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Analgesic, anti-inflammatory and antipyretic activities of the petroleum ether fraction from the ethanol extract of *Desmodium podocarpum*

Zhan-Zhou Zhu[a], Ke-Jia Ma[a,b], Xia Ran[a,c], Hong Zhang[a,*], Cheng-Jian Zheng[a], Ting Han[a], Qiao-Yan Zhang[a], Lu-Ping Qin[a,**]

[a] *Department of Pharmacognosy, School of Pharmacy, Second Military Medical University, No. 325, Guohe Road, Shanghai 200433, PR China*
[b] *College of Pharmacy, Jiamusi University, Jiamusi 154007, PR China*
[c] *School of Life Science, East China Normal University, Shanghai 200062, PR China*

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ABSTRACT

Ethnopharmacological relevance: Desmodium podocarpum is a plant that has been used in the folk medicine to treat febrile diseases, cough and bleeding wounds. However, there is no scientific basis or reports in the modern literature regarding its effectiveness as an analgesic, anti-inflammatory and antipyretic agent.
Aims of the study: The objective of this study is to evaluate the analgesic, anti-inflammatory and antipyretic activities of the petroleum ether fraction (PEF) from the ethanol extract of *Desmodium podocarpum*.
Materials and methods: PEF (50, 100, 200 mg/kg) was estimated for its pharmacological properties by using the acetic acid-induced writhing test, the hot plate test, the Carrageenan-induced rat paw edema model, the dimethylbenzene-induced mouse inflammation model, and the lipopolysaccharide (LPS)-induced rat fever model. In addition, the acute toxicity of PEF was also studied.
Results: PEF significantly and dose-dependently inhibited the writhing responses in mice, increased reaction time of mice in the hot plate test, reduced carrageenan-induced paw edema in rats and the dimethylbenzene-induced ear edema in mice, and attenuated LPS-induced fever in rats. No death of mice was observed when orally administered PEF up to 4.2 g/kg.
Conclusions: These findings suggest that PEF possesses evident analgesic, anti-inflammatory and antipyretic activities, and has a favorable safety, which supports the use of *Desmodium podocarpum* as an analgesic, anti-inflammatory and antipyretic drug in the folk medicine.

1. Introduction

Desmodium podocarpum DC., a type of shrub belonging to the family Leguminosae, is widely found in Yunnan, Gansu, Guizhou and other Southwest Provinces in China. In Chinese folk medicine, the whole plant of *Desmodium podocarpum* is used in the treatment of febrile diseases, cough and bleeding wounds for its functions of clearing away heat and toxic materials and cooling blood (Editorial committee of Chinese Materia Medica, 1999).

There are many extracts from plants of this genus *Desmodium* that have been proven to possess anti-inflammatory, analgesic and antipyretic activities in many animal models. For example, the ethanol extract of the leaves of *Desmodium adscendens* (Sw.) DC. var. adscendens, a medicinal plant in the African traditional medicine, could induce hypothermia and had an analgesic effect in mice (N'gouemo et al., 1996). The aqueous extract of *Desmodium adscendens* also had anti-anaphylactic properties in guinea pigs, which could reduce the lung histamine content in a dose-dependent manner, elicited a dose-dependent reduction in the amount of spasmogens released, and inhibited histamine-induced contraction of ileal muscle (Addy and Dzandu, 1986). The methanol extract from *Desmodium triflorum* DC. decreased the acetic acid-induced writhing responses in mice and the licking time on the late phase in the formalin test, and inhibited λ-carrageenan-induced paw edema in mice. The anti-inflammatory mechanism of *Desmodium triflorum* might be related to the decrease in the level of malondialdehyde (MDA) in the edema paw via increasing the activities of superoxide dismutase (SOD) and glutathione reductase (GRd) in the liver, and the reduction in the nitric oxide (NO) level via regulating the interleukin-1β (IL-1β) production and the level of tumor necrosis factor-α (TNF-α) in the inflamed tissues (Lai et al., 2009). The aqueous extract of root and aerial parts of *Desmodium gangeticum* DC. was also found to have significant anti-inflammatory and analgesic activities in experimental animals (Rathi et al., 2004).

Although a number of plants belonging to the genus *Desmodium* have been investigated on their chemical components and

** Corresponding author. Tel.: +86 21 81871300.
* Corresponding author. Tel.: +86 21 81871305.
E-mail addresses: lpqin@smmu.edu.cn (L.-P. Qin)
zhanghong@smmu.edu.cn (H. Zhang).

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pharmacological effects, there seems to be no report on the analgesic, antipyretic and anti-inflammatory activities of the plant *Desmodium podocarpum*. In this study, the above activities of the petroleum ether fraction (PEF) from the ethanol extract of *Desmodium podocarpum* were evaluated in mice and rats to substantiate and expand its clinical applications. In addition, we determined acute oral toxicity of PEF which exhibited a good security.

