Antiallergic activity of Andrographolide

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Andrographolide, a diterpene lactone, isolated from *Andrographis paniculata* significantly decreased degranulation of mast cells of rats and reduced the liberation of histamine from the cells when tested *in vitro* at concentration of 30,100 and 300 ug/ml. Moreover, andrographolide was found to produce an increase in delayed hypersensitivity in mice.

LEAVES and roots of *Andrographis paniculata* have been used for medicinal purposes in various conditions such as disorders of bowel and liver, colic, undignosed fever, loss of appetite and helminthic infestations. Further, this plant has been used in general debility, dysentery and dyspepsia[1]. The present study aims to examine the immunomodulatory effect of one of the isolated active principles, andrographolide, a diterpene lactone, claimed to possess anti-inflammatory and antipyretic[2], diabetic[3] and hepatoprotective effects[4].

Andrographolide was obtained from *Andrographis Paniculata* leaves[5]. Albino rats (100-180 g) and mice (25-30 g) of either sex procured from Laboratory Animal Resource Section of the Institute, were housed in groups of 5-10 animals in polypropylene cages.The animals were maintained on standard diet and acclimatized for one week and fasted overnight with free access to water prior to the experiments. Control animals were treated orally with vehicle (propylene glycol in saline) while experimental animals received either test material (andrographolide in propylene glycol) of reference drugs (levamisole, sodium cromoglycate) as suspension in propylene glycol. Following immunopharmacological tests have been employed.

Effect of andrographolide on mesenteric mast cells was studied in albino rats of either sex (six in each group)[6]. The rats were scarificed by cervical dislocation, the abdomen was opened and mesentery along with 2-3 cm of adjoining jejunal-ileal loop was taken out. Tissues were transferred to a petri dish containing oxygenated Ringer-Locke solutions, pH 7.4 and incubated for 10 min with andrographolide (30,100 and 300 µg/ml)sodium cromoglycate (15 µg/ml) or propylene glycol in saline. Tissues incubated in saline were used as normal controls. After 10 min the tissues were challenged with compound 48/80 (0.4 µg/ml) for another 10 min. After second incubation, the mesenteric strips were removed, mounted on a glass slide and dried overnight. Prior to staining, the mesenteric strips were separated from adjoining intestinal loops. The mesenteric mast cells were stained[7].

The effect on andrographolide on compound 48/80-induced histamine liberation from mast cells was assessed in male albino rats (130-180 g) The rats were killed by decapitaion. Saline buffer (8.0 ml) containing (mM) NaCl-(137), KCl- (2.7), $MgCl_2$-(1.00), Glucose- (5.6) and HEPES [2-(4-(2-hydroxyethyl)-1-piperazinyl)-ethane sulphonic acid] (10.00); pH 7.4 was injected into the peritoneal cavity. The body was gently massaged for 2 min and the peritoneal fluid was collected and centrifuged at 220 g. The pellet was washed twice with buffer. The cellular suspension (0.6 ml, final volume containing 40000-50000 mast cells) was preincubated for 25 min at 37° with andrographolide (30,100 and 300 µg/ml) or sodium cromoglycate (15 µg/ml). calcium (5×10^4 M) was added 1 min before addition of compound 48/80 (0.4 µg/ml) which was used to induce histamine liberation. The reaction was stopped after 10 min by

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Table 1: Effect of andirographolide on liberation of histamine from mesenteric mast cells of rats

Treatment	Dose (µg/ml)	Histamine ng/ml	
		Mean ± SE	% Inhibition
Control	—	808.34 ± 15.96	—
Sodium cromoglycate	15	133.34 ± 11.78**	83.42
Andrographolide	30	712.5 ± 7.98**	11.78
	100	629.16 ± 10.48**	22.12
	300	525.00 ± 10.76**	35.03

n = 4 animals in each group; **P < 0.01 Dunnett's 't' test

Table-2: Effect of andrographolide on cell-mediated immunity

Treatment	Dose (mg/kg)	Change in paw size	
		24 h Mean ± SE (mm)	% Increase
Control	—	0.77 ± 0.03	—
Levamisole	15	1.51 ± 0.05**	+ 96.10
Andrographolide	3	0.89 ± 0.04	+ 15.58
	10	0.95 ± 0.03	+ 23.38
	30	1.12 ± 0.04**	+ 45.45

n = 10 animals in each group; ** P< 0.01 Dunnett's 't' test

adding 1 ml of cold buffer. The tubes were kept at 4° and centrifuged. The supernatant was collected and histamine concentration was measured fluorometrically[8].

