Indian Journal of Pharmacology 2000; 32: 21-24 **RESEARCH PAPER**

ASSESSMENT OF THE ANTI-INFLAMMATORY EFFECTS OF *SWERTIA CHIRATA* IN ACUTE AND CHRONIC EXPERIMENTAL MODELS IN MALE ALBINO RATS

SHIVAJI BANERJEE, TAPAS KUMAR SUR, SUVRA MANDAL*, PRABHASH CHANDRA DAS*, SRIDHAR SIKDAR

Department of Pharmacology, Dr. B.C. Roy PG Institute of Basic Medical Sciences, 244B, Acharya J.C. Bose Road, Calcutta-700 020.

*Chemical Research Unit, CCRAS, Regional Research Institute (Ayurveda), Block CN 4, Sector V, Salt Lake City, Calcutta-700 091.

Manuscript Received: 28.6.99 Revised: 6.8.99 Accepted: 1.11.99

SUMMARY

Objectives: To study the anti-inflammatory effect of xanthone derivative (1,5-dihydroxy-3,8-dimethoxy xanthone) of *Swertia chirata* (SC-I) in acute, sub-acute and chronic experimental models in male albino rats.

Methods: Aerial parts of *Swertia chirata* were extracted with organic solvent and purified by chromatographic procedure. SC-I was studied in carrageenin-induced hind paw oedema in rats and the paw volume was measured plethysmometrically at 0 and 3 h after injection. The compound was subjected to turpentine oil-induced granuloma pouch in rats. The pouch was opened on day 7 under anaesthesia and the exudate collected by a syringe was measured. The drug was also investigated in formalin-induced oedema models in rats. Degree of inflammation was measured plethysmometrically on day 1 and 7 and compared with control and standard drug, diclofenac. All the drugs were administered orally.

Results: The higher dose of SC-I significantly reduced carrageenin-induced pedal oedema (57%) and formalin-induced pedal oedema in rats (58%). SC-I also decreased exudate volume (35%) in turpentine oil-induced granuloma formation in comparison to control.

Conclusion: 1,5-dihydroxy-3,8-dimethoxy xanthone of *Swertia chirata* showed significant anti-inflammatory action in acute, sub-acute and chronic experimental models in rats.

KEY WORDS *Swertia chirata* granuloma pouch oedema inflammation diclofenac 1,5-dihydroxy-3,8-dimethoxy xanthone

INTRODUCTION

Swertia chirata Buch-Ham (Fam. *Gentianaceae*) is widely used in India to treat fever and malaria[1]. It is also used to treat liver diseases[1]. In addition it is reported to have anti-inflammatory activity[1-3]. Xanthone derivatives like mangostin, isomangostin and mangostin triacetate[3] are known to possess significant anti-inflammatory activities. The total xanthones of *Swertia* species produce significant CNS stimulant action[4-5]. Reports also suggest that, several varieties of xanthones show potent anti-platelet activity[6,7], anti-cancer[8], anti-fungal[9] and antimalarial effects[10].

In our laboratory it was observed that crude benzene extract of *Swertia chirata* exert anti-inflammatory action in acute model[2,3]. In the present study, we assessed the anti-inflammatory effects of one of the major xanthone derivatives, 1,5-dihydroxy-3,8-dimethoxy xanthone (SC-1) using *in vivo* pharmacological experimental models.

MATERIALS AND METHODS

Plant material and drug (SC-I) preparation: The aerial parts of fresh unadulterated *S. chirata* were collected from the local market, Calcutta and authenticated by Dr. S.R. Das, Survey Officer, Regional Research Institute (Ayurveda) Govt. of India, Calcutta. The air dried and powdered aerial parts of *S.chirata* were sucessively extracted with petrol in a soxhlet. The extract obtained was made free of any solvent by distillation. Then it was purified by silica

Correspondence: Shivaji Banerjee, e-mail: impuffer@cal2.vsnl.net.in

gel chromatography[11]. In the benzene eluents the xanthone SC-I (1,5-dihydroxy-3,8-dimethoxy xanthone, m.p. 185ºC, yellowish crystalline needles from petrol: benzene=9.1) was obtained. The structure of SC-I was confirmed by spectral analysis (UV, IR, NMR)[12]. The test drug SC-I was used as an emulsion in 5% suspension with gum acacia and administered orally at the dose of 25 and 50 mg/kg.

Experimental procedure: *In vivo* anti-inflammatory effect of 1,5-dihydroxy-3,8-dimethoxy xanthone (SC-I) isolated from *S. chirata* was assessed using adult male albino rats of Wistar strain (body weight 150 ± 5 g). The animals were grouped in cages in an air conditioned room at the temperature of 22 ± 1ºC with 12 h light and dark cycle. The animals were maintained with pellet diet and water *ad libitum*. They were further segregated into groups of 10 for different experimental schedule (acute, sub-acute and chronic).

Carrageenin-induced pedal oedema in rat: Animals were divided into four groups comprising ten animals in each group. In all groups, acute inflammation was produced by subplantar injection of 0.1 ml of freshly prepared 1% suspension of carrageenin in normal saline in the right hind paw of the rats and paw volume was measured plethysmometrically at 0 and 3 h after carrageenin injection[13]. Animals were premedicated either with vehicle (5% gum acacia), or SC-I (25 and 50 mg/kg) or diclofenac (10 mg/kg) orally[14] two hours before injection. Mean increase in paw volume was measured and percentage inhibition was calculated.

Turpentine oil-induced granuloma pouch in rat: Subcutaneous dorsal granuloma pouch was made in ether anaesthetized rats by injecting 2 ml of air, followed by injection of 0.5 ml of turpentine oil into it[15,16]. All drugs were administered orally one hour prior to turpentine oil injection and continued for seven consecutive days. On day 7, the pouch was opened under anaesthesia, the amount of exudate was taken out with a syringe, the volume was measured and compared with those of the control and standard group.

Formalin-induced oedema in rat hind paw: 0.1 ml of 2% formalin was injected into the subplantar area of right hind paw of ether anaesthetised rat[17]. All drugs were given orally one hour prior to formalin injection and continued for 7 consecutive days. Degree of inflammation was measured plethysmometrically on days 1 and 7.

Table 1. Effect of SC-I in carrageenin-induced rat hind paw oedema.

Treatment	Dose (mg/kg, *p.o.*)	Paw volume increase after 3 h (ml)	Percentage of inhibition
Control		0.63 ± 0.04	-
SC-I	25	0.42 ± 0.01[a]	21
SC-I	50	0.27 ± 0.06[a]	57
Diclofenac	10	0.23 ± 0.03[a]	63

Values are expressed as mean±SEM; number of animals used are 10 in each group; [a]P <0.001.

Statistical analysis: It was performed using Student's unpaired ' *t* ' test and P values less than 0.05 were considered significant. Data are represented as mean±SEM.

RESULTS

Carrageenin-induced pedal oedema: Anti-inflammatory effect of SC-I against carrageenin-induced inflammation is shown in Table 1. It significantly reduced the paw volume (P <0.001) as compared to the control rats. Diclofenac showed similar type of reduction (P <0.001).

Turpentine oil-induced granuloma pouch: SC-I also significantly reduced the exudate volume (P <0.001) in turpentine oil-induced granuloma pouch dose dependently (Table 2), which was comparable with the effect of diclofenac (P <0.001).

Formalin-induced oedema: Table 3 shows that SC-I was also effective in chronic inflammation. Formalin-induced pedal oedema was inhibited significantly by SC-I (P <0.05 and P <0.001) as compared to the control rats. Diclofenac also exerted inhibitory action on oedema formation.

DISCUSSION

Inhibition of carrageenin-induced inflammation in rats is one of the most suitable test procedures to screen anti-inflammatory agents. The development of carrageenin-induced oedema is bi-phasic, the first phase is attributed to the release of histamine, 5-HT and kinins, while, the second phase is related to the release of prostaglandins[18-20]. Xanthone fraction of *S. chirata* (SC-I) is highly effective in inhibiting

Table 2. Effect of SC-I in turpentine oil-induced granuloma pouch in rat.

Treatment	Dose (mg/kg, *p.o.*)	Volume of exudate (ml)	Percentage of inhibition
Control		2.26 ± 0.07	-
SC-I	25	1.90 ± 0.09[a]	16
SC-I	50	1.48 ± 0.12[b]	35
Diclofenac	10	0.96 ± 0.08[b]	58

Values are expressed as mean±SEM; number of animals used are 10 in each group; [a]P <0.01, [b]P <0.001.

Table 3. Effect of SC-I in formalin-induced rat hind paw oedema.

Treatment	Dose (mg/kg, *p.o.*)	Paw volume increase on day 7 (ml)	Percentage of inhibition
Control		1.13 ± 0.12	-
SC-I	25	0.82 ± 0.18[a]	27
SC-I	50	0.47 ± 0.14[b]	58
Diclofenac	10	0.46 ± 0.12[b]	59

Values are expressed as mean±SEM; number of animals used are 10 in each group; [a]P <0.05, [b]P <0.001.

carrageenin-induced oedema formation in rats. Xanthone derivatives of *Swertia* species possesses CNS-stimulant, anti-cancer and anti-microbial activity. It was also reported that xanthones inhibited IgE-mediated intestinal anaphylaxis reaction in rat[21].

Granuloma pouch technique was modified[16] using croton oil as irritant. An aseptic inflammation resulting in large volume of haemorrhage exudate is elicited which resembles the subacute type of inflammation. Instead of croton oil, turpentine oil or carrageenin can be used as an irritant. Therefore, turpentine oil-induced granuloma pouch offer a model for exudative type of inflammation. Though, the chemical mediators of this type of response is unknown, protein synthesis is necessary for the formation of granuloma[22]. SC-I of *S. chirata* has shown potential inhibitory action on exudate formation. Kinin is said to be the main mediator of granuloma, as it both vasodilate and increase vascular permeability in the early stages of inflammation. It may also be responsible for the vascular flushing that occurs in the carcinoid syndrome. Besides, kinin formation is also implicated in endotoxin shock, hereditary angioneurotic oedema, anaphylaxis, arthritis and acute pancreatitis[23]. Gene sequence identity between a portion of this kininogen molecule, a major acute phase protein and a proteinase inhibitor suggests that kininogen inhibit acid proteases in an area of inflammation and may prolong the actions of the kinins[24]. Keeping all these in view it may therefore be said that SC-I may possess anti-kinin like activity.

It is well known that inhibition of formalin-induced pedal oedema in rats is one of the most suitable test procedure to screen anti-arthritic and anti-inflammatory agents as it closely resembles human arthritis[25]. Injection of formalin subcutaneously into hind paw of rats produces localized inflammation and pain. The nociceptive effect of formalin is biphasic, an early neurogenic component followed by a later tissue mediated response[26]. Thus formalin-induced arthritis is a model used for the evaluation of an agent with probable anti-proliferative activity. This experiment is associated with the proliferative phase of inflammation. Results with 1,5-dihydroxy-3,8-dimethoxy xanthone of *S. chirata* indicated that SC-I is quite compatible with those of the standard drug diclofenac. Therefore, the drug appears to be effective against formalin-induced arthritis. These findings justifies the usefulness of SC-I in the treatment of inflammation associated diseases like arthritis.

It is concluded that 1,5-dihydroxy-3,8-dimethoxy xanthone (SC-I) of *S. chirata* possesses significant anti-inflammatory activity, which is comparable to diclofenac.

ACKNOWLEDGMENT

The authors are grateful to UGC, New Delhi for their financial assistance (Sanction No.: F.3-2/96/SR-II dated 6.12.1996).

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