African Journal of Biotechnology Vol. 10(53), pp. 11023-11027, 14 September, 2011
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB11.332
ISSN 1684–5315 © 2011 Academic Journals

Full Length Research Paper

Chemical composition, anti-inflammatory and analgesic activities of the petrol ether extract of *Pinellia cordata*

Baokang Huang[1]*, Zhiyong Chu[2], Weiping He[1] and Luping Qin[1]

[1]Department of Pharmacognosy, School of Pharmacy, Second Military Medical University, Shanghai, China.
[2]Naval Medical Research Institute, Shanghai, China.

Accepted 11 August, 2011

The analgesic and anti-inflammatory effect of the petrol ether extract of *Pinellia cordata* (PEPC) was investigated using the xylene-induced ear edema and acetic acid-induced writhing test in mice. PEPC was administered intragastrically (i.g.) at 200, 400 and 600 mg/kg. It had significant anti-inflammatory and analgesic activities with dose dependent manner. The chemical components of PEPC were analyzed by gas chromatography-mass spectrometry (GC/MS), and the main components were melissane (20.1%), τ-sitosterol (13.9%) and linoleic acid (10.1%). These data may afford scientific basis on the traditional use of the plant for painful and inflammatory conditions.

Key words: Anti-inflammatory, analgesic activity, *Pinellia cordata*, petrol ether extract, gas chromatography-mass spectrometry (GC/MS).

INTRODUTION

There are multiple reports of natural products having antimicrobial, anti-inflammatory and analgesic activities, and the essential oils, sesquiterpenes and flavonoids might be their active components (Akin et al., 2010; Lawal et al., 2010; Lu et al., 2009; Owolabi and Omogbai, 2007). In China, many traditional Chinese medicines have been used to treat pains or inflammation caused by conditions such as arthritis and tendinitis.

Pinellia cordata is a medicinal plant (Araceae family) native to southeast China. The plant is marked with arrowhead leaves and grows along forest stream or steep rock near stream (Figure 1). The stem tuber of *P. cordata* (Chinese name “Dishuizhu”) contains a variety of constituents, including alkaloids, which have a variety of pharmaceutical properties. Though it is a little poisonous, it was traditionally used as a magical herb in east China for treating all kinds of pain, stomachache, snake bite poisoning, injury, arthritis, rheumatism, cancerous tumors and skin diseases. Capsules containing this crude drug were clinically used as analgesic and anti-inflammatory agents in Zhejiang Province (Chiang Su New Medicinal College, 2003; Institute of Botany, 1979). The present study was therefore planned to investigate the chemical composition, analgesic and anti-inflammatory effects of the petrol ether extract of *P. cordata*.

*Corresponding author. E-mail: hbkcn@163.com. Fax: +86021-81871301.

MATERIALS AND METHODS

Adult ICR mice weighing 18-22g were obtained from Animal Center of Second Military Medical University. Mice were housed in cages and maintained in an air-conditioned room with controlled temperature (21 ± 1℃) and humidity (55 ± 15%), and were kept on a 12 h light/dark cycle. They were fed with standard diet and water and acclimated 3 days before they were used. All procedures involving animals were conducted in accordance with the European Community guidelines for the use of experimental animals and approved by the Second Military Medical University Committee on Animal Care and Use. The animals were deprived of food for 15 h before the experiment, with free access to drinking water. Unless indicated, equal number of female and male animals was used in each experiment. In all experiments, animals were randomly divided into groups of ten animals, and each animal was used only once in the experiment.

Protocol

Each group was vehicle treated (intragastrically, i.g.), with PEPC (200, 400 and 600 mg/kg). As a reference, indomethacin (10 mg/kg, i.g.) (Chengdu Pharmaceutical Factory, Chengdu, China) was used as the standard drug. 2% Tween 20 solution (TW, Merck) in physiological saline was used as a vehicle. The dosages of PEPC were estimated based on our preliminary experiment and

recommended oral dosage for the folk use in human. Acetic acid and xylene (Sinopharm chemical reagent Co. Ltd., China) were used as the inducer drug.

Herbal material and its extract

P. cordata was collected in August 2009 from Zhejiang Province, China and was authenticated by Professor Hanchen Zheng, Department of Pharmacognosy, Second Military Medical University. A voucher specimen (No. H038#) was deposited in the herbarium of the Department of Pharmacognosy, School of Pharmacy, Second Military Medical University. Air-dried powdered material weighing 1 kg was macerated and soaked in three volumes (w/v) of petroleum ether (30 to 60℃) for 24 h at room temperature. The process was repeated three times and filtrates were pooled and concentrated at 45℃ under reduced pressure in a rotary evaporator and yielded 22.1 g of petroleum ether extract (PEPC). PEPC was kept in a refrigerator and was freshly dissolved in the vehicle before each experiment.

Gas chromatography-mass spectrometry (GC/MS) analysis

GC/MS analysis was carried out on a Thermo Focus DSQ GC–MS instrument (Thermo, USA). The analytes were separated on a 30 m × 250 μmHP-5 capillary column coated with a 0.25 μm film of 5% phenyl methylpolysiloxane. Split ratio was 30:1. The column oven temperature was programmed at 15℃ min^{-1} from an initial temperature of 60 to 300℃, which was held for 5 min. The injection and ion source temperatures were 250℃. Helium (99.999%) was used as carrier gas at a flow rate of 1 $ml.min^{-1}$. The ionizing electron energy was 70 eV and the mass range scanned was 40 to 400 amu in full-scan acquisition mode. Compounds were identified using the NIST Mass Spectral Search Program (National Institute of Standards and Technology, USA.)

Assay of xylene -induced inflammation in mice

One hour after giving the medicine for the last time, the mice's right ear was embrocated with xylene according to the method described by literature (Zhou et al., 2008). The edema was measured 1 h after xylene treatment, after which mice were sacrificed and a plug was excised from both the ears. The ear swelling rate (SR) was measured as follows:

$SR = (W_R - W_L) / W_L$,

Where, W_R is the weight of right (treated) ear and W_L is the weight of the left (untreated) ear. The anti-inflammatory activity was expressed as inhibitory ratio (IR) or percent tumefaction reduction in treated mice compared to the controls.
The IR of the treated group was calculated as follows:

$IR = (A - B) \times 100 / A$,

Where, A is the edema of vehicle group and B is the edema of drug group. Each value was the mean of individual determinations in 10 mice.

Abdominal constriction induced by acetic acid

The writhing test was carried out following the method described by literature (Rabanal et al., 2005). Mice were randomly divided into each group (n = 10), and groups of overnight fasted mice were treated with PEPC, vehicle or indomethacin 1 h before an intraperitoneal injection of 0.7% acetic acid at 0.1 ml/10 g body weight. The number of writhing was counted for each animal, starting 3 min after acetic acid injection over the period of 12 min.

Statistical analysis

Results were expressed as mean ± S.E.M. Statistical analyses were performed with one-way ANOVA followed by Student's t-test. *P* values less than 0.05 were considered to be statistically significant.

RESULTS AND DISCUSSION

The chemical components of the petrol extracts of *P. cordata* were analyzed by GC/MS, and 26 compounds were identified. The main compounds were melissane (20.1%), τ-sitosterol (13.9%), linoleic acid (10.1%), nonacosane (8.6%), hexadecanoic acid (7.7%), campesterol (6.7%) and heptacosane (5.8%) (Table 1). In the acetic acid-induced writhing test, PEPC showed obvious inhibition of the writhing responses induced by acetic acid in a dose-dependent manner, with inhibitory ratio of 19.5%, 42.7% and 53.0%, respectively (Figure 1), while the indomethacin induced 61.6% writhing inhibition. PEPC showed highest inhibitory ratio at the dose of 600 mg/kg. In the writhing response, acetic acid causes pain by liberating PGs and many others that excite pain nerve endings (Choi et al., 2006).

However, constriction induced by acetic acid was considered as a non-selective anti-nociceptive model, and for the chemical diversity of the natural products, herb extracts possesses both peripheral and central analgesic activity (Sánchez-Mateo et al., 2006). The crude drug of *P. cordata* has been used to treat abdominal pain, waist ache and headache in folk tradition. In order to clarify whether the activities are due to the blockade of the effect or the release of PGs that excite pain nerve endings, more animal models including formalin, hot plate and tail flick test are needed in further study. In the xylene-induced ear edema test, the positive control drug indomethacin at 10 mg/kg exhibited significant inhibitory effect. The inhibition was up to approximately 28.7%. PEPC exerted obvious anti-inflammatory activity in a dose-dependent manner. At the dose of 200, 400 and 600 mg/kg, the edema reductions were 2.6, 14.2 and 30.8%, respectively. At the dose of 600 mg/kg, the potency of PEPC was comparable to that of 10 mg/kg indomethacin (Figure 2). Indomethacin is a known nonselective COX inhibitor. In xylene-induced ear edema acute inflammation model, PEPC might acts in part through the inhibition of the inflammatory mediators of the acute phase of inflammation like indomethacin.

The results demonstrate the anti-inflammatory properties of PEPC and provided evidence of this herbal medicine for the treatment of inflammatory diseases in Chinese. The PEPC is a complex mixture of fatty acids and sterols, which comprises a great part of the fraction. It was reported that stigmasterol and sitosterol could



Figure 1. The plant of *Pinellia cordata* N. E. Brown.

Table 1. Compounds identified by GC/ MS from the petrol ether extracts of rhizoma of *Pinellia cordata* N. E. Brown.

RT	Compound	Mr	MF	%
3.09	Hexanal	100	$C_{16}H_{12}O$	0.3
4.71	(Z)-2-Hexanal	112	$C_7H_{12}O$	0.5
7.34	(E)-2-Decenal	154	$C_{10}H_{18}O$	0.3
7.75	2,4-Decadienal	152	$C_{10}H_{16}O$	0.4
9.58	Diethyl phthalate	222	$C_{12}H_{14}O_4$	0.3
9.79	Apiol	222	$C_{12}H_{14}O_4$	0.2
11.32	Methyl palmitate	270	$C_{17}H_{34}O_2$	0.8
11.51	Hexadecanoic acid	256	$C_{16}H_{32}O_2$	7.7
11.66	Ethyl palmitate	284	$C_{18}H_{36}O_2$	0.4
12.17	Methyl octadeca-9,12-dienoate	294	$C_{19}H_{34}O_2$	0.9
12.19	Methyl elaidate	296	$C_{19}H_{36}O_2$	0.8
12.36	Linoleic acid	280	$C_{18}H_{32}O_2$	10.1
12.46	Stearic acid	284	$C_{18}H_{36}O_2$	2.9
12.56	Palmitic acid butyl ester	312	$C_{20}H_{40}O_2$	1.1
13.05	Tricosane	324	$C_{23}H_{48}$	0.9
13.32	Isopropyl linoleate	322	$C_{21}H_{38}O_2$	3.4
13.88	Pentacosane	352	$C_{25}H_{52}$	2.7
14.71	Heptacosane	380	$C_{27}H_{56}$	5.8
15.29	Bis(2-ethylhexyl) sebacate	426	$C_{26}H_{50}O_4$	3.3
15.77	Nonacosane	408	$C_{29}H_{60}$	8.6
16.76	Z-10-octadecen-1-ol acetate	310	$C_{20}H_{38}O_2$	1.2
17.14	Melissane	422	$C_{30}H_{62}$	20.1
18.64	1,30-Triacontanediol	454	$C_{30}H_{62}O_2$	1.2
19.00	Campesterol	400	$C_{28}H_{48}O$	6.7
19.36	Stigmasterol	412	$C_{29}J_{48}O$	3.3
19.88	Unknown	412	$C_{29}H_{48}O$	1.7
20.09	T-sitosterol	414	$C_{29}H_{50}O$	13.9

Mr, Relative molecular mass; MF, molecular formula; 5%, relative contents.

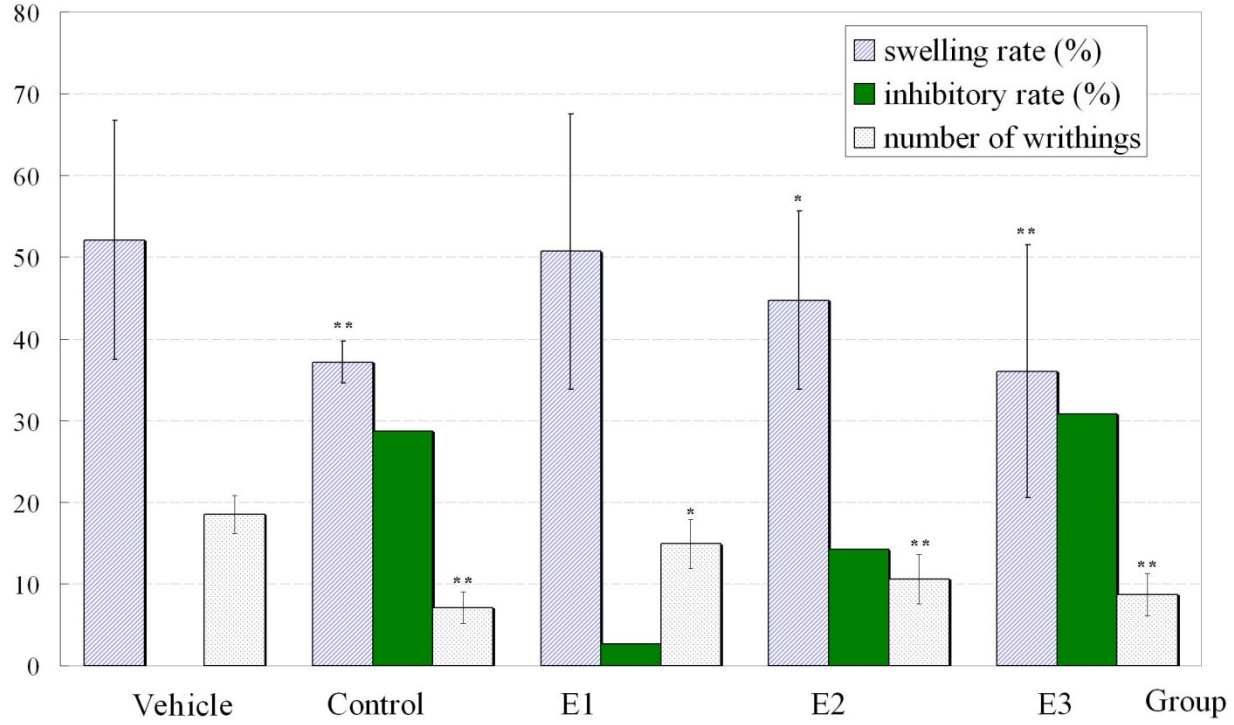


Figure 2. Effect of PEPC on xylene-induced ear edema and acetic acid-induced writhing responses in mice. C = Control groups (mice were treated with 2% Tween 20 solution, 10 ml/kg, i.g.); D = group treated with indomethacin (10 mg/kg, i.g.); E1, E2 and E3 = groups treated with the different dose of PEPC by weight, respectively (200, 400 and 600mg/kg, i.g.). The number of writhing was demonstrated in the figure. The ear swelling rate and inhibitory rate were shown as percentage. Data are expressed as mean ± S.E.M (n =10). *P<0.05, **P<0.01, vs. control, Student's t-test.

significantly and dose-dependently reduce formalin-induced pain; acetic acid induced abdominal constrictions (Al-Yousuf et al., 2002; Erazo et al., 2006). Oils with higher a-linolenic acid content produced a greater inhibition in carrageenan and arachidonic acid induced paw edema models (Singh et al., 2008). Due to complexity of the chemical compounds of PEPC, the activity of the PEPC might be related to the combination of the major chemical compounds such as linolenic acid and sitosterol. However, to interpret the mechanism and the relationship between chemical composition and pharmacological effects, more animal models, such as rat paw edema model and hot plate test, should be conducted by using single compounds including alkaloids from *P. cordata*,

Conclusion

Our results indicate that PEPC has analgesic and anti-inflammatory effects, which may provide some pharmacological evidence for its folkloric use and further exploitation. These activities might be related to the combination of the major chemical compounds such as linolenic acid and sitosterol in the PEPC. In further exploring the activity and mechanism of action of PEPC, the active principle could be isolated and further molecular and pharmacological experiments warranted.

REFERENCES

Akin M, Demirci B, Bagci Y, Baser KHC (2010). Antibacterial activity and composition of the essential oils of two endemic Salvia sp. from Turkey. Afr. J. Biotechnol., 9: 2322-2327.

AL-Yousuf MH, Ali BH, Bashir AK, Tanira MO, Blunden G (2002). Central nervous system activity of *Leucas inflata* Benth. in mice. Phytomedicine 9: 501-507.

Chiang Su New Medicinal College (2003). Dictionary of Chinese Crude Drugs, Shanghai Scientific Technology Press, Shanghai.

Choi JH, Jung BH, Kang OH, Choi H, Park PS, Cho SH, Kim YC, Sohn DH, Park H, Lee JH, Kwon DY (2006). The anti-inflammatory and anti-nociceptive effects of ethyl acetate fraction of *Cynanchi Paniculati* Radix. Biol. Pharm. Bull., 29: 971–975.

Erazo S, Delporte C, Negrete R, Garcia R, Zaldivar M, Iturra G, Caballero E, Lopez JL, Backhouse N (2006). Constituents and biological activities of *Schinus polygamus*. J. Ethnopharmacol., 107: 395-400.

Institute of Botany (1979). Academia Sinica Flora of China, Science Press, Beijing.

Lawal U, Ibrahim H, Agunu A, Abdulahi Y (2010). Anti-inflammatory and analgesic activity of water extract from *Ipomoea asarifolia* Desr (Convolvulaceae). Afr. J. Biotechnol., 9(51): 8877-8880.

Lu Y, Zhong CX, Wang L, Lu C, Li XL, Wang PJ (2009). Anti-inflammation activity and chemical composition of flower essential oil from *Hedychium coronarium*. Afr. J. Biotechnol., 8(20): 5373-5377.

Owolabi OJ, Omogbai EKI (2007). Analgesic and anti-inflammatory activities of the ethanolic stem bark extract of *Kigelia africana* (Bignoniaceae). Afr. J. Biotechnol., 6: 582-585.

Rabanal RM, Bonkanka CX, Hernández-Pérez M, Sánchez-Mateo CC (2005). Analgesic and topical anti-inflammatory activity of *Hypericum canariense* L. and *Hypericum glandulosum* Ait. J. Ethnopharmacol., 107: 591-596.

Sánchez-Mateo CC, Bonkanka CX, Hernández-Pérez M, Rabanal RM (2006). Evaluation of the analgesic and topical anti-inflammatory effects of *Hypericum reflexum* L. fil. J. Ethnopharmacol., 107: 1-6.

Singh S, Nair V, Jain S, Gupta YK (2008). Evaluation of anti-inflammatory activity of plant lipids containing a-linolenic acid Indian. J. Exp. Biol., 46(6): 453-456.

Zhou MH, Wang HG, Suolangjiba, Kou JP, Yu BY (2008). Antinociceptive and anti-inflammatory activities of *Aquilaria sinensis* (Lour.) Gilg. Leaves extract. J. Ethnopharmacol., 117: 345-350.