

***invitro* Antihistaminic and Anticholinergic activity of *Actiniopteris radiata* (Sw.) Link. on isolated rat ileum**

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Abstract: The ethanolic extract of whole plant of *Actiniopteris radiata* (Sw.) Link was tested to evaluate the spasmolytic effect on isolated rat ileum. The crude extract at a dose of 2, 4, 10 mg/ml dose dependently relaxed the rat ileum which was contracted by agonist like histamine and acetylcholine.

Keywords : *Actiniopteris radiata*, Asthma, contraction , Acetylcholine, histamine.

Introduction

Asthma is one of the common global health problem among the individual of all ages. About 5-10% population were suffered due to asthma globally and the prevalence is increasing day by day mostly in children. Today numbers of synthetic antihistaminic and anticholinergic drugs are available for symptomatic control of asthma along with their quit disturbing side effect. So the herb is best alternative in treatment along with safety and security.

The *Actiniopteris radiata* (Sw.) Link. belonging in to Actiniopteridaceae family is plant with grate medicinal value. According to the Ayurvedic text it used as styptic and anthelmintic^{1, 2}, treatment of bronchitis and gynecological disorders³, tuberculosis, astringent, anti-inflammatory, antipyretic, alleviates vitiated blood, cough, asthma and bronchitis^{4,5,6,7}, also increases increase fertility in woman and in spermatorrhoea⁸. Phytochemically the plant contains Quercetin-3-rutinoside (Rutin), hentriacontane, hentriacontrol, β -sitosterol palmitate, β -sitosterol-D(+)-glucoside, glucose and fructose alkaloids, glycosides, flavonoids, tannin, phenols^{9,10,11}.

Materials and methods

Plant material

Actiniopteris radiata (Sw.) Link whole plant was collected from hilly areas of Satpuda ranges, Chopda,

Dist. Jalgaon Maharashtra, India in July 2009 and taxonomically identified and authenticated at the BSI (Botanical Survey of India, Pune). A voucher specimen (No. ACTR1) deposited at BSI. The extraction was done using continuous soxhlet extraction using pet ether (60-80°C), ethyl acetate, ethanol (95%), methanol respectively. The bioactive ethanol extract (17.47%) shown presence of alkaloids, flavonoids, glycosides, sterols and triterpenoids.

Animal

Male wistar rats (average wt. 200–250 g) were housed in groups of five under standard laboratory conditions of temperature ($25 \pm 2^\circ\text{C}$) and 12/12 hr light/dark cycle. Animals had free access to standard pellet diet and water *ad libitum*. The Institutional Animals Ethics Committee approved the protocol vid no. NIB/IACE/09-10/83.

Experimental Procedure¹²

Rats were sacrificed and a segment from ileum (2 cm) was dissected from the terminal ileum and mounted in an organ bath containing Tyrode solution (10 ml) between two stainless steel hooks under 0.5 to 1 g initial tension. The lower hook was fixed at the bottom of the organ bath and upper one was connected to an isotonic transducer. The Tyrode solution composition (pH 7.4) was (concentration in gm/lit.) NaCl 8.0, KCl

0.2, CaCl₂ 0.2, MgCl₂ 0.1, NaHCO₃ 1.0, NaH₂PO₄ 0.05, and Glucose 1.0 gm/liter. It was continuously aerated and maintained at 37 ± 0.5°C The equilibrium period was 60 min and the bath solution was refreshed every 15 min. After equilibrium period, a dose response curve for histamine and acetylcholine in variant molar concentrations, by maintaining 45 min time cycle was taken separately in following groups,

- Group-I –Control (Histamine & Ach)
- Group-II- Vehicle (PEG-400, 5 % /ml)
- Group-III- Test extract (2mg/ ml)
- Group-IV- Test extract (4mg/ ml)

Group-V- Test extract (10mg/ ml)

Statistical Analysis

Ileum contractions induced by agonist were assumed as 100% and reductions induced by extract calculated. Percentage of ileum relaxation or contraction was expