ANTI-INFLAMMATORY AND ANALGESIC ACTIVITY OF LEAVES OF *SPILANTHES ACMELLA* (ELSA) IN EXPERIMENTAL ANIMAL MODELS

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Summary

The aim of the study was to evaluate the anti-inflammatory and analgesic activity of the ethanolic extract of leaves of *Spilanthes acmella* (ELSA) in experimental animal models. Carrageenan induced rat paw edema model, Granuloma pouch method and Adjuvant arthritis method were used for acute, sub-acute and chronic inflammation respectively. For central and peripheral analgesic activity tail flick method and Glacial Acetic Acid Induced Writhing Test were used. ELSA showed significant anti-inflammatory activity against acute, subacute and chronic inflammation as well as central and peripheral analgesic activity.

Keywords : *Spilanthes acmella,* flavonoids, anti-inflammatory,analgesic.

Introduction

Spilanthes acmella (Bengali- Akarkara, Assamese-Pirazha, Manipuri-Maanja-lei, Telegu- Maratitige) is an indigenous herb belonging to the family compositae. It is grown as an annual herb throughout the tropics [1]. It is found throughout India and up to 5,000 ft. in the Himalayas and other mountains [2]. The flowers and leaves of *Spilanthes acmella* (SA) have been used as folk medicine for stammering, toothache, stomatitis and throat complaints. In India roots, leaves and flowers heads of Akarkara are used as medicine. The Indian traditional healers use the flower heads of SA in dental and gum care. It is one of the major ingredients in popular herbal tooth powders and paste. In Srilanka, the flowers are chewed or used in the form of a tincture for toothache and to stimulate flow of saliva (sialogogue). SA have been popularly known as toothache plant [3]. The plant has been well documented for its use as spices, antiseptic, antibacterial, antifungal, antimalarial [4], immunostimulating and diuretic activity [3] and is used as remedy for flu cough, rabies disease and tuberculosis.

It also possessed excellent antimicrobial activities against red halophillic cocci from salt cured fish [4]. The crushed plant is used in rheumatism. The leaves of SA have been reported to contain flavonoid compounds [1]. Its flowers contain amino acids, alkaloids, N-isobutylamides (spilanthol, undeca-2E, 7Z, 9E-trienoic acid isobutylamide and undeca-2E-en-8, 10-diyonic acid isobutylamide); essential oil containing the major constituents as limonene (23.6%), β-caryophyllene (20.9%) (Z)-β-ocimine (14%), germacrene D (10.8%), myrcene (9.5%); a mixture of C22 to C35 normal hydrocarbons. A number of other N-isobutylamides such as 2E-N-(2-methylbutyl)-2-undecene-8, 10-diynamide; 2E, 7Z-N-isobutyl-2, 7-tridecadiene-10,12-diynamide and 7Z-N-isobutyl-7-tridecene-10,12-diyamide from SA have been reported. Air dried whole plant of SA contain Myricyl alcohol, α-and β-amyrins, β-sitosterol, stigmasterol and other compounds [3]. A very few study have been done on its anti-inflammatory and analgesic activity. Therefore, the present study have been undertaken to evaluate its anti-inflammatory and analgesic activity.

Material and methods

Preperation of the extract

Fresh leaves of SA were collected in the month of may-june from Assam Medical College and Hospital campus (AMCH), Dibrugarh and authenticated by Dr. M. Islam, Prof., department of life science, Dibrugarh University. The leaves were cleaned, air dried at room temperature, powdered by mechanical grinder and then ethanolic extract was prepared using 95% ethanol by percolation method [5] followed by steam evaporation. A net yield of 25gm was obtained by percolating 450gm of dry powder of the leaves.

Animals

Healthy albino rats of Wister strain (150-180gm) and Swiss albino mice (25-30gm) of either sex were used for the experiment which were obtained from the central animal house, AMCH, Dibrugarh. Before commencing the work permission from the Institutional Animal Ethical Committee (Regd. No.634/02/a/CPCSE) was obtained.

Acute oral toxicity

Albino rats of either sex were used for acute oral toxicity test according to the OECD guidelines 425 [6] and no mortality was recorded upto the maximum dose of 2000 mg/kg. Arbitrary dose of 500 mg/kg and 100 mg/kg were selected for the study.

Anti-inflammatory activity

For Anti-inflammatory activity against acute, sub-acute and chronic inflammation, healthy albino rats of either sex weighing 150–180 gm were divided into three groups with six animals in each group. Aspirin 100 mg/kg was taken as the standard drug [7].

Group-A (control) received 3% gum acacia 10 ml/kg p.o.
Group-B (Test) received ELSA 500 mg/kg p.o.
Group-C (standard) received Aspirin 100 mg/kg p.o.

(1) Acute Inflammation

The anti-inflammatory activity against acute inflammation was tested by carrageenan induced rat paw oedema method [8]. After overnight fasting acute inflammation was produced by sub-planter injection of 0.1 ml of freshly prepared 1 % carrageenan suspension in normal saline in the left hind paw [9] of rats in each group. The animals were treated with single dose of respective drug 1 hour before carrageenan injection. The paw volume was measured plethysmometrically [10], just before carrageenan injection i.e. at '0'hour and then at 3rd hour after carrageenan injection. Increase in paw edema was measured as the difference between the two readings and the percentage of inhibition of paw edema was calculated.

(2) Sub-Acute Inflammation

The anti-inflammatory activity against sub-acute inflammation was tested by Granuloma pouch method [11]. Rats were anaesthetized with ether and subcutaneous dorsal air pouches were prepared at the backs, by injecting 20 ml of air, after proper shaving and disinfection. Then 1 ml of 20 % carrageenan suspension in sesame oil was injected into each pouch. 48 hours later air was withdrawn from the pouch and 72 hours later any resulting adhesions were broken. The animals were treated with respective drugs for four days starting from the day of pouch formation. On the 5th day, the animals were sacrificed and exudates were measured. The average values of the exudates and the percentage inhibition was then calculated for all the groups.

(3) Chronic inflammation:

The anti-inflammatory activity against chronic inflammation was tested by Adjuvant arthritis method in albino rats [12]. On day 1, the animals were injected into the sub-plantar region of the left hind paw with 0.1 ml of Complete Freund's Adjuvant. Dosing with the test compound or the standard drug to the respective groups was started on the same day and continued for 12 days. Paw volume of both sides were recorded on the day of injection. On day 5, the volume of the injected paw was measured again, indicating the primary lesion. On day 21, non injected paw volume was determined again and the poly-arthritis severity was graded on a scale of 0-4: 0=no swelling; 1=isolated phalanx joint involvement; 2=involvement of phalanx joint and digits; 3=involvement of the entire region down to the ankle; 4=involvement of entire paw, including ankle. The maximum joint score was 12 including 3 secondary arthritis paws for each rat [13]. An 'Arthritic Index' was calculated as the sum of the scores as indicated above for each animal.

Central analgesic activity

The central analgesic activity was tested by tail flick method in albino rats [14] After overnight fasting healthy albino rats of either sex were divided into three groups with six animals in each group.The tail flick latencies (reaction time) of the animals were assessed by analgesiometer (Elite). Basal reaction time to radiant heat was taken by placing the tip (last 2 cm) of the tail on the radiant heat source. Tail withdrawal from the heat (flicking response) was taken as the end point. A cut of period of 10 seconds was observed to prevent damage to the

tail. The mean reaction time were recorded at pre-drug, 15 , 30 , 60 , 90 , 120 , 150 and 180 min after administration of vehicle or drugs. Pethidine was taken as the standard drug[8]. All the drugs were administered subcutaneously (s.c).

Peripheral analgesic activity

The peripheral analgesic activity was tested by Glacial Acetic Acid Induced Writhing Test in albino mice [15]. Healthy albino mice of either sex weighing 20–30 gms were fasted overnight. The animals were divided into three groups with six animals in each group. One hour after administration of the drugs, induction of writhing was done in mice by giving intraperitoneal injection of acetic acid (1%) at a dose of 10 ml/kg body weight. The number of writhing responses were counted and recorded for 20 minutes. Aspirin was taken as the standard drug[7].

