

ANTIINFLAMMATORY AND ANALGESIC ACTIVITY OF TOPICAL ADMINISTRATION OF *SIEGESBECKIA PUBESCENS*

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ABSTRACT

Several topical formulations containing methanolic extract of *Siegesbeckia pubescens* was investigated for antiinflammatory and analgesic activities in rat. The effects were studied using carrageenan-induced edema and formalin testing. Piroxicam gel and methyl salicylate ointment were studied as positive control for antiinflammatory and analgesic activity, respectively. The edema inhibition of the preparations containing extract at the doses of 1-5% w/w were significantly different from the control group. The antiinflammatory effect of *Siegesbeckia pubescens* 4-5% w/w was similar to the effect of piroxicam gel 3 h after carrageenan injection. The analgesic activity of topical preparation with more than 4% w/w was observed in the late phase. The topical analgesic activity of the extract was less than the analgesic activity of methyl salicylate ointment. The results of the present study further confirm the use of *Siegesbeckia pubescens* traditionally for the treatment of painful inflammatory conditions and can be useful for the treatment of local inflammation.

Keywords: *Siegesbeckia pubescens*; antiinflammatory activity; analgesic activity; topical administration.

INTRODUCTION

Siegesbeckia pubescens (SP) is an annual herb widely distributed in Central China and its aerial part has been used traditionally to treat arthritis, hypertension, malaria, neurasthenia, and snakebite (CPC, 2005). Pharmacological studies have shown its antibacteria, antiinflammatory and hypotensive activity after oral application and intraperitoneal administration (Qian *et al.*, 2000; Xu *et al.*, 2001; Park *et al.*, 2007). There are no available reports on the effect of topical application of SP. Therefore, the aim of the present study was to investigate antiinflammatory and analgesic activity of topical creams containing SP methanolic extract.

MATERIALS AND METHODS

Plant material

The aerial part of SP used in this study were collected from its natural habitat in Hubei province, China, in autumn 2006. The plant was identified and authenticated by Professor Changgong Zhang, School of Pharmacy, Huazhong University of Science and Technology, China. A voucher specimen (No. 06005-S) was preserved at the herbarium of School of Pharmacy, Huazhong University of Science and Technology.

Preparation of extract

The aerial parts of plant were dried in the shade and powdered so that all the material could be passed through a mesh not larger than 0.5 mm. Powdered plant material (500 g) was soaked in 1 L of methanol for 2 days, and filtered. The filtrate was evaporated to dryness under

reduced pressure and weighed; the yield of the extract was 6.43% w/w.

Preparation of topical formulation

Creams of the dried extract of SP were prepared using lipophilic cream base. Lipophilic cream base composed of cetostearyl alcohol: wool alcohols: white soft paraffin (0.5: 6: 93.5) (German Pharmacopoeia, DAB 1999) were prepared as outlined in the monographs.

The cream formulation contained (1, 2, 3, 4 and 5)% w/w dried extract of SP. This was achieved by levigating the dry extract with the cream base in a mortar and pestle until a smooth cream was obtained. The creams were then filled into 5 g aluminum collapsible tubes (Qingdao Shuangqing Tool Cart Co., Ltd., China) using laboratory-made cream-filler.

Formalin test

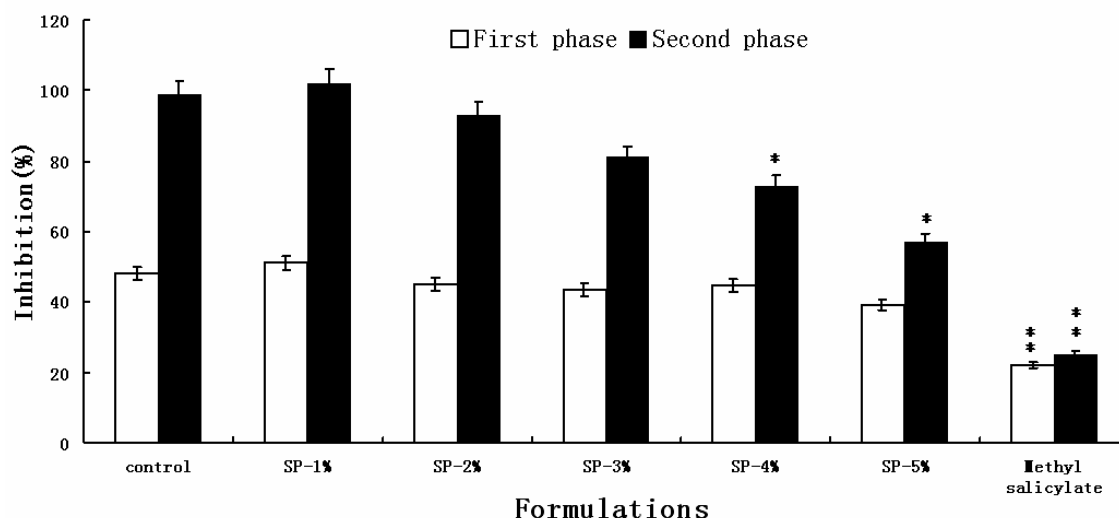
0.3 g of cream containing 1–5% of SP methanolic extract were applied to the dorsal surface of the left hind paw by gently rubbing 50 times with the index finger. Rats of the control groups received only the cream base. Methyl salicylate ointment 30% (The Mentholatum (Zhongshan) Pharmaceuticals Co, Ltd., Batch No. 06020266, China) applied in the same way was used as reference. Fifteen minutes later, the antinociceptive activity was determined using the formalin test described by Hunskaar and Hole (1987). Twenty microliter of 5% formalin was injected subcutaneously into the right hind paw of mice. The time (in seconds) spent in licking and biting responses of the injected paw was taken as an indicator of pain response. Responses were measured for 5 min after formalin injection (early phase) and 20-30 min after formalin injection (late phase).