2. Materials and methods

2.1. Plant material and extraction

The whole plant of *Desmodium podocarpum* was collected in Jiande of Zhejiang Province in July 2009 and identified by Professor Lu-Ping Qin, a pharmacognosist from the Department of Pharmacognosy, School of Pharmacy, Second Military Medical University (Shanghai, China). A voucher specimen of *Desmodium podocarpum* was deposited with the number SY3245 in the Herbarium of the Department of Pharmacognosy, School of Pharmacy, Second Military Medical University (Shanghai, China). The dried plant (500 g) was pulverized with a motor-driven grinder to prepare the extract. After refluxing extraction with 8 L 85% (v/v) aqueous ethanol at 55 °C four times for 0.5 h each time, the extract was filtered and then the solvent was evaporated to get the ethanol extract of *Desmodium podocarpum* (DPE, 59.54 g) under reduced pressure in a rotary evaporator. Further, the crude ethanol extract of the whole plant was sequentially fractionated into five sub-extracts explicitly, namely, petroleum ether, dichloromethane, ethyl acetate (EtOAc), n-butanol, and remaining water extracts. The petroleum ether fraction (PEF) was concentrated under reduced pressure to obtain a residue (16.24 g) for bioactivity determination.

2.2. GC–MS analysis

GC–MS analysis was performed for determination of PEF composition with a Finnigan Voyager gas chromatograph fitted with a fused silica VF-5 ms capillary column (3 m × 0.25 mm; coating thickness 0.25 μm, VARIAN, USA) using the following temperature program. The initial temperature was 50 °C, raised to 180 °C at 15 °C/min, and then it was raised to 300 °C at 5 °C/min and held for 15 min. The injector temperature was 240 °C and the MS source Helium was used as the carrier gas at a flow rate of 1.0 ml/min with a split ratio of 50:1 (v/v). The gas chromatograph was coupled to a Finnigan Voyager mass selective detector. The ionization source temperature was 250 °C.

2.3. Animals

ICR mice (20–25 g), male Sprague-Dawley (SD) rats (200–220 g) and male Wistar rats (200–220 g), obtained from the Experimental Animal Center of the Second Military Medical University (Shanghai, China), were housed in a regulated environment (20 ± 2 °C), with a 12 h light/dark cycle (08:00––20:00, light). The animals were deprived of food for 15 h before the experiment, with free access to drinking water. Each animal was used only once in the experiment. All animal treatments were strictly in accordance with international ethical guidelines concerning the care and use of laboratory animals, and all the experiments were carried out under the approval of the Committee of Experimental Animal Administration of the University. Each experimental group consisted of 10 animals.

2.4. Drugs and reagents

The following reagents and drugs were used: ethanol (AR), dimethylbenzene (AR) and acetic acid (AR) (Sinopharm chemical reagent Co. Ltd., China), aspirin, ibuprofen, indomethacin, dexamethasone, paracetamol (Chengdu Pharmaceutical Factory, Chengdu, China), lipopolysaccharide, and carrageenan (Sigma, St. Louis, MO, USA).

2.5. Treatment

PEF, aspirin, ibuprofen, indomethacin, dexamethasone and paracetamol were respectively dissolved in distilled water prior to administration. Four groups of animals ($n = 10$) were orally administered 50, 100, 200 mg/kg PEF and 350 mg/kg DPE, respectively, by intubation. The positive group of animals ($n = 10$) were respectively given aspirin (100 mg/kg), ibuprofen (200 mg/kg), indomethacin (5 mg/kg), dexamethasone (5 mg/kg) and paracetamol (100 mg/kg) in different experiments. Another group of animals (negative control group, $n = 10$) were given distilled water, and it was run concurrently with the drug-treated groups, all of which were given in a volume of 10 ml/kg body weight irrespective of dose.

2.6. Analgesic test

The peripheral analgesic activity of PEF was evaluated in male mice using the acetic acid-induced writhing test (Garcia et al., 2004), while central analgesic activity of PEF against thermal stimuli was studied in female mice using the hot plate test (Franzotti et al., 2000).

In the writhing test, male mice ($n = 10$) were orally administered DPE, PEF or aspirin (100 mg/kg), respectively, before 1 h of intraperitoneal injection of acetic acid (1%, 10 ml/kg). The number of writhing reflexes was counted during the following 15 min and the experiment was repeated twice.

In the hot plate test, a transparent glass cylinder with 23 cm high and 23 cm diameter was used to keep the mouse on the heated surface of the plate. The temperature of the hot plate was maintained at 55 ± 0.5 °C by using a thermo-adjustable water-circulating pump. The reaction time was noted by observing either the licking of the hind paws or the jumping movements to avoid thermal pain. After pre-treatment latencies were determined before one day of the experiment, only mice that showed initial nociceptive responses between 5 and 30 s were selected for the experiment. The post-treatment reaction time of each animal was recorded after 30, 60, 90 and 120 min of administration of DPE, PEF or ibuprofen (200 mg/kg), respectively.

2.7. Anti-inflammatory test

The anti-inflammatory activity of PEF was evaluated with both carrageenan-induced rat paw edema model (Winter et al., 1962) and dimethylbenzene-induced mouse inflammation model (Zheng et al., 2009).

In the rat paw edema test, male Wistar rats were used and acute inflammation was produced by subplantar injection of 0.1 ml of freshly prepared 1% (w/v) carrageenan in normal saline into the right hind paws of rats. Paw volume was measured plethysmometrically using a paw edema calcimeter (YLS-7A Shandong Academy of Medical Science device station, Shandong) at 0, 0.5, 1, 2, 3, 4 and 6 h after carrageenin injection. Animals were orally premedicated with DPE, PEF or indomethacin (5 mg/kg) before 0.5 h of injection. The mean increase in paw volume was measured and inhibitory percentage was calculated. The edema rate of rats was calculated as follows:

$$\text{Edema rate}\,(\%) = \frac{V_t - V_0}{V_0} \times 100$$

where V_0 is the volume before carrageenan injection (ml); V_t is the volume at t h after carrageenan injection (ml).