RESEARCH PAPER

Received 7 September 1994
Indian. J. Pharm. Sci., 1995, 57(3) pp. 121-125

Analgesic, Antipyretic and Antiulcerogenic Effects of Andrographolide

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Andrographolide from Andrographis panicultata (30, 100, and 300 mg/kg, oral) has been studied for its analgesic, antipyretic and antiulcerogenic activities. Andrographolide did not show any analgesic activity in hot plate test in mice while it showed significant (P< 0.05) analgesic activity in acetic acid-induced writhing in mice and Randall Selitto's test in rats at 300 mg/kg dose. Andrographolide, (100 and 300 mg/kg, oral) produced significant (P < 0.05) antipyretic effect after 3h of administration in Brewer's yeast-induced pyrexia in rats. Andrographolide also possessed significant (p < 0.05) anti-ulcerogenic activity at 100 and 300 mg/kg doses, aspirin induced ulceration in rats.

ANDROGRAPHIS PANICULATA is a medicinal herb and leaves and roots of this plant have been used in disorders of bowel and liver, colic, and undiagnosed fever and loss of appetite.[1] Further, this plant forms one of the chief constituents of household medicine "Alui" extensively used for general debility, dyspepsia and dysentery amongst adults and infants.[2] In addition to the hepato protective effect[3,4] of andrographolide (a diterpene lactone from **A. paniculata** leaves), diuretic[5] and antiinflammatory activities have also been observed previously in this laboratory.[6] The present communication deals with the analgesic and antipyretic activities of andrographolide. Antiulcertogenic effect of andrographolide has also been investigated since leaves of **A. paniculata** are used in dyspepsia in traditional system of medicine.[2]

MATERIALS AND METHODS

Isolation of antrographolide: Andrographolide was obtained from **A. paniculata** leaves with slight modification of the method reported in the literature.[7] One kg of powdered dried leaves of **A. paniculata** were extracted with chloroform in a Soxhlet extractor. Chloroform extract was concentrated and left overnight to obtain a crystalline mass. The mass was filtered, dried, crystallized and re-crystallized from ethanol to obtain white crystals (m.p. 220°C). The crystals were identified to be andrographolide (yield 4.7 g) by mixed melting point with authentic sample of andrographolide.

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Pharmacological Studies

Andrographolide was suspended in propylene glycol and given orally to experimental rats (120-165 g) and mice (15-20 g) in doses of 30, 100 and 300 mg/kg for different pharmacological experiments. Control group of animals received comparable volume of vehicle (propylene glycol in normal saline). One group of animals was given standard drug, orally. Six to ten animals were taken in each group unless otherwise indicated. Following pharmacological activities were carried out.

(i) Analgesic activity: The analgesic was testedby three different methods which are given below.

(a) Hot Plate test: Eddy's hot plate was maintained at 55 ± 0.5°C. Reaction time was noted by observing the licking of the hind paws[8] of male mice

(18-20 g). After taking the initial reaction time, each group of mice was administered test compound (30, 100 and 300 mg/kg) or pethidine (5 mg/kg, i.p.) The reaction time of each mouse was recorded just before and at 15, 30, 60, 90, 120, 180 and 360 min after drug administration. The increase in reaction time against control group was calculated.

(b) Acetic acid-induced writhing: Writhing response was induced in female mice (15-20 g) by i.p. injection of 3% acetic acid (300 mg/kg)[9] Test and reference drug (aspirin, 300 mg/kg) were administered orally 1h before acetic acid injection. The mice were placed signly in glass jars and number of writhing movements were counted for 20 min following acetic acid injection.

(c) Randall Selitto test: Albino rats of either sex (120-165 g) were subcutaneoulsy injected 0.1ml of 20% Brewer's yeast suspension in to plantar surface of right hind paw.[10] The drugs were administered 1h before yeast injection. The pain threshold was calculated by means of analgesiometer (UGO BASILE), 1 and 3h after yeast injection. The per cent change in pain threshold in comparison to control group was determined.

(ii) Antipyretic activity: Male albino rats (120-165 g) were given s.c., 20 ml/kg of 20% aqueous suspension of sterilized dry yeast powder. After 18h, drugs were administered orally to the animals showing an increase of 0.5°C or more in rectal temperature.[11] Rectal temperature was determined by digital telethermometer, immediately before and 18h after Brewer's yeast injection (Oh) to determine the pyretic response to yeast. Temperature taken prior to the drug administration (Oh) of febrile animals, served as pre-drug control. At 1,3 and 6h after oral dosing, rectal temperature were recorded to calculate percentage inhibition for each animal with its own yeast-induced fever as 100%.

(iii) Antiulcerogenic activity: Aspirin-induced ulceration method[12] was followed in male albino rats (5 groups of 6 animals, each). Rats were pre-treated orally with single dose of andrographolide (30, 100 and 300 mg/kg/day) and standard drug cimetidine (100 mg/kg/day) for 3 days. A single dose of aspirin (300 mg/kg, orally) was given daily half an hour after saline or drug treatment. For three days food was withheld for 2h after aspirin treatment. On the third day, 4h after aspirin administration, rats were sacrificed with anaesthetic ether, stomachs were removed and cut along greater curvature. The gastric juice was transferred to a test tube and centrifuged at 3200 g for 20 min. The volume of the supernatant was measured and total acid was estimated by titration method[13] in terms of mEq/ litre. After dissecting the stomachs, they were kept in diluted formation solution (2.5%) and checked a few minutes later for ulcerogenic activity.[14]

(iv) Statistical analysis: Dunnett's 't' test was employed for statistical analysis of data[15] and the level of significance was observed at $p < 0.05$.

RESULTS

(i) Analgesic activity

(a) Hot Plate test: The mean reaction time to thermal stimulus- induced pain ranged between 2.15 to 2.95 sec at different periods of observation in vehicle-treated control mice. Antrographolide (30, 100 and 300 mg/kg) did not significantly increase this reaction time up to an observation period of 360 min. The reference drug, pethidine hydrochloride (5 mg/kg) significantly ($p < 0.05$) increased the reaction time from 30 minutes (3.57 ± 0.36 sec) and persisted upto an observation period of 360 min (5.02 ± 0.32 sec).

(b) Acetic acid-induced writhing: In the control group of mice, acetic acid-induced writhing count was 68.0 ± 4.70 **(Table-1)**. Administration of andrographolide (300 mg/kg) and reference drug, aspirin (300 mg/kg) significantly ($p < 0.05$) inhibited the writhing counts to 48.33. ± 3.34 and 10.17 ± 4.15, respectively. The per cent reduction in writhing

Table-1: Effect of andrographolide on acetic acid-induced writhing in mice and paw pressure (Randal-Selitto test.) in rats

Drug	Dose (mg/kg)	Acetic acid-induced writhing No. of writhings/ 20 min/mouse	Pressure on paw in g	
			1h Pain threshold Mean ± S.E.	3h pain threshold Mean ± S.E.
Control	—	68.0 ± 4.70	41.92 ± 3.68	66.42 ± 6.33
Aspirin	300	10.17 ± 4.15*	157.58 ± 11.82*	204.25 ± 11.05*
Andro-grapholide	30	60.17 ± 4.45	43.17 ± 6.92	73.50 ± 9.53
	100	55.83 ± 4.17	54.67 ± 8.86	87.92 ± 9.07
	300	48.33 ± 3.34*	76.83 ± 11.56*	107.83 ± 9.40*

n=6 animals in each group; * $p<0.05$

movement was 28.93 by andrographolide (300 mg/kg) and 85.04 by aspirin (300 mg/kg).

(c) Randall Selitto test: Effect of single oral dose of andrographolide on yeast-induced pain threshold in rats is summarised in Table-1. Andrographolide at dose level of 30 and 100 mg/kg did not increase pain threshold at 1 and 3h as compared to respective control values (41.92 ± 3.68 g at 1h, 66.42 ± 6.33 g at 3h). However, andrographolide (300 mg/kg) as well as aspirin (300 mg/kg) significantly ($p < 0.05$) increased the pain threshold.

(ii) Antipyretic activity: The experimental rats showed a mean increase of about 1.45°C in rectal temperature 18h after yeast injection **(Table-2)**. Andrographolide at 30 mg/kg had not significant antipyretic effect. However, at doses of 100 and 300 mg/kg, the compound produced significant ($p < 0.05$) antipyretic effect 3h after its administration which persisted up to 6h of observation period. The antipyretic effect of andrographolide at the highest dose level tests (300 mg/kg, oral) was comparable to aspirin (300 mg/kg, oral).

(iii) Antiulcer activity: The effect of andrographolide and cimetidine on antiulcer activity is summarised in **Table-3**. Mean score of ulcer severity was significantly ($p < 0.05$) decreased by andrographolide (100 and 300 mg/kg) and cimetidine ($p < 0.05$) in comparison to control (3.57 ± 0.26). Effective concentration of andrographolide producing 50% inhibition of ulcer was 951.48 mg/kg with 95% confidence limits of 645.65 - 1412.54 mg/kg.

Andrographolide (100 and 300 mg/kg) and cimetidine (100 mg/kg) produced a significant ($p<0.05$) decrease in gastric juice and total acid content as compared to controls (Table-3). However, the degree of reduction in gastric juice or total acid content was significantly greater in cimetidine-treated rats when compared to andrographolide-treated rats.

DISCUSSION

Andrographolide was tested for both narcotic and non-narcotic analgesic activities. While the hot plate method was employed to assess the central effect of compound in producing analgesia, acetic

Table-2: Effect of andrographolide on Brewer's yeast-induced pyrexia in rats.

Drug	Dose (mg/kg)	Rectal Temperature in oC (mean + S.E.) Before drug (h) a -18	b 0	After drug (h) 1	3	6
Control	—	37.58 ± 0.07	39.0 ± 0.07 (+1.42)[c]	39.05 ± 0.08 (+0.05) [3.52]	39.07 ± 0.08 (+0.07) [4.93]	39.07 ± 0.08 (+0.07) [4.93]
Aspirin	300	37.68 ± 0.15	39.17 ± 0.17 (+1.49)[c]	38.53 ± 0.14* (-0.64) [42.95]	38.38 ± 0.14* (-0.79) [53.02]	38.27 ± 0.15* (-0.90) [60.40]
Andro-grapholide	30	37.53 ± 0.07	38.97 ± 0.07 (+1.44)[c]	38.85 ± 0.09 (-0.12) [8.33]	38.82 ± 0.09 (-0.15) [10.42]	38.83 ± 0.09 (-0.14) [9.72]
	100	37.57 ± 0.12	39.08 ± 0.16 (+1.51)[c]	38.92 ± 0.20 (+0.16) [10.59]	38.47 ± 0.20* (-0.61) [40.39]	38.33 ± 0.21* (-0.75) [49.67]
	300	37.77 ± 0.08	39.18 ± 0.13 (+1.41)[c]	38.65 ± 0.11 (-0.53) [37.59]	38.40 ± 0.13* (-0.78) [55.32]	38.35 ± 0.14* (-0.83) [58.87]

n= Six animals in each group; * P < 0.05
a: temperature just before yeast injection
b: temperature just before drug administration
c: change of temperature following yeast injection

Figures in parenthese idicate difference in temeperature at Oh and respective time interval
Figures in bracket indicate the per cent temperature reduction

acid writhing and Randall Selitto's methods were used to test the peripheral analgesic activity of andrographolide[16]. The results of the present study showed a weak peripheral analgesic activity of andrographolide as compared to aspirin.

It is well known that most of the non-steroidal anti-inflammatory drugs possess antipyretic activity. The results obtained in the present study reveal that 300 mg/kg of andrographolide was comparable to aspirin (300 mg/kg) in its antipyretic activity. Although, no direct experimental evidence for the ability of andrographolide to interfere with prostaglandin synthesis has been presented, the possibility of an inhibitory effect on prostaglandin syntthetase in a manner similar to that of aspirin can not be ruled out. This is supported indirectly by the well known fact that pyrogen either activates prostaglandin synthesis or facilitate substrate availability for the enzyme.[17] In any event, the synthesis of prostaglandin in hypothalamus is enhanced. Therefore, it appears that antipyretic activity of andrographolide may be related to the inhibition of prostglandin synthesis in hypothalamus. It possesses antiulcerogenic activity to an extent of 31.51% as against 85.43% observed

Table-3: Effect of andrographolide on ulcerogenic index, gastric juice secretion and total acid secretion in rat stomach

Drug	Dose (mg/kg)	Ulcerogenic index		Gastric juice	
		Mean Score of severity (Mean ± S.E.)	% Inhibition	Volume ml/100g (Mean ± S.E)	Total acid mEq/L (Mean ± S.E)
Control	—	3.57 ± 0.26	—	0.82 ± 0.06	0.76 ± 0.04
Cimetidine	100	0.52 ± 0.14*	85.43	0.25 ± 0.03*	0.20 ± 0.04*
Andro-grapholide	30	3.23 ± 0.17	9.52	0.70 ± 0.04	0.66 ± 0.03
	100	2.67 ± 0.21*	25.30	0.54 ± 0.04*	0.50 ± 0.04*
	300	2.29 ± 0.28*	36.51	0.37 ± 0.04*	0.33 ± 0.04*

n=six animals in each group; *p<0.05

with the standard drug, cimetidine. Antiulcerogenic activity, although limited, is further substantiated by the observation that andrographolide caused significant decrease in total acidity and gastric acid juice secretion.

The promising analgesic and antipyretic action of andrographolide coupled with antiulcerogenic action indicate that compounds merits further investigation.

ACKNOWLEDGEMENT

Authors wish to thank the Director for providing the necessary facilities. The help rendered by Dr. V. Raviprakash, Senior Scientist of this Division in the preparation of the manuscript is gratefully acknowledged.

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