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In vitro anti-inflammatory, analgesic and acute toxicity studies of ethanol extracts of *Andrographis paniculata* Nees and *Spilanthus acemella* Murr.

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ABSTRACT

The present study was carried out to evaluate the anti-inflammatory, analgesic and acute toxicity effects of Andrographis paniculata Nees and Spilanthus acemella Murr crude ethanolic extracts. The antiinflammatory activity was measured by carrageenan induced rat paw edema and analgesic activity was measured by acetic acid writhing test methods. 300mg/kg of S. acemella extract shown significant ($p<0.05$) antiinflammatory while 300mg/kg A. paniculata extract shown significant ($p<0.05$) analgesic activity as compared to diclofenac sodium. The ethanolic extracted has shown no toxic effect upto 1500 mg/kg body weight of Wister male rats. The results were obtained in a dose dependent manner. It may be concluded that the ethanolic extract of Andrographis paniculata Nees and Spilanthus acemella Murr has antiinflammatory and analgesic potential and have no toxic effect may be used as a future herbal medicine.

Key words: Antiinflammatory, analgesic, *Andrographis paniculata* Nees, *Spilanthus acemella* Murr, Wister albino rats.

INTRODUCTION

Pain is an unpleasant sensation no doubt, but on the whole it is usually beneficial to man (or animal). Pain is mainly a protective mechanism for the body. It occurs whenever any tissues are being damaged, and it causes the individual to react to remove the pain stimulus [Guyton and Hall, 2006].

Analgesics relieve pain as a symptom, without affecting its cause [Tripathi, 1999]. Currently available analgesic drugs such as opiates and NSAIDs are not useful in all cases due to their side effect profile. Opiate analgesic such as morphine has strong addictive potential and other side effects including respiratory depression, drowsiness, decreased gastrointentinal motility, nausea and several alterations of endocrine and autonomic nervous system while NSAIDs are well known for their ability to produce gastrointestinal bleeding, ulceration etc [Mate *et al*., 2008; Almeida *et al*., 2001]. Therefore, search for new analgesic drugs with promising pharmacological actions has become an urgent need. 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 antiinflammatory, analgesic and acute toxic effect of various extracts of *Andrographis paniculata* and *Spilanthus acemella*.

MATERIALS AND METHODS

Collection of plant material and extraction

Plant material collected from pot cultivated plants in Rajasthan University campus. Plant material was authenticated as *Andrographis paniculata* (RUBL 20873) and *Spilanthus acemella* (RUBL 20903) by Herbarium, Department of Botany, Rajasthan University, Jaipur, Rajasthan, India. Colleted plants were shade dried and grinded with pestle mortar. The resultant was then subjected for ethanol extraction on soxhlet apparatus. The extracts were then concentrated in vacuum under reduced pressure using rotary flash evaporator and dried in desiccators. The extracts were stored in refrigerator until used.

Experimental animals

Wistar albino rats weighing 175-225 g of either sex were obtained from Department of Zoology, University of Rajasthan, Jaipur, Rajasthan, India. They were acclimatized for 10 days under standard housing conditions (24° ±1°C; 45- 55% Relative Humidity with 12:12h light/dark cycle). The animals had free access to rat food (Hindustan Lever Ltd., Mumbai, India) and water. The animals were habituated to laboratory conditions for 48h prior to experimental protocol to minimize any nonspecific stress. The experimental protocol was approved by the Institutional Animal Ethics Committee of Government College of Pharmacy, Jaipur, (Rajasthan), India and animals were maintained under standard conditions in the animal house approved by Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA).

Analgesic activity

Acetic acid induced writhing method: The analgesic activity of the samples was evaluated using acetic acid induced writhing method in mice [Ahmed *et al*., 2004] Writhing was induced in rat (n = 6) by intraperitoneal injection (10ml/kg) of 0.6% acetic acid. The number of writhing was counted over a 20 min period as previously reported[7]. Animals in each group were treated through oral route 30 min before injection of acetic acid with extracts of selected plants at different concentrations (100, 200 and 300mg/kg body weight) and standard drug Diclofenec sodium (12.5mg/kg body weight). The control group received only 4% acacia gum solution (10ml/kg body weight).

Antiinflammatory activity

Carrageenan-induced paw edema model: Carrageenan-induced hind paw edema model [Winter *et al*., 1962; Adeyemi *et al.*, 2002] was used for determination of antiinflammatory activity. 120min after the oral administration of plant extracts (100, 200 and 300 mg/kg body weight) or dosing vehicle, each rat was injected with freshly prepared suspension of 0.15 ml/l0 g of 0.6% acetic acid in physiological saline (154 nM NaCl) into subplantar tissue of the right hind paw. The control group received 25µL saline solutions. The paw volume was measured after 180min by the mercury displacement method using a plethysmometer (Labco, India). The percentage inhibition of paw volume in treatment group was compared with the carrageenan control group. Diclofenac sodium (12.5mg/kg p.o.) was used as reference drug. The difference between the two readings was taken as the volume of edema, and the percentage anti-inflammatory activity was calculated using following equation:

Percentage anti-inflammatory activity = $(1 - Vi/V) \times 100$,

Where V is the paw volume 3h after the carrageenan injection and Vi is the initial paw volume.

Acute toxicity

The acute toxicity of the extract was determined by the method of Reed and Meunch [1938] on Wistar albino rats.

36 Wistar albino rats of either sex weighing 175-225 g were divided into 6 groups, each containing six animals. The rats were fasted for 18 hours, with water and libitum. The animals were administered with the suspension of 95% alcoholic extract in 1% Tween 80 solutions by oral route. Group 1,2,3,4 and 5 were administered orally with a dose of 50,100, 500, 1000 and 1500 mg/kg body weight respectively. Group 6 was given 1% Tween 80 in distilled water and kept as control. The number of animals dead in each group, after 72hours of administration of the drug was recorded and results were tabulated [Turner, 1965].

Statistical analysis

The results of statistical analysis for animal experiment were expressed as mean ± SEM and were evaluated by ANOVA followed by Dunnet's multiple comparisons. The results obtained were compared with the vehicle control group. The p<0.05, 0.001 were considered to be statistically significant.

Table 1 Anti-inflammatory activity of ethanol extracts of *Andrographis paniculata* Nees and *Spilanthus acemella* Murr. on Wister male rats

Treatment/dose (mg/kg)		Paw volume increase in ml			% inhibition of paw edema
		60min	120min	180min	
Carageenan%	10ml/kg	0.42±0.02	0.56±0.03	0.67±0.01	0.00
Diclofenec sodium	12.5mg/kg	0.27±0.02	0.22±0.01	0.19±0.02	71.64
A. paniculata	100mg/kg	0.64±0.04	0.59±0.02	0.58±0.03	13.43
	200mg/kg	0.46±0.03	0.42±0.04	0.38±0.02	43.28
	300mg/kg	0.35±0.01	0.33±0.01	0.24±0.04	64.17
S. acemella	100mg/kg	0.54±0.02	0.48±0.03	0.47±0.01	29.85
	200mg/kg	0.32±0.03	0.24±0.03	0.23±0.02	65.67
	300mg/kg	0.26±0.01	0.21±0.03	0.18±0.03	73.13

Data presented as mean ±SEM, n=5 for all groups. a, b and c are significantly different from control at P < 0.05, P < 0.01 and P < 0.001 respectively in a students' t –test.

Table 2 Analgesic activity of ethanol extracts of *Andrographis paniculata* Nees and *Spilanthus acemella* Murr. on Wister male rats

Group	Drug	Route of administration	Number of writhing movement (mean SEM) 20 minutes	Protection
Control	10ml/kg	Intra peritoneal	63.0±0.70	-
Diclofenec sodium	12.5mg/kg	Intra peritoneal	12.6±0.83	80.00
A. paniculata	100mg/kg	Intra peritoneal	43.3±0.45	31.26
	200mg/kg	Intra peritoneal	29.8±0.56	52.70
	300mg/kg	Intra peritoneal	20.5 ± 1.2	67.46
S. acemella	100mg/kg	Intra peritoneal	30.2± 1.2	52.02
	200mg/kg	Intra peritoneal	28.3± 1.2	55.08
	300mg/kg	Intra peritoneal	23.7± 1.2	62.38

Table 3 Acute toxicity study of aqueous extracts of *Andrographis paniculata* Nees and *Spilanthus acemella* Murr.

Group	Dose (mg/kg)	No. of animal	No. of survival	No. of death	Percentage of mortality	LC_{50}
Andrographis paniculata Nees.						
1	50	6	6	0	0	-
2	100	6	6	0	0	-
3	500	6	6	0	0	-
4	1000	6	6	0	0	-
5	1500	6	6	0	0	-
6	Control	6	6	0	0	-
Spilanthus acemella Murr.						
1	50	6	6	0	0	-
2	100	6	6	0	0	-
3	500	6	6	0	0	-
4	1000	6	6	0	0	-
5	1500	6	6	0	0	-
6	Control	6	6	0	0	-

RESULTS

Antiinflammatory activity of both the plant extracts was significant when compare to standard Diclofenec sodium (12.5mg/kg bw). Activity is totally dose dependant and increased with dose concentrations. Ethanolic extract of *S. acemella* at 300mg/kg bw had showed more inhibition of paw edema (73.13%) after 180 minutes of treatment than diclofenec sodium (71.64%) and 300mg/kg bw of *A. paniculata*. The results are shown in table 1.

Analgesic activity of ethanolic extracts was dose dependent and comparable to commercial drug. The maximum protection against writhing movement was observed in *A. paniculata* at 300mg/kg bw (67.46) than *S. acemella* (62.38) at same concentration. (Table 2)

The selected plant extracts were orally administered and has no toxic effect upto 1500mg/kg body weight of Wistar albino rats. No morality was observed after 72 hrs of administration of extracts. (Table 3)

DISCUSSION

Acetic acid induced writhing in mice attributed visceral pain finds much attention of screening analgesic drugs [Hasan *et al*., 2010]. The crude extracts of both the plants showed significant analgesic action compared to the reference drug diclofenac sodium but *A. paniculata* at 300mg/kg was found to exhibit higher analgesic activity (67.46 %) than 300mg/kg of *S. acemella* (62.38%). Pain sensation in acetic acid induced writhing method is elicited by triggering localized inflammatory response resulting release of free arachidonic acid from tissue phospholipid [Ahmed *et al*., 2006] via cyclooxygenase (COX). The increase in prostaglandin levels within the peritoneal cavity then enhances inflammatory pain by increasing capillary permeability [Zakaria *et al*., 2008]. The acetic acid induced writhing method was found effective to evaluate peripherally active analgesics. The plant extracts of *A. paniculata* and *S. acemella* exhibited both types of pain inhibition. The analgesic effect of the plants in both models suggests that they have been acting through central and peripheral mechanism [Sabina *et al*., 2009].

In conclusion, we can confirm that the ethanolic extracts of *A. paniculata* and *S. acemella* are endowed with both central and peripheral analgesic properties. It was found that the observed analgesia in *S. acemella* was demonstrated by the active constituents, spilanthol an N- isobutylamide [Wongsawatkul *et al.,* 2008] while in *A. paniculata* activities due to Andrographolide a diterpene [Amroyan *et al*., 1999; Shen *et al.,* 2002].

However, further study is needed in order to understand the precise mechanism. In future experiments, studies with purified fractions of the extract can be conducted for further pharmacological and toxicological characterization, such as the research of the mechanisms involved in the central and peripheral analgesic effect.

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