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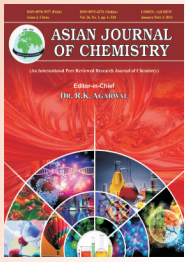

Studies on Characterization of Active Constituents in *Prunella vulgaris* L. and Mechanism of their Antihypertensive Effect

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Prunella vulgaris L. is widely distributed around China, which is used for facial paralysis, muscle and bone pain, soothing liver qi and dispersing liver stagnation. Scholars have done extensive researches on the chemical composition, pharmacological efficacy and quality control of *Prunella vulgaris* L. and have isolated and purified many compounds from it, which can be divided into triterpenoids and their saponins, steroids, flavonoids, coumarins, *etc*. The object of the study is to isolate, purify and characterize the active constituents in *Prunella vulgaris* L. and to study their antihypertensive effect. Reflux extraction was used to extract active constituents, column chromatography and preparative TLC were used to purify compounds and spectroscopy was used to analyze the structures of the compounds. The antihypertensive effect of *Prunella vulgaris* L. in SHR rats was observed by intragastric administration. As results five compounds were isolated, namely 2α,3α-24-trihydmxyursa-12-en-28-oic acid-28-O-β-D- glucopyranosly ester, chrysophanol, rotundic acid 28-O-α-D-glueopyranosyl(1 → 6)-β-D-g1ueopyranoside, A-spinasterol and β-sitosterol. Three weeks after administration at the test dose, compared with the blank control group, each *Prunella vulgaris* L. ethanol extract group could significantly reduce the systolic and diastolic blood pressures in SHR rats, the differences were statistically significant and showed certain dose-effect relationship. In conclusion *Prunella vulgaris* L. has certain antihypertensive effect.

Keywords: *Prunella vulgaris* L., rotundic acid 28-O-α-D-glueopyranosyl(1 → 6)-β-D- g1ueopyranoside, Antihypertension.

INTRODUCTION

Spica Prunellae is the dried ear of Labiatae perennial herb *Prunella vulgaris* L., which is widely distributed around China, the plant has relatively low requirements on living environment and can grow in slopes, meadows, stream sides, roadsides and other moist grounds. *Prunella vulgaris* L. had received much attention of the ancient physicians, as early as in the "Southern Yunnan Materia Medica"[1], *Prunella vulgaris* L. was recorded as "bitter and slightly pungent in taste, slightly warm in nature, enters the liver meridian, removes liver wind and promotes meridian circulation. Used for facial paralysis, muscle and bone pain, soothing liver qi and dispersing liver stagnation. Scholars at home and abroad have done extensive researches on the chemical composition, pharmacological efficacy and quality control of *Prunella vulgaris* L. and have isolated and purified many compounds from it, which can be divided into triterpenoids and their saponins, steroids, flavonoids, coumarins, *etc.*[2-6]. Modern clinical studies have shown that *Prunella vulgaris* L. has antihypertensive, hypoglycemic, antibacterial, antiinflammatory, antiallergic and antiviral effects[7-10].

In this experiment, the active constituents in *Prunella vulgaris* L. were extracted, purified and isolated and their structures were characterized, meanwhile, their antihypertensive effects and mechanisms were explored, laying the foundation for further study of compounded formulation and pharmacological effects of *Prunella vulgaris* L.

EXPERIMENTAL

Bruker AV 400 MHz superconducting NMR spectrometer; 200-300 mesh column chromatography silica gel (Qingdao Haiyang Chemical Factory); Sephadex LH-20 column chromatography gel (Pharmacia Corporation); RP-18 F254 TLC plates; Buchi Rotavapor R. 200 rotary evaporator, Panlab noninvasive blood pressure measurement system (RWD Life Science Co., Ltd., Shenzhen), all of the reagents were analytically pure or chemically pure.

Drugs and animals: *Prunella vulgaris* L. was purchased from the medicine company, which was identified as *Prunella vulgaris* L. in the genus Prunella of the family Labiatae; Captopril Tablets (Sino-American Shanghai Squibb Pharmaceuticals Ltd.) SHR rats, male, weighing 200-250 g, aged 10 weeks, were purchased from Shanghai Laboratory Animal Center (SLAC). Rats were kept in separate cages and fed and watered *ad libitum*.

Extraction and purification of active constituents in *Prunella vulgaris* L.: 5 kg of *Prunella vulgaris* L. was taken and extracted by reflux extraction with 95 % ethanol three times, each time lasted 2 h, the filtrates were then combined, ethanol was evaporated and the remaining was concentrated to an appropriate volume. The *Prunella vulgaris* L. extract was taken and extracted with petroleum ether, ethyl acetate and *n*-butanol, respectively, then the solvents were evaporated under reduced pressure.

The ethyl acetate extract was taken, loaded on column chromatography and gradient-eluted with petroleum ether-ethyl acetate solvent, the same fractions were combined, then three compounds (compound **3**, **4** and **5**) were isolated by means of preparative TLC and preparative HPLC. The *n*-butanol extract was taken, loaded on column chromatography and gradient-eluted with chloroform-methanol solvent, after combining the same fractions, the eluent was secondary-purified using Sephadex LH-20 column chromatography gel and preparative HPLC to isolate two compounds (compound **1** and **2**).

Experiment on antihypertensive effect of *Prunella vulgaris* L.

Preparation of test solution: The ethanolic extract of *Prunella vulgaris* L. was taken, prepared into the test solution with a concentration of 1 g crude drug per 1 mL aqueous extract solution and stored in the refrigerator, which was prepared into the required concentrations during the experiment.

Animal grouping and administration

The SHR rats were randomly divided into five groups, namely the blank control group, captopril control group and *Prunella vulgaris* L. high-, medium- and low-dose groups, each group contained 10 rats. The rats were intragastrically administered distilled water, captopril (10 mg/kg), *Prunella vulgaris* L. ethanol extracts (5, 10 and 15 mg/g) once per day for 3 consecutive weeks, respectively.

Index detection: Every week, tail artery blood pressure was measured in awake rats using Panlab noninvasive blood pressure measurement system.

Statistical processing: Experimental data were processed using SPSS 13.0 software, all data were expressed as -x ± s. Indices among groups were compared using one-way ANOVA and pairwise comparison was performed using t test, difference was considered statistically significant when $p < 0.05$.

RESULTS AND DISCUSSION

Compound 1: White powder (methanol), positive Liebermann-Burchard reaction. Blue in 5 % sulfuric acid-ethanol system. ^{1}H NMR (pyridine-d_5, 500 MHz) δ: 0.88, 0.96, 0.97, 0.99, 1.14, 1.25 (each 3H, s, CH_3), 3.83 (1H, d, J = 11.5 Hz, 24-Ha), 4.07 (1H, d, J = 11.5 Hz, 24-Hb), 4.49 (1H, m, 2-H), 4.54 (1H, br.s, 3-H), 5.43 (1H, d, J = 8.2 Hz, H_{glu}-1); ^{13}C NMR (pyridine-d_5, 500 MHz) δ: 42.2 (C-1), 66.3 (C-2), 73.5 (C-3), 45.4 (C-4), 49.7 (C-5), 18.5 (C-6), 32.3 (C-7), 39.1 (C-8), 47.2 (C-9), 38.4 (C-10), 23.7 (C.1I), 122.5 (C-12), 135.6 (C-13), 42.3 (C-14), 27.4 (C-15), 28.6 (C-16), 47.2 (C-17), 41.5 (C-18), 46.4 (C-19), 30.7 (C-20), 34.3 (C-21), 32.3 (C-22), 23.5 (C-23), 65.3 (C-24), 16.5 (C-25), 16.7 (C-26), 26.4 (C-27), 176.4 (C-28), 33.5 (C-29), 23.8 (C-30), 95.5 (C-l'), 74.4 (C-2'), 78.7 (C-3'), 71.5 (C-4'), 79.6 (C-5'), 62.8 (C-6'). The above data were basically consistent with the reported literature[11], so the compound was identified as 2α, 3α-24-trihydmxyursa-12-en-28-oic acid-28-O-β-D- glucopyranosly ester.

Compound 2: Red powder, turned red in sodium hydroxide solution. ^{1}H NMR ($CDCl_3$, 500 MHz) δ: 12.19 (1H, Ar-OH), 12.11 (1H, Ar-OH), 7.87 (1H, dd, J = 8.5, 1.4 Hz, H-5), 7.72 (1H, t, J = 13.5 Hz, 8.2 Hz, H-6), 7.62 (1H, d, J = 1.5 Hz, H-4), 7.32 (1H, dd, H-7), 7.15 (1H, d, J=1.5Hz, H-2), 2.44 (3H, s, Ar-CH_3). The above data were consistent with the reported literature[12], so the compound was identified as chrysophanol.

Compound 3: White powder, blue in 5 % sulfuric acid ethanol system. ^{1}H NMR (pyridine-d_5, 500 MHz) δ: 2.75 (1H, s, H-18), 0.85, 0.94, 1.12, 1.14, 1.32, 1.62 (each 3H, s, H-29, 24, 27, 26, 25, 23), 1.05 (3H, d, J = 7 Hz, H-30), 5.64 (1H, brs, H-12), 6.15 (1H, d, J = 5.5 Hz, H_{glu}-1'), 5.64 (1H, d, J = 8 Hz, H_{glu}-1"); ^{13}C NMR (pyridine-d_5, 500 MHz) δ: 38.5 (C-1), 29.4 (C-2), 73.4 (C-3), 42.7 (C4), 48.5 (C-5), 18.8 (C-6), 33.2 (C-7), 40.5 (C-8), 47.1 (C-9), 38.2 (C-10), 24.2 (C-11), 123.3 (C-12), 135.2 (C-13), 42.5 (C-14), 29.2 (C-15), 26.4 (C-16), 48.5 (C-17), 54.3 (C-18), 72.5 (C-19), 42.5 (C-20), 26.5 (C-21), 37.7 (C-22), 68.1 (C-23), 22.5 (C-24), 16.5 (C-25), 17.6 (C-26), 24.3 (C-27), 177.2 (C-28), 26.4 (C-29), 16.4 (C-30), 93.5 (C-1'), 73.2 (C-2'), 78.5 (C-3'), 70.5 (C-4'), 79.5 (C-5'), 68.3 (C-6'), 100.3 (C-1"), 78.7 (C-2"), 75.4 (C-3"), 72.2 (C-4"), 71.9 (C-5"), 63.5 (C-6"). The above data were consistent with the reported literature[13], so the structural characterization of compound 3 was rotundic acid 28-O-α-D-glueopyranosyl (1 → 6)- β-D-g1ueopyranoside.

Compound 4: White crystals. ^{1}H NMR (pyridine-d_5, 500 MHz) δ: 5.12, 5.11 (dd, J = 10.5, 17.2 Hz, H-22, 23), 5.14 (1H, d, J = 5.0 Hz, H-7), 3.04 (1H, m, H-3), 1.05 (3H, d, J = 8.5 Hz, H-21), 0.91 (3H, d, J = 10.5 Hz, H-26), 0.85 (3H, t, J = 12 Hz, H-29), 0.85 (3H, d, J = 11.5 Hz, H-27), 0.84 (3H, s, H-19), 0.50 (3H, s, H-18). After comparing with the literature[14], the spectral data for compound **4** were basically consistent with A-spinasterol. In addition, TLC R_f value was the same with the reference substance and the mixed melting point of the two did not drop, so the compound was identified as A-spinasterol.

Compound 5: Acicular crystals (petroleum ether), colorless, m.p. 192-193 °C. Liebermann-Burchard reaction was positive, suggesting that the compound should be steroidal compounds. TLC identification was performed on compound **5** and β-sitosterol reference, the results showed that the R_f values in three development systems were completely identical and the mixed melting point with reference substance did not drop, so the compound was identified as β-sitosterol.

Effect of *Prunella vulgaris* L. ethanol extract on blood pressure in SHR rats: The experimental results showed that the blood pressures of SHR rats in each group were relatively close before administration, of which the systolic and diastolic blood pressures were around 170, 123 mm Hg, respectively, the differences were not statistically significant ($P > 0.05$); one week after administration, the systolic and diastolic blood pressures of SHR rats in *Prunella vulgaris* L. experimental groups and captopril group were slightly lower than the blank control group. After 2-3 weeks of administration, the systolic

and diastolic blood pressures of SHR rats in each experimental group were significantly lower compared with the blank control group, the differences remained statistically significant ($P < 0.05$) (Figs. 1 and 2).

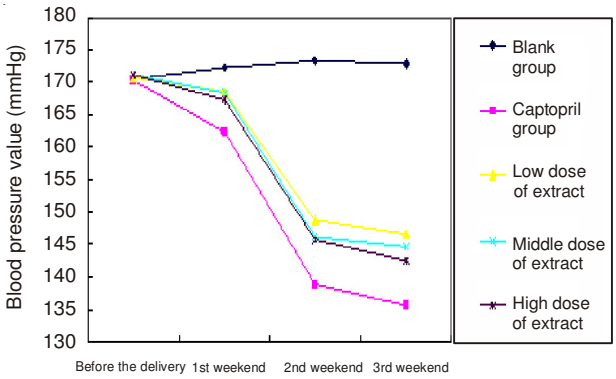

Fig. 1. Effect of each experimental group on blood pressure in SHR rats (systolic blood pressure, unit: mmHg)

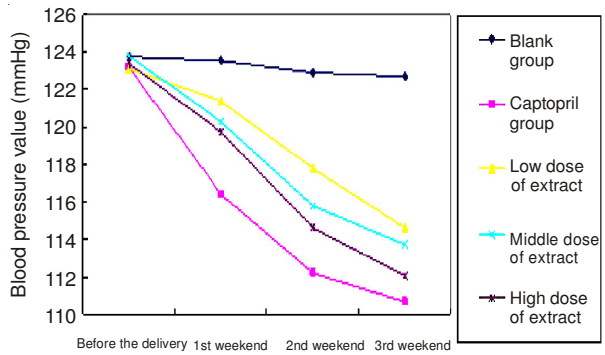

Fig. 2. Effect of each experimental group on blood pressure in SHR rats (diastolic blood pressure, unit: mmHg)

There is no disease such as high blood pressure in traditional Chinese medicine theory, high blood pressure is often attributed to the categories of dizziness, headache and liver wind and associated with stroke, heart palpitation, edema and other diseases. In "The Inner Canon of Yellow Emperor", there is the description of its pathogenesis as "all the syndromes of trembling and dizziness are pertaining to the liver."

Traditional Chinese medicine theory holds that hypertension is caused by imbalance of yin and yang of internal organs, disorder of qi and blood, disturbance in qi movement, endogenous wind-fire and phlegm stagnation due to emotional disorders, improper diet, over work, insufficient natural endowment, vigorous or feeble constitution, *etc*. Therefore, early hypertension can be treated by calming liver to stop endogenous wind.

Prunella vulgaris L. is bitter and slightly pungent in taste, slightly warm in nature, enters the liver meridian, removes liver wind and has the effects of soothing liver qi and dispersing liver stagnation, which is a traditional Chinese medicine used in the treatment of hypertension. In this experiment, the ethanol extract of *Prunella vulgaris* L. was extracted and the ethyl acetate and *n*-butanol extracts were isolated, then column chromatography, TLC and preparative HPLC were used to purify the compounds, meanwhile, the structures of the isolated compounds were identified, a total of five compounds were isolated, namely 2α,3α-24-trihydmxyursa-12-en-28-oic acid-28-O-β-D-glucopyranosly ester, chrysophanol, rotundic acid 28-O-α-D-glueopyranosyl(1 → 6)-β-D-g1ueopyranoside, A-spinasterol and β-sitosterol.

Liang *et al.*[15] studied the antihypertensive effect of *Prunella vulgaris* L. extract in spontaneously hypertensive rats. Their study showed that 6 w after the intragastric administration of a certain dose of *Prunella vulgaris* L. extract, the systolic and diastolic blood pressures of low-, medium- and high-dose groups were all significantly lower than the blank control group, indicating that *Prunella vulgaris* L. extract has an antihypertensive effect in SHR rats. Meanwhile, serum NO, ET and Ang II levels of SHR rats were determined after treatment by *Prunella vulgaris* L. And the results showed that NO level was significantly elevated, while serum levels of ET and Ang II decreased, suggesting that *Prunella vulgaris* L. extract may achieve hypotension by changing the contents of the three.

The results of present experiment showed that three weeks after administration at the test dose, each *Prunella vulgaris* L. ethanol extract (5, 10 and 15 mg/g) group could all significantly reduce the systolic and diastolic blood pressures in SHR rats when compared with the blank control group, the differences remained statistically significant ($P < 0.05$) and showed certain dose-effect relationship. The antihypertensive mechanism of action of *Prunella vulgaris* L. needs further study.

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