHRs received a single oral administration of Act and Isoact (10 mg/Kg SHR), and changes in SBP and DBP were recorded over 24 h. We previously reported that the blood pressure (SBP and DBP) of SHR was changeable during 24-h (Lin et al., 2006; Han et al., 2011). Therefore, a comparison at a fixed time (such as 2, 4, 6, 8, and 24 h) between blank and sample instead of before and after oral administration itself was used. It was found that Act, but not Isoact, effectively reduced SBP and DBP of SHR compared to the blank (distilled water) group. SBP was significantly reduced in the Act group at 2, 4 and 6 h by 18.8, 16.5 and 14.9 mmHg, respectively, but the reduction of SBP (3.3 mmHg) at 24 h after Act treatment was not sta-

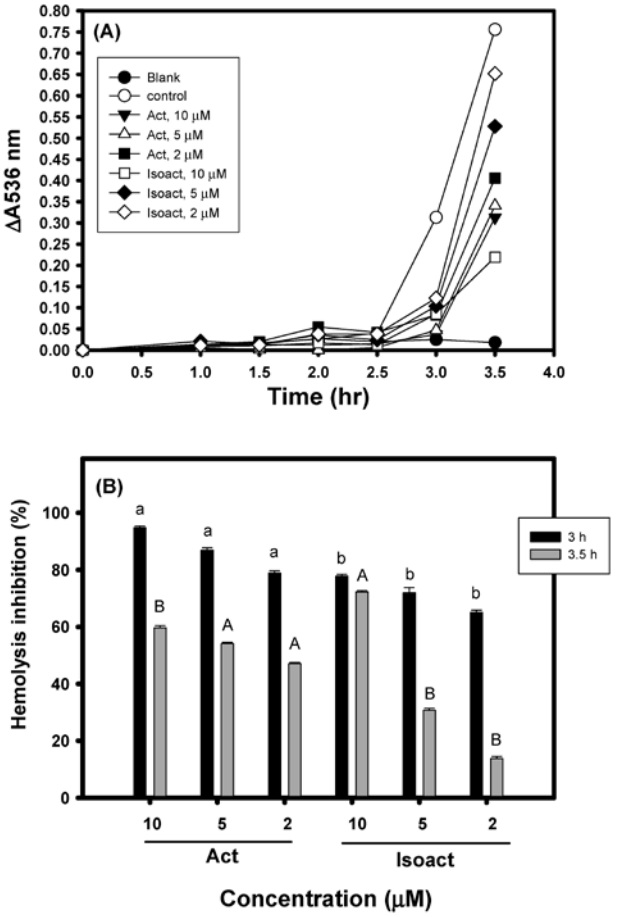

Figure 6. (A) Inhibition of different concentrations (2, 5, and 10 μM) of acteoside (Act) and isoacteoside (Isoact) on hemolysis of rat red blood cells induced by 2,2'-azo-bis(2-amidinopropane)dihydrochloride (AAPH). Each mixture was centrifuged at 1000×*g* for 10 min at fixed time (at 0, 1, 1.5, 2.0, 2.5, 3.0, and 3.5-h), and the supernatant was measured at 536 nm. Deionized water was used instead of AAPH solution or sample solution, respectively, as a blank group or as a control group. (B) The hemolytic inhibition (%) of Act and Isoact at 3 h or at 3.5 h was calculated as follows: (ΔA536 $nm_{control}$ – ΔA536 nm_{sample}) ÷ ΔA536 $nm_{control}$ × 100. Values at each time point not sharing an alphabetic letter are significantly different ($P < 0.05$).

tistically significant (Figure 7A). As shown in Figure 7B, DBP was decreased in the Act group at 2, 4 and 6 h (by 8.5, 7.6 and 12.0 mmHg, respectively), although only the result obtained at 6 h was statistically significant ($P < 0.05$). It was noted that Act and captopril (as positive control) had similar effects on SBP at early stages (2, 4 and 6 h) after oral administration.

DISCUSSION

Active oxygen species and free radical-mediated reactions are involved in degenerative or pathological processes such as aging, cancer, coronary heart disease and Alzheimer's disease (Ames, 1983; Gey, 1990; Smith et al., 1996). Several reports have focused on screening the antioxidant activities from natural resources. In the present study, we first compared the antioxidant activities of Act, Isoact and 6-*O*-acetylact. Act and Isoact are structural isomers in which the constituent of caffeic acid moiety is bonded to the C-4 hydroxyl group of glucose in the former and to the C-6 hydroxyl group of glucose in the latter. While the constituents of rhammose and 3',4'-dihydroxyphenylethanol, respectively, are bonded to the C-3 and C-1 hydroxyl groups of the glucose moiety in the same Act and Isoact molecule, 6-*O*-acetylact is an Act derivative in which acetic acid is bonded to the C-6 hydroxyl group in the glucose moiety (Figure 1). Koo et al. (2006) reported that Act and its aglycones exhibited DPPH-scavenging activities, and the order of these activities (expressed as IC_{50}) were Act (1.28 μM) > caffeic acid (2.22 μM) > 3',4'-dihydroxyphenylethanol (7.72 μM) > α-tocopherol (15.1 μM). In the present report (Figure 2), the order of DPPH scavenging activities (expressed as IC_{50}) is 6-*O*-acetylact (9.55 μM) ≈ Isoact (9.48 μM) > Act (11.4 μM). These values were comparable to or bet-

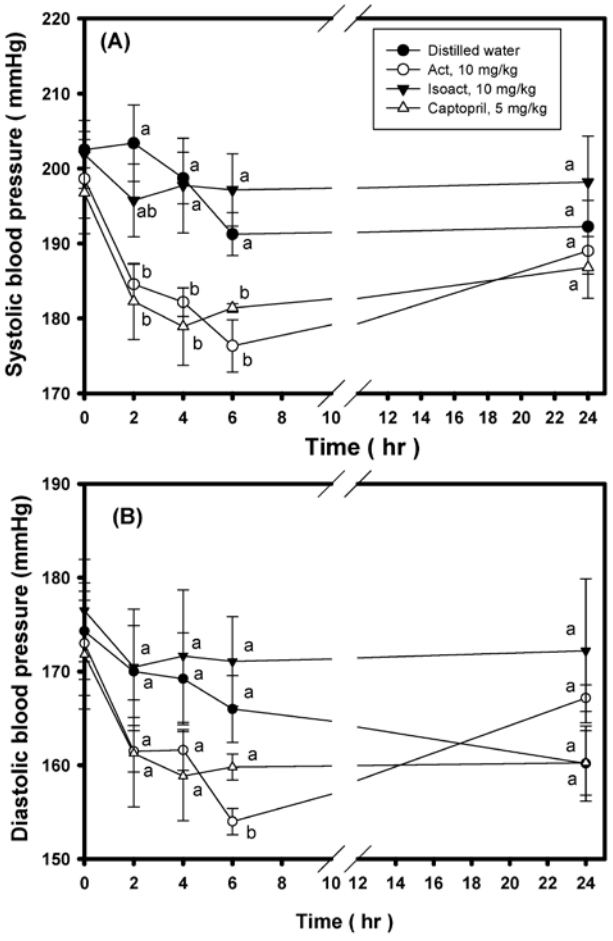

Figure 7. Effects of acteoside (Act) and isoacteoside (Isoact) (10 mg/Kg body weight) or captopril (5 mg/kg body weight) on systolic blood pressure (A) and diastolic blood pressure (B) of spontaneous hypertensive rats (SHR) after a single oral administration for 24 h. Distilled water (0.5 ml) was used as a blank. Values at each time point not sharing an alphabetic letter are significantly different ($P < 0.05$).

ter than those of ascorbic acid (IC_{50} of 13.1 μM) and BHT (IC_{50} of 18.5 μM) for scavenging DPPH radicals. The higher IC_{50} of Act in the present report might be from the final concentration of 60 μM DPPH in stead of 30 μM used of Koo et al. reported (2006). As DPPH radical assay belongs to the electron-transfer reaction (Huang et al., 2005), it is speculated that the derivative in C-6 hydroxyl group of the glucose moiety, such as acetyl group in 6-*O*-acetylact and caffeic acid moiety in Isoact, may more easily provide an electron-transfer reaction than those of the C-6 free hydroxyl group in the glucose moiety of Act for the DPPH scavenging assay.

Act, 6-*O*-acetylact and Isoact have been reported to possess superoxide-scavenging activity in vitro using the xanthine/xanthine oxidase system to generate superoxide radicals (Wang et al., 1996; Gao et al., 1999). However, our present study indicated that Act exhibited xanthine oxidase inhibitory activities (Figure 3). Therefore, the superoxide radical was generated by using PMS-NADH system in the present report (Liu et al., 2004; Lin et al., 2008) instead of xanthine/xanthine oxidase system. Indeed, using the xanthine/xanthine oxidase system to generate superoxide radical, Wang et al. (1996) obtained an IC_{50} of 63 μM Act against superoxide radicals, and this value is close to that for inhibition of xanthine oxidase activity by Act, as reported in the present study (53.3 μM). It was reported that caffeic acid exhibited xanthine oxidase inhibitory activities (Chiang et al., 1994). It may be speculated that the superoxide-scavenging activities using xanthine/xanthine oxidase generating system of some phytochemicals with structures associated with caffeic acid, such as Act or Isoact (Wang et al., 1996; Gao et al., 1999), may have been contributed, at least in part, on xanthine oxidase inhibitions. Interestingly, Wang et al. (1996) found that Act (0.5 and 2.5 mM) did not inhibit xanthine oxidase activity which as determined by an oxygen electrode for oxygen consumptions during xanthine oxidation. Thus, it is important to bear in mind that different methods for assaying superoxide-scavenging activity may produce contradictory data.

Li et al. (1993) have reported a similar extent of inhibition of Act and Isoact against AAPH-mediated hemolysis in the RBC of mice. However, we showed here that Act exhibited much higher inhibitory activity against AAPH-induced hemolysis in RBC of rats than did Isoact (Figure 6). It is unclear whether such an inconsistency may have been due to the difference in rodent species.

Kang et al. (2003) reported that Act exhibited ACE inhibitory activities, and the IC_{50} was 373.3 μg/mL which was calculated to be 598 μM using Hip-His-Leu as substrates. In the present report, Act showed higher ACE inhibitory activities than did Isoact, and the IC_{50} of the former was calculated to be 472 μM by area in the HPLC chromatograms (Figure 5). Act has been reported to exert nitric oxide-mediated relaxing effects on endothelium-intact aortic rings of SD rats (Wong et al., 2001). Although Act has been shown to be antihypertensive in Wistar rats, the study employed intravenous injection of Act into anaesthetized rat in order to rule out the adsorption factor (Ahmad et al., 1995). Herein, we administrated Act orally to SHRs and determined changes in blood pressure. We found that the oral administration of Act, but not of Isoact, exhibited antihypertensive effects by lowering SBP and DBP over 24 h after a single administration (Figure 7). Act at a dose of 10 mg/kg body weight had an effect close to that of captopril in the dose of 5 mg/kg SHR in lowering SBP and was better than captopril at lowering DBP.

In conclusion, Act exhibited antioxidant activities, ACE inhibitory activities, and antihypertensive effects on SHRs. The results presented here will benefit the effort to develop herbal medicines or related products using Act, which has been found in many plants and herbal medicines, for antioxidant protection and therapeutic effects in the future.

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洋丁香苷及其結構類似物之抗氧化與抗高血壓活性評估

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洋丁香苷 (Acteoside, Act)，是許多植物或中草藥中的活性成分。本試驗利用洋丁香苷及兩種結構類似物，異洋丁香苷 (isoacteoside, Isoact) 與 6- 乙酸基洋丁香苷 (6-*O*-acetylacteoside, 6-*O*-acetylact)，進行抗氧化、抗血管收縮素轉化酶、抗溶血體外試驗及以自發性高血壓鼠進行抗高血壓之動物試驗。結果顯示，三種試驗樣品皆能有效清除 DPPH 自由基與超氧自由基。因為異洋丁香苷與 6- 乙醯洋丁香苷有類似清除自由基活性，因此後面試驗只以洋丁香苷與異洋丁香苷進行比較。兩者皆具有抑制黃嘌呤氧化酶與血管收縮素轉化酶活性；在抑制 2,2'-azo-bis(2-amidinopropane)dihydrochloride 誘發溶血試驗上，洋丁香苷效果優於異洋丁香苷。以自發性高血壓鼠進行抗高血壓之動物試驗，洋丁香苷與異洋丁香苷分別以 10 mg/Kg 劑量單一餵食一次觀察 24 小時血壓變化。結果顯示，在此劑量上只有洋丁香苷具有明顯調降高血壓鼠收縮壓與舒張壓活性。這些結果顯示，含有洋丁香苷的中草藥可能可以做為抗氧化與血壓調節之保健食品。

關鍵詞：洋丁香苷；抗高血壓活性；血管收縮素轉化酶 (ACE)；抗氧化；溶血。