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Table 1: Effect of topical administration of SP methanolic extract on carrageenan-induced paw edema in rats

Treatment ^a	N	Paw volume (mL) ^b at time after carrageenan injection				
		Initial	1 h	2 h	3 h	4 h
Base cream	7	1.04 ± 0.01	1.55 ± 0.03	1.68 ± 0.03	1.74 ± 0.04	1.70 ± 0.02
SP-1%	7	1.01 ± 0.03	1.33 ± 0.04* (37.25)	1.40 ± 0.02* (39.06)	1.45 ± 0.06* (37.14)	1.4 ± 0.01* (40.90)
SP-2%	7	1.06 ± 0.07	1.32 ± 0.04* (49.02)	1.43 ± 0.03* (42.18)	1.47 ± 0.06* (41.43)	1.41 ± 0.04* (46.97)
SP-3%	7	1.02 ± 0.02	1.24 ± 0.06** (56.86)	1.33 ± 0.03** (51.56)	1.40 ± 0.01* (45.71)	1.36 ± 0.05* (48.48)
SP-4%	7	1.05 ± 0.04	1.22 ± 0.05** (66.67)	1.28 ± 0.03** (64.06)	1.33 ± 0.02** (60.04)	1.28 ± 0.04** (65.15)
SP-5%	7	1.04 ± 0.03	1.15 ± 0.06** (78.43)	1.20 ± 0.04** (75.00)	1.21 ± 0.04** (75.71)	1.20 ± 0.03** (75.76)
Piroxicam gel 0.5%	7	1.03 ± 0.06	1.10 ± 0.01** (86.27)	1.13 ± 0.04** (84.38)	1.14 ± 0.03** (84.29)	1.12 ± 0.05** (86.36)

n: Number of animals

^a 0.3 g of preparation were applied to the plantar surface of the right hind paw by gently rubbing 50 times with the index finger.^b Values are mean ± S.E.M. (percent reduction). * p < 0.05, ** p < 0.01.**Fig. 1:** Antinociceptive effect of topical administration of SP methanolic extract on the first phase and the second phase of formalin-induced edema in rat. Values represent the mean ± S.E.M. *p < 0.05, **P < 0.01 vs. control value.

STATISTICAL ANALYSIS

ANOVA followed Student-Newman-Keuls test was used to determine significant differences between groups and P<0.05 was considered significant.

RESULTS AND DISCUSSION

The results of antiinflammatory activity after topical administration of SP methanolic extract are reported in Table 1. Statistical analysis showed that the edema inhibition of preparations containing extract are significantly different from control group at all the

concentrations tested. The results showed that the antiinflammatory effect of the formulation containing 5% of the SP methanolic extract was similar to the effect of gel containing 0.5% of piroxicam.

The effects of SP cream on formalin test have been shown in fig. 1. The groups which received cream containing 4 and 5% of extract produced significant (P < 0.05) inhibition in the late phase of formalin induced pain, respectively. The positive control methyl salicylate ointment produced significant (P < 0.01) inhibition in both phases.

The formalin test is a valid and reliable model of nociception and is sensitive for various classes of analgesic drugs. Formalin test produced a distinct biphasic response and different analgesics may act differently in the early and late phases of this test. Therefore, the test can be used to clarify the possible mechanism of antinociceptive effect of a proposed analgesic (Tjolsen *et al.*, 1992). Centrally acting drugs such as opioids inhibit both phases equally but peripherally acting drugs such as aspirin, indomethacin and dexamethasone only inhibit the late phase. The late phase seems to be an inflammatory response with inflammatory pain that can be inhibited by antiinflammatory drugs (Hunnskaar and Hole, 1987; Rosland *et al.*, 1990). The effect of SP on the late phase of formalin test suggests that its activity may be resulted from its peripheral action when compared with indomethacin activity in this respect. Based on the results of this study, we suggest that the antinociceptive effect of SP may be attributed to inhibition of prostaglandin release and other mediators involved in this test (Farsam *et al.*, 2000).

CONCLUSION

From these overall results, we can conclude that topical formulations containing at least 4-5% of SP methanolic extract possesses both antiinflammatory and analgesic effect which can be useful for the treatment of local inflammation. Phytochemical investigations of this plant showed the presence of organic acid and alphanol as well as diterpenoids (Xiong *et al.*, 1992; Xu *et al.*, 2001). There is a need for further studies in order to isolate the active ingredients in the plant that is responsible for its biological activities and to elucidate the mechanism of action of these active ingredients.

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