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Biologic Activities of Lysimachiae Herba II-Analgesic and antiinflammatory effects of ethyl acetate fraction and a phenyl propanoid component

Jongwon Choi*, Jong-Cheol Park[1], and Chung Kyu Lee

College of Pharmacy, Kyungsung University, Pusan 608-736 and
[1]Department of Oriental Medicine Resources, Sunchon National University, Sunchon 540-742, Republic of Korea

Abstract – The methanolic extract of Lysimachiae christinae Herba, which has been used for diuretic, calculi remover and jaundice remedy in oriental countries, was found to possess analgesic and antiinflammatory effect in Freund's complete adjuvant treated rat. From ethyl acetate fraction of the herb a phenyl propanoid isoferulic acid was isolated as an active principle.

Key words – Lysimachiae christinae Herba, isoferulic acid, antiinflammatory effect, arthritis.

Introduction

Lysimachiae christinae Herba has long been used to induce diuresis, remove calculi and treat jaundice in oriental countries (Huang, 1993; Namba, 1993). Biological study was much less carried out except that on the inhibitory activity of Na^+/K^+-ATPase by 6-tridecylresorcylic acid and grevillol of *L. japonica* (Shoji *et al.*, 1984). In previous paper, we reported that the methanolic extract of the herb revealed hepatic protecting effect in galactosamine treated rat by preventing liver damage in metabolizing enzyme system (Kim, *et al.*, 1996). The authors initiated a study to elucidate the effect of fractions and its column chromatographical isolate on analgesic, antiinflammatory and immunopotentiating activities in Freund's complete adjuvant treated rats.

Materials and methods

Herb material and animals – Lysimachiae christinae Herba which was imported from China, was purchased in a local market in Pusan, Korea, and identified pharmacognostically. A voucher specimen is deposited in the laboratory of C. Park. Male ICR mice(18~22 g) and Sprague-Dawley rats(140~160 g) were donated from Korean Experimental Animals Corp. in Daejon, Korea, and were adapted for lab atmosphere for more than one week.

Apparatus – The mp was determined on a Thomas Hoover 6406 apparatus and was not corrected. The IR spectra were recorded on a Hitachi 2703 spectrophotometer in KBr tablet and the UV spectra were run with CE 599 Universal automatic scanning spectrophotometer. The EI-MS spectra were taken with a Hewlett-Packard 5985B GC/MS spectrometer operating at 70 eV. The NMR spectra were recorded with a Bruker AM-200 spectrometer with TMS as an internal standard and chemical shifts were given as δ (ppm).

Extraction, fractionation and isolation – The herb material (2.4 kg) was extracted with 95% methanol to give methanolic extract

*Author for correspondence.

(MeOH ex., 175 g). From the MeOH ex., fractions of n-hexane, chloroform, ethyl acetate (EtOAc) and water were obtained as previously reported (Kim *et al.*, 1996).

Isolation and identification of Compound 1 – The EtOAc fr. (11 g) was applied to silica gel (30 g) column chromatography (solvent system : chloroform-MeOH-water=25-8-5, lower phase, and chloroform-MeOH-water =7-3-1, lower phase) to give 23 eluates with different TLC(solvent system: chloroform-MeOH =1-2) pattern. Eluates 12~23 were subjected to column chromatography on Sephadex LH-20 with MeOH-water(2-1 to 1-0) repeatedly to afford a pure phenyl propanoid Compound 1.

Preparation of dosage form – MeOH ex., EtOAc fr. and Compound **1** were suspended 1% CMC-Na solution and administered orally 250, 150 and 10 mg/kg for five days, respectively.

Analgesic activities – Ten mice of each group were orally administered with Lysimachiae samples one hour prior to the intraperitoneal injection of 0.7% acetic acid solution(0.1 ml/10 g) and the number of writhing syndrome was counted for 10 mins according to the method of Whittle (1949). Other ten mice of each group, which were treated as above, were put on hot plate (55~60 ℃, Ugo Basile, Italy) and the time of endurance was checked. The effect of aminopyrine (100 mg/kg) was compared as standard.

Induction of arthritis with Freund's complete adjuvant (FCA) and sample treatments – FCA(Sigma, USA, 0.05 ml) was injected right hind paw of rat at day 0 and sample was treated for five days from day 10 to day 14. Animals were sacrificed on day 15 for further tests.

Measurement of antiinflammatory activity – Five rats of each goup, which were treated with FCA, were administered with samples orally once a day for five days from the 10th day of adjuvant treatment. Then the thickness of edema was measured with Plethysmometer(Ugo Basile, Italy) and the regression rate of edema were calculated (Tsurufji *et al.*, 1979). For rheumatic arthritis (RA, Hara *et al.*, 1979), and C-reactive protein (CRP, Corrall, *et al.*, 1981) tests, six rats of each group were treated as mentioned above and were checked with RA77 kit (Eiken Chem. Co., Japan) and CRP-slide kit (Eiken Chem. Co., Japan), respectively. The effect of indomethacin (20 mg/kg) was compared as standard.

Measurements of variations in blood – The variations in blood components or enzyme activity, such as the numbers of erythrocytes, leucocytes and platelets and the levels of total protein, cholesterol and lactate dehydrogenase caused by sample traetment in FCA treated animals were checked by K-1000 cell counter (Sysmex, Japan), protein analysis kit (Sigma, USA), LDH kit (Asan, Korea, Marbach and Weil, 1967) and cholesterol kit (Asan, Korea, Allain *et al.*, 1974), respectively.

Preparation of serum and enzyme source – Pretreated animals were anesthized with CO_2 gas and blood was collected from abdominal artery. A part of whole blood was stood in CBC bottle to separate serum for 30 mins. Perfuged liver organ was isolated and homogenized with about four volumes of phosphate buffer (pH 7.5). Subsequent centrifugation at 10,000×g for 10 mins produced supernatant, which was centrifuged again at 105,000×g to divide cytosolic supernatant (for the assay of glutathione S-transferase activity and other contents) and microsomal precipitate (for the assays of other enzyme activities). The protein contents were measured by Biuret's method of Hatcher and Anderson (1969) using Folin reagent with bovine serum albumin (Sigma, Fr.IV) as standard.

Measurements of hepatic enzyme activities and components – The methods for measurements for hepatic enzyme activities or components level are as follows: lipid peroxide level according to Ohkawa (1979), ac-

tivities of superoxide dismutase and glutathione peroxidase according to Marklund *et al.* (1974) and Paglia and Valentine (1967) and protein analysis according to Lowry (1951) with bovine serum albumin (Sigma, Fr. V) as standard.

Results and discussion

Identification of compound 1 – Isolated compound **1** (mp 230~2 ℃, needle crystal) showed aromatic ring and carboxyl group in IR spectra (ν_{max}) at 3404, 1671, 1629, 1512, 1266 and 1134 cm^{-1}, respectively. According to EI-MS spectra, molecular peak[m/z 194($C_{10}H_{10}O_4$), relative intensity; 100], and several other peak at 179(M-CH_3, 43.6), 177(M-OH, 11.1), 133(177-COOH-H, 22.0), 105(10.0), 77 (15.0) and 51(16.0). In ^{1}H-NMR spectrum, trans-olefinic proton was shown at δ 7.45 and 6.24, with coupling constant of 15.9 Hz, and methoxy group at 3.79, which mean C_6-C_3 structure with aromatic carboxylic acid. And the ^{13}C-NMR specrtrum shows that the compound is quite agreed with isoferulic acid (Pouchert and Behnke, 1993) and is different from authentic ferulic acid (δ 167.7, 149.0, 147.9, 144.3, 125.7, 122.9, 115.6, 115.4, 111.2 and 55.6, Table 1).

Analgesic and antiinflammatory effects – The methanolic extract and its ethyl acetate fraction of Lysimachiae christinae Herba showed significant analgesic effects indicated by the method of acetic acid and hot plate. And also showed antiinflammatory effects in FCA treated rats. The antiinflammatory effect was also comfirmed by decrease in rheumatic arthritis (RA) factor and C-reactive protein (CRP) which are barometers of arthritis. Such effects were repeated by the constituent compound **1** at the level of 10 mg/kg (Tables 2 and 3).

Effects on blood components and enzyme activity – The hematological components which are elevated by induction of arthritis were significantly reduced by treatments of samples or component **I** as shown in Table 4. The level of total protein and acti-

Table 1. Spectral data of Compound **1**

IR (KBr), cm^{-1}	EI-MS, m/z (rel.int.)	^{1}H-NMR, δ, (200 MHz, DMSO-d_6)	^{13}C-NMR, δ, (50.3 MHz, DMSO-d_6)
3404, 1671, 1629, 1512, 1266, 1134	194(M^+, 100.o); 179(43.6); 111(11.1); 133(22.0); 105(10.0); 77(15.0) and 51(16.0)	3.79(3H, s, OCH3); 6.24(1H, d, J= 15.91 Hz, H-8); 6.93(1H, J=8.8 Hz, d, H-6) and 7.45 (1H, J=15.91 Hz, d, H-7)	55.0(OCH_3); 112.0 (C-8); 114.1(C-2); 120.9(C-6); 127.1 (C-1); 144.1(C-7); 149.6(C-4) and 167.7(C-2)

Table 2. Analgesic effect of Lysimachiae christinae Herba on writhing syndrome due to 0.7% acetic acid and endurance time stood on hot plate in mice

Treatment[1]	Dose (mg/kg)	No. of Writhing[2] (10 mins)	% of Control	Endurance time[2] (seconds)	% of Control
Control		46.0±4.24[a]	100.0	25.8±5.29[a]	100.0
MeOH ex.	250	32.5±3.11[b]	70.7	56.3±2.15[b]	218.2
EtOAc fr.	150	28.6±4.36[b,c]	62.2	63.4±5.36[b,c]	245.7
Compound 1	10	22.5±2.16[c]	48.9	71.1±4.74[c]	275.6
Aminopyrine	100	10.3±2.63[d]	22.4	146.0±11.09[d]	565.9

[1]Ten mice were orally administered with Lysimachiae materials one hour prior to the intraperitoneal injection of 0.7% acetic acid solution or to putting on hot plate (55~60 ℃). [2]Values are expressed as mean±S.D. of 10 experiments. Values sharing the same superscript letter are not significantly different each other($p<0.05$) by Duncan's multiple range test.

Table 3. Effect of Lysimachiae christinae Herba on the paw edema and rheumatic arthritis (RA) and C-reactive protein (CRP) tests in rats treated with Freund's complete adjuvant

Treatment[1]	Dose (mg/kg)	Thickness[2] of edema (mm)	% of Control	RA test[3] (%)	CRP test[3] (%)
Control		74.4±5.58[a]	100.0	87.9	76.4
MeOH ex.	250	55.7±4.35[b]	74.9	54.3	50.3
EtOAc fr.	150	43.9±3.86[c]	59.0	48.7	45.6
Compound 1	10	36.4±2.96[d]	48.9	36.5	38.4
Indomethacin	20	38.8±2.76[c,d]	47.8	39.7	40.6

[1]Five (for edema) or six (for RA and CRP tests) rats were treated with Freund's complete adjuvant and Lysimachiae materials were administered orally once a day for five days from the 10th day of adjuvant treatment. [2]Values are expressed as mean±S.D. of five experiments. Values sharing the same superscript letter are not significantly different each other($p<0.05$) by Duncan's multiple range test. [3]Values are expressed as mean of five experiments.

Table 4. Effect of Lysimachiae christinae Herba on the hematological values in rats treated with Freund's complete adjuvant

Treatment[1]	Dose (mg/kg)	RBC[2] ($\times 10^6$/μl)	WBC[2] ($\times 10^3$/μl)	Platelet[2] ($\times 10^3$/μl)
Normal		7.4±1.16[a]	14.4±2.51[a]	122.8±12.8[a]
Control		12.8±1.36[b]	28.7±5.02[b]	200.6±15.9[b]
MeOH ex.	250	9.6±1.32[c]	21.5±4.22[c]	176.5±20.6[b,c]
EtOAc fr.	150	8.4±1.02[a,c]	18.8±2.07[a,c]	168.7±18.5[c]
Compound 1	10	7.8±0.70[a,c]	17.5±1.98[a,c]	156.9±14.9[c]
Indomethacin	20	8.1±0.92[a,c]	18.5±1.27[a,c]	163.4±15.2[c]

[1]Five rats were treated with Freund's complete adjuvant and Lysimachiae materials were administered orally once a day for five days from the 10th day of adjuvant treatment. [2]Values are expressed as mean±S.D. of five experiments. Values sharing the same superscript letter are not significantly different each other ($p<0.05$) by Duncan's multiple range test.

vity of lactate dehydrogenase were also recovered to near the normal state especially by Compound **1** (Tables 5 and 6). On the other hand the decreased level of cholesterol by arthritis induction was recovered by treat-

Table 5. Effect of Lysimachiae christinae Herba on the serum total protein and cholesterol concentration in rats treated with Freund's complete adjuvant

Treatment[1]	Dose (mg/kg)	Total protein[2] g/dl	Cholesterol[2] mg/dl
Normal		6.72±0.25[a]	79.6±7.28[a]
Control		8.29±0.36[b]	63.8±5.00[b]
MeOH ex.	250	7.89±0.19[b,c]	68.9±5.94[b,c]
EtOAc fr.	150	7.65±0.12[c,d]	73.6±4.72[a,c]
Compound 1	10	7.26±0.16[d]	75.4±4.90[a,c]
Indomethacin	20	7.43±0.20[d]	74.6±3.27[a,c]

[1]Ten rats were treated with Freund's complete adjuvant and Lysimachiae materials were administered orally once a day for five days from the 10th day of adjuvant treatment. [2]Values are expressed as mean± S.D. of ten experiments. Values sharing the same superscript letter are not significantly different each other ($p<0.05$) by Duncan's multiple range test.

Table 6. Effect of Lysimachiae christinae Herba on the serum lactate dehydrogenase (LDH) activity in rats treated with Freund's complete adjuvant

Treatment[1]	Dose (mg/kg)	LDH Activity[2] Wroblewski unit/L	% of Normal
Normal		724.6±46.00[a]	100.0
Control		1230.2±87.96[b]	169.8
MeOH ex.	250	1006.5±65.78[c]	138.9
EtOAc fr.	150	909.3±58.43[c,d]	125.5
Compound 1	10	856.4±62.45[d]	118.2
Indomethacin	20	884.2±50.65[d]	122.0

[1]Five rats were treated with Freund's complete adjuvant and Lysimachiae materials were administered orally once a day for five days from the 10th day of adjuvant treatment. [2]Values are expressed as mean±S.D. of ten experiments. Values sharing the same superscript letter are not significantly different each other ($p<0.05$) by Duncan's multiple range test.

Table 7. Effect of Lysimachiae christinae Herba on the hepatic lipid peroxide content in rats treated with Freund's complete adjuvant

Treatment[1]	Dose (mg/kg)	Contents of MDA[2] (nmole/g of tissue)	% of Control
Normal		18.5±0.81[a]	100.0
Control		41.3±2.16[b]	223.2
MeOH ex.	250	33.4±2.45[c]	180.5
EtOAc fr.	150	25.9±2.36[d]	140.0
Compound 1	10	26.3±1.89[d]	142.2
Indomethacin	20	24.3±2.17[d]	131.4

[1]Ten rats were treated with Freund's complete adjuvant and Lysimachiae materials were administered orally once a day for five days from the 10th day of adjuvant treatment. [2]Values are expressed as mean of malonedialdehyde±S.D. of ten experiments. Values sharing the same superscript letter are not significantly different each other ($p<0.05$) by Duncan's multiple range test.

Table 8. Effect of Lysimachiae christinae Herba on the hepatic superoxide dismutase(SOD) and glutathione peroxidase (GP) activities in rats treated with Freund's complete adjuvant

Treatment[1]	Dose (mg/kg)	SOD activity[2] Unit/mg protein	GP activity[3] Unit/mg protein/min
Normal		9.58±0.208[a]	240.5±19.66[a]
Control		3.79±0.359[b]	116.7±14.71[b]
MeOH ex.	250	6.74±0.184[c]	186.1±15.37[c]
EtOAc fr.	150	7.43±0.319[d]	208.1± 9.72[c,d]
Compound 1	10	7.58±0.412[d]	210.9±12.45[c,d]
Indomethacin	20	7.63±0.387[d]	213.2±11.26[d]

[1]Five rats were treated with Freund's complete adjuvant and Lysimachiae materials were administered orally once a day for five days from the 10th day of adjuvant treatment. [2,3]Values are expressed as mean±S.D. of five experiments and values sharing the same superscript letter are not significantly different each other ($p<0.05$) by Duncan's multiple range test. [2]50% inhibition rate of oxidation of pyrogallol and [3]oxidized NADPH n mole.

ment of samples near to normal state (Table 5).

Effects on lipid peroxide, superoxide dismutase and glutathione peroxidase – The increased level of lipid peroxide by arthritis induction in liver tissue was significantly lowered by samples (Table 7) and decreased activities of superoxide dismutase and glutathione peroxidase were somewhat recovered (Table 8). But, in contrast, the treatment of samples didn't affect to the activities of superoxide dismutase and glutathione peroxidase.

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