Statistical analysis

Statistical analysis was done using one way ANOVA followed by Dunnet's test. Significance level of <0.05 was considered significant[16].

Results

In carrageenan induced rat paw edema model, ELSA (500 mg/kg, p.o) and aspirin showed significant anti-inflammatory activities against acute, sub-acute and chronic inflammation when compared to the control group ($p<0.01$) . However the anti-inflammatory activity of aspirin was more than ELSA (table 1).

ELSA produced significant analgesia both centrally and peripherally as compared to the control. ELSA (100 mg/kg, p.o) and aspirin produced significant ($P<0.01$) increase in the tail- flick reaction time as compared to the control. The increase in reaction time in the aspirin treated group was more than test group. Peak analgesic effect of ELSA was observed after 90 min of administration (table 2).

In the acetic acid induced writhing test, ELSA (100 mg/kg,p.o) and aspirin produced significant decrease ($p<0.05$ and $p<0.01$ respectively) in the number of wriths as compared to the control. The decrease by aspirin was more than ELSA (table3).

Discussion

In case of acute inflammation, the probable mechanism of action of carrageenan induced edema is bi-phasic, the first phase is attributed to the release of histamine, serotonin and kinins in the first hour; while, the second phase is related to the release of prostaglandin like substances in 2-3 hours [17] . Drugs that inhibit carrageenan induced paw edema, may act through inhibition of leukocyte migration and prostaglandin synthesis [18]. The suppression of the first phase may be due to inhibition of the release of early mediators, such as histamine and serotonin, and the action in the second phase may be explained by an inhibition of cyclo-oxygenase [19].

Table 1 : ANTI-INFLAMMATORY ACTIVITY OF LEAVES OF *SPILANTHES ACMELLA* ON CARRAGEENAN INDUCED RAT PAW EDEMA

Group	Drug, Dose (p.o)	Acute	Sub-acute	Chronic		
		Mean increase in paw vol (ml) (% inhibition in parentheses)	Mean vol of exudates on 5th day (ml) (% inhibition in parentheses)	Mean increase in paw vol (ml) (% inhibition in parentheses)		Arthritis Index
				5 th day	21 st day	21 st day
A (control)	3% gum acacia 10 ml/kg	0.51 ± 0.01	3.94 ± 0.03	1.12 ± 0.02	0.35 ± 0.03	10 ± 0.65
B (test)	ELSA 500 mg/kg	0.21[a] ± 0.01 (58.82)	2.6[a] ± 0.01 (40.72)	0.81[a] ± 0.01 (27.67)	0.09[a] ± 0.01 (74.28)	4.16[a] ± 0.13
C (standard)	Aspirin 100 mg/kg	0.17[a] ± 0.02 (66.67)	1.97[a] ± 0.02 (50.00)	0.72[a] ± 0.03 (35.75)	0.02[a] ± 0.01 (94.28)	2.83[a] ± 0.24
F		99.81	231	60.12	93.3	79.83
df		2, 15	2, 5	2, 15	2, 15	2, 15
p		<0.01	<0.01	<0.01	<0.01	<0.01

Values are expressed as Mean ± SEM; n=6 in each group ; [a]p<0.01 when compared to the control ; ANOVA followed by Dunnet's Multiple Comparision Test.

Table 2: CENTRAL ANALGESIC ACTIVITY OF LEAVES OF *SPILANTHES ACMELLA* ON THE TAIL FLICK RESPONSE IN ALBINO RATS

Group	Drug, Dose (s.c)	Pre- drug reaction time (sec)	15 min	30 min	60 min	90 min	120 min	150 min	180 min
A (control)	3% gum acacia 10 ml/kg	3.8 ± 0.18	3.5 ± 0.12	3.4 ± 0.10	3.6 ± 0.09	3.56 ± 0.09	3.55 ± 0.12	3.34 ± 0.12	3.32 ± 0.12
B (test)	ELSA 100 mg/kg	3.94 ± 0.21	4.22 ± 0.16[b]	4.45 ± 0.9[b]	4.83 ± 0.09[a]	6.53 ± 0.14[b]	5.83 ± 0.24[b]	5.35 ± 0.19[a]	4.73 ± 0.18[b]
C (standard)	Pethidine 5 mg/kg	3.80 ± 0.07	4.6 ± 0.17[b]	4.66 ± 0.17[b]	4.86 ± 0.24[a]	7.85 ± 0.47[b]	7.67 ± 0.39[b]	7.22 ± 0.38[b]	4.87 ± 0.38[a]
	F df p	2.57 2, 15 >0.05	24.2 2, 15 <0.01	33.59 2, 15 <0.01	16.08 2, 15 <0.01	48.36 2, 15 <0.01	41.72 2, 15 <0.01	40.6 2, 15 <0.01	12.08 2, 15 <0.01

Values are expressed as Mean ± SEM; n = 6 in each group; [a]p < 0.05, [b]p< 0.01 when compared with control; ANOVA followed by Dunnet's Multiple Comparision Test.

Table 3: PERIPHERAL ANALGESIC ACTIVITY OF LEAVES OF *SPILANTHES ACMELLA* ON GLACIAL ACETIC ACID INDUCED WRITHING RESPONSE IN ALBINO MICE

Group	Drug, Dose (p.o)	Number of writhing movements in20 min	% protection
A (control)	3% gum acacia 10 ml/kg	72.33 ± 5.23	–
B (test)	ELSA 100 mg/kg	25.83 ± 3.66[a]	62.95
C (standard)	Aspirin 100 mg/kg	10.67 ± 1.80[b]	85.25
	F df p	70.31 2, 15 <0.01	

Values are expressed as Mean ± SEM; n = 6 in each group; [a]p < 0.05; [b]p < 0.01 when compared with control; ANOVA followed by Dunnet's Multiple Comparision Test.

On preliminary phytochemical screening aqueous extract of *Spilanthes acmella* was found to contain flavonoid compounds. Flavonoids are known to target prostaglandins which are involved in the late phase of acute inflammation and pain perception [1]. Sub-acute and chronic inflammation involves infiltration of macrophages, neutrophils and proliferation of fibroblasts [20]. Flavonoids has got anti-proliferative activity which is found to cause a decrease in the weight and volume of contents of granuloma [21]. Tail- flick test to thermal stimulation and acetic acid induced writhing test are models of pain that mainly involve central and peripheral mechanisms respectively[22]. Acetic acid – induced writhing has been used as a model of chemonociceptive induced pain, which peripherally increase PG-E2 and PG-F2 [23]. Essential oils' constituents such as (-)-linalool antagonize different pain responses elicited by exposure to chemical stimulus such as acetic acid- induced, by a thermal stimulus or by a tissue injury produced by formalin injection [24]. In both the experimental pain models ELSA revealed the anti- nociceptive effect. Thus the antiinflammatory and analgesic activity of *Spilanthes acmella* may be due to the presence of flavonoid compounds and esential oils though it has to be confirmed.

Thus the present study revealed that *Spilanthes acmella* possesses significant anti-inflammatory and analgesic activities. However, further studies on other models and clinical trials are required to confirm these results and to establish the exact mechanism of action and the active principles involved in the anti-inflammatory and analgesic activity.

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References

(1) A. Chakraborty, R. K. B. Devi, S. Rita, Kh. Sharatchandra, Th. I. Singh. Preliminary studies on anti-inflammatory and analgesic activities of *Spilanthes acmella* in experimental animal models. Indian J Pharmacol 2004; 36 (3): 148-150.

(2) R. N. Chopra, S.L. Nayar, I.C. Chopra. Glossary of Indian Medicinal plants. 5th ed. New Delhi, National Institude of Science Communication (CSIR), 1999; 232.

(3) Sheela Chandra, H .P. Sharma, Ramesh Chandra, S. Jha. Medicinal Herbs-Spilanthes species: a review. Pharmbit 2007; XV (1): 17-22.

(4) Ang Boon Haw and Chan Lai Keng. Micropropagation of *Spilanthes acmella* L., a bio-insecticide plant, through proliferation of multiple shoots. J. Appl. Hort. 2003; 5(2): 65-68.

(5) Extracta Liquida-Liquid Extracts. Pharmaceutical Formulas. London, The Chemist and Druggist, 1950; 183.

(6) OECD (Organization for Economic Cooperation and Development). OECD Guidelines for Testing of Chemicals [Internet]. France: OECD Publishing; 2006 July 11. Section 4, Health Effects: Test No. 425: Acute Oral Toxicity: Up-and-Down Procedure; [Adopted 2006 March 23, cited 2008 September 27] ; p. 1—27. Available from: http://www.oecdbookshop.org/oecd/index. asp?lang=en.

(7) Ghosh MN. Toxicity studies. Fundamentals of experimental pharmacology. 3rd ed. Kolkata (India), S. K. Ghosh & Others, 2005; 190-197.
(8) Goyal R. K. Screening of drugs acting on central nervous system. Practicals in pharmacology. 6th ed. Ahmedabad (India), B.S.Shah prakashan, 2007; 121–137.

(9) Kulkarni S. K. Experiments on intact preparations (in vivo studies). Handbook of experimental pharmacology. 3rd ed. Delhi (India), Vallabh prakashan, 1999; 111–171

(10) Chattopadhyay R. N., Chattopadhyay R. R., Roy S. Maitra S. K. A simple method for plethysmometric measurement of paw volume of small laboratory animals in the evaluation of anti–inflammatory effect. Bull. Calcutta School Trop Med 1986; 34: 5–8.
(11) Agrawal S. S., Paridhavi M. Screening methods used for herbal drugs. Herbal Drug Technology. Hyderabad (India), Universities press, 2007; 583–587.
(12) Gerhard Vogel H. Antiarthrotic and immunomodulatory activity. Drug discovery and evaluation. 2nd ed. Germany, Springer publication, 2002; 802–805.
(13) Gu WZ, Sydney R. Brandwein. Inhibition of type II collagen–induced arthritis in rats by triptolide. Int J Immunopharmacol 1998; 20: 389–400.

(14) D'Armour FE, Smith DL. A method for determining loss of pain sensation. J Pharmacol Exp Ther 1941; 72: 74–79.

(15) Witkin LB, Hebner CF, Gaddi F, O'Keefe E, Spilaletta P, Plumer AJ. Pharmacology of 2–amino–indane hydrochloride (SU–8629). A potent non narcotic analgesic. J Pharmacol Exp Ther. 1961; 133: 400-08.
(16) Rao KV. Multiple comparison test procedures. In: Rao KV, Balakrishna (eds). Biostatistics. 1st ed. New Delhi, India, Jaypee Brothers Medical Publishers, 1999; 273-284.
(17) Brooks PM, Day RO. Non steroidal anti-inflammatory Drugs difference and similarities. J N Engl Med 1991; 324:1716-1725.

(18) Di Rosa M, Willoughby DA. Screens for anti-inflammatory drugs. J Pharm Pharmacol 1971; 23:297-298.

(19) Mani Ganesh, Mani Vasudevan, Kaliappan Kamalakannan, et al. Anti-inflammatory and Analgesic Effects of Pongamia glabra Leaf Gall Extract. Pharmacologyonline 2008; 1: 497-512.
(20) Grover JK. Experiments in Pharmacy and Pharmacology. New Delhi, CBS Publishers and Distributors, 1990; 176-180.
(21) Koganov MM, Dues OV, Tsorin BL. Activities of plant-derived phenols in a fibroblasts cell culture model. J Natural Products 1999; 62:481-483.
(22) P. Khosla, Bhanwra Sangeeta, J. Singh, R.K. Srivastava. Antinociceptive activity of Azadirachta indica (neem) in rats. Indian J Pharmacol 2000; 32: 372-374
(23) Jyothi.G, William M Carey, Ravi Kumar B, Krishna Mohan G. Antinociceptive and Antiinflammatory Activity of Methanolic Extract of Leaves of *Shorea Robusta.* Pharmacologyonline 2008; 1: 9-19.
(24) A. Vendruscolo, I. Takaki, L. E. Bersani-Amado, et al. Antiinflammatory and antinociceptive activities of Zingiber officinale Roscoe essential oil in experimental animal models. Indian J Pharmacol 2006; 38(1): 58-59.