ANTI-INFLAMMATORY ACTIVITY OF *Spermacoce articularis* Linn ON CARRAGEENAN INDUCED PAW EDEMA IN WISTAR MALE RATS

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Summary

Petroleum ether, chloroform, ethyl acetate, water, benzene extracts of *Spermacoce articularis* were investigated for anti-inflammatory activity in carrageenan induced paw edema in wistar male rats, and compared to a positive control drug, Celecoxib. These extracts were given (ip) in a concentration of 150 mg/kg b.w. before carrageenan injection. *S. articularis* in petroleum ether, chloroform, ethyl acetate, water, benzene extracts showed 27.14, 55.26, 53.93, 74.5 and 53.5% inhibition on carrageenan induced rat paw edema respectively at the end of three hours. All the extracts showed significant anti-inflammatory effect compared to the control. However, these extracts were found to be less effective than celecoxib. The present study indicates that *S. articularis* has significant antiinflammatory effect. The maximal edema inhibitory effect may be due to the presence of ursolic acid.

Key Words: *Spermacoce articularis,* Anti-inflammatory, Ursolic acid, Carrageenan, Celecoxib.

Introduction

Herbal medicines derived from plant extracts are being increasingly utilized to treat a wide variety of clinical diseases, though relatively little knowledge about their mode of action is available. There is a growing interest in the pharmacological evaluation of various plants used in Indian traditional systems of medicine. Thus, the present investigation was carried out to evaluate the anti-inflammatory potential of *Spermacoce articularis* Linn in experimental animal models.

S. articularis (Syn: Shispida, Borreria hispida, Borreria articularis), commonly known as Poaia, belongs to the family Rubiaceae. It is originally a native to the temperate and tropical Asia region. It is now cultivated in many parts of the world. The leaves are used in the treatment of conjunctivitis, earache, inflammation of eyes, intermittent fever, etc., by many tribes of India[2]. The plant is used in Sidha medicine[1].

On the basis of these common uses of this plant in traditional folk medicine and its above reported activities in the literature, we have evaluated the anti-inflammatory effect of various extracts of *S. articularis*.

Material and Methods

Collection of plant materials

Arial parts of *S. articularis* were collected from Tirunelveli of Tamilnadu state, India. The plant was identified and authenticated. A voucher specimen (No. 503/02) is maintained in the Herbarium, Department of Pharmaceutical Sciences, BIT, (Mesra, India). The plant parts were cleaned, dried under shade and powdered by a mechanical grinder. The pulverized plant was extracted successively with petroleum ether, chloroform, ethyl acetate and distilled water. It was also extracted separately with benzene [3]. The extracts of *S. articularis* were administered as a suspension in 2% PEG-400 to the animals. Phytochemical studies

Freshly prepared *S. articularis* extract was subjected to phytochemical screening tests for the detection of various constituents using conventional protocol[4], [5].

Animals

Albino rats of Wistar strain (120-200 g) of either sex were procured from the central animal house of the institute. They were housed in standard polypropylene cages and kept under controlled room temperature (24 ± 20C; relative humidity 60-70%) in a 12 h light-dark cycle. The rats were given a standard laboratory diet and water ad libitum. Food was withdrawn 12 h before and during the experimental hours. All experimental protocols were approved by the institutional animal ethics committee. Drugs

The following chemicals and drugs were used: carrageenan (Sigma-Aldrich), petroleum ether, chloroform, ethyle acetate, benzene, silica gel G and silica gel GF 254 (SD Fine Chemicals) and celecoxib (Dr. Reddy's Laboratories Ltd., India).

Antiinflammatory study

The animals were divided into groups as shown in Table 1. Acute inflammation was produced by subplantar injection of 0.1 ml of 1% suspension of carrageenan with 2% PEG – 400 in normal saline, in the right hind paw of the rats[6], one hour after oral administration of the drugs. The paw volume was measured plethysmometrically by the method of *Chattopadhyay, et al.,*[7] at '0' and '3' hours after the carrageenan injection. LD_{50} value of 750 mg/kg, *i.p.* was reported by *Dhar, et al*., [8]. The animals were treated with petroleum ether, chloroform, ethyl acetate, water and benzene extracts of *S. articularis* (150 mg/kg., *i.p.*). Saline (10 ml/kg., *i.p.*) treated animals served as control and celecoxib 10 mg/kg., *i.p.* suspended in 2% PEG – 400 was used as standard drug. Mean increase in paw volume was measured and the percentage of inhibition was calculated.

Statistical Analysis

Statistical analysis was done by unpaired Student's 't' test. A $p\ value < 0.01$ was considered significant.

Results

The results of the animal experiments are shown in Table 1. In this acute inflammation model, chloroform, ethyl acetate, water and benzene extracts of *S. articularis* (150 mg/kg., *i.p.*) and the standard drugs produced significant inhibition of paw edema as compared to the control. All the extracts were found to be less effective than celecoxib. However, the benzene extract of *S. articularis* (150 mg/kg., *i.p.*) showed more significant inhibition among these extracts. The results were found to be highly significant ($P<0.01$) in comparison to the control. Preliminary phytochemical analysis of the extracts of *S. articularis* revealed the presence of ursolic acid and triterpenes.

Table 1. Effect of various extracts of *S. articularis* and standard drug on carrageenan-induced rat paw edema.

Treatment Group	Dose (mg/kg., *i.p.*)	Increase in paw volume (mean ± SEM) in ml.	% inhibition of paw oedema
Control (N/Saline)	10 ml/kg	3.85 ± 0.216	-
Petroleum ether extract	150	2.75 ± 0.163	27.14
Chloroform extract	150	1.36 ± 0.124*	55.26
Ethyl acetate extract	150	1.42 ± 0.149*	53.93
Distilled water extract	150	1.42 ± 0.161*	53.3
Benzene extract	150	1.0 ± 0.127*	74.5
Celecoxib	100	0.82 ± 0.124*	81.26

Values are mean ± SEM.
n = 6 animals in each group.
* $p < 0.01$ when compared to control.

Discussion

Carrageenan-induced hind paw edema is the standard experimental model of acute inflammation. Carrageenan is the phlogistic agent of choice for testing antiinflammatory drugs as it is not known to be antigenic and is devoid of apparent systemic effects. Moreover, the experimental model exhibits a high degree of reproducibility.[6] Carrageenan-induced edema is a biphasic response. The first phase is mediated through the release of histamine, serotonin and kinins whereas the second phase is related to the release of prostaglandin and slow reacting substances which peak at 3 h.[9] The increase in the paw volume following carrageenan

administration in the control (3.85 ± 0.216 ml) and celecoxib treated group (0.82 ± 0.124 ml) is comparable to the findings of previous workers. The *S. articularis* extracts produced significant inhibition of carrageenan-induced paw edema. The inhibition was however, less than that of the standard drug, celecoxib.

The results of the present study suggest that the chloroform, ethyl acetate, water and benzene extracts of *S. articularis* in doses of 150 mg/kg., *i.p.* significantly suppressed carrageenan-induced paw edema in rats.

On preliminary phytochemical screening the extracts of *S. articularis* were found to contain ursolic acid, methyl ursolate, uvaol [3] and other triterpenes [5], which are probably responsible for cyclooxygenase enzyme inhibiting activity. Further studies may reveal the exact mechanisms of action responsible for the antiinflammatory activities of *S. articularis*.

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