Delayed type of hypersensitivity (DTH) test was employed to study the immunostimulant activity. Groups of 10 mice received by oral route andrographolide (3,10 and 30 mg/kg per day), levamisole (50 mg/kg day) and propylene glycol in saline (control), respectively for a period of days starting from one day prior to first subcutaneous injection of sheep red blood cells (SRBC). Mice were immunized by injecting 20 µl of 5 x 10^9 SRBC/ml. s.c. into the right hind paw. Seven days later, the thickness of the left hind paw was measured using a screw gauge and mice were then challenged by injecting 20 µl of 5 x 10^9 SRBC/ml intradermally into the left hind paw (0 time). Foot thickness was measured again at 24 and 48 h after this challenge[9]. The difference between pre (0 time) and post challenge foot thickness, expressed in mm, was taken as a measure for DTH.

Compound 48/80, a histamine liberator from mast cells, induced 84.0% degranulation of mesenteric mast cells in vehicle-treated rats. Sodium cromoglycate a standard inhibitor of histamine release, significantly protected the mast cells from compound 48/80-induced degranulation which is evident from 33% of degranulation observed. Similarly, the degranulation in andrographolide-exposed mast cells

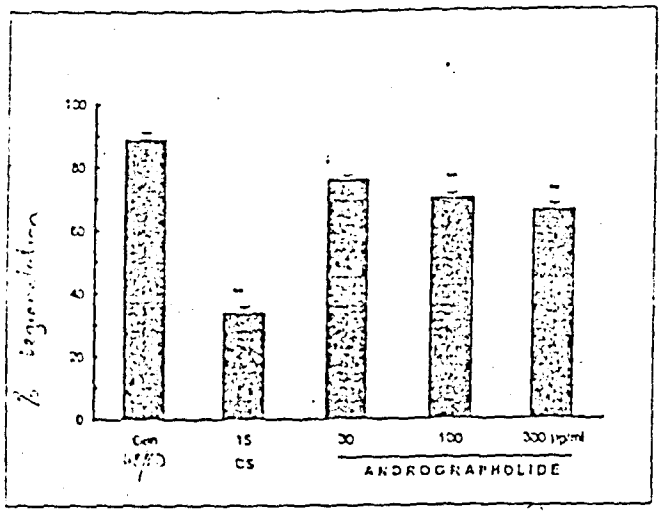

Fig. 1: Effect of andrographolide and sodium cromoglycate (CS) on mesenteric mast cell degranulation induced by compound 48/80. **P <0.01

was significantly less than controls and ranged between 65-76% (Fig-1).

Histamine release from vehicle-treated mast cells in response to compound 48/80 was 808.34 ± 15.96 ng/ml. Histamine release from sodium cromoglycate-treated mast cells was significantly inhibited (83.42%) as compared to controls. Andrographolide (10-300 µg ml), similarly inhibited the histamine release significantly, the extent of inhibition ranging between 12-37% (Table-1).

Andrographolide (30 mg/kg) and levamisole (15 mg/kg), significantly increased DTH reaction by 45.54% and 96.10%, respectively (Table -2). A similar immunostimulant effect has been reported from alcoholic extract of *A. paniculata* and andrographolide in mice[10].

The inhibition of compound 48/80-induced degranulation of mast cells and consequent inhibition of release of histamine by andrographolide is of significance. Sodium cromoglycate, a compound which is known to inhibit the release of histamine and other autacoids from sensitized mast cells and to inhibit antigen-induced bronchospasm is used in the treatment of bronchial asthma. Observations on andrographolide, identical to sodium cromoglycate, necessitate to assess the efficacy of andrographolide in asthmatic conditions. The membrane stabilizing action of andrographolide may be a contributory factor for inhibition of histamine release. In conclusion, the present study reveals that andrographolide significantly decreases degranulation of mast cells with consequent reduction in the liberation of histamine. In addition, the compound produces immunostimulant activity in DTH reaction in mice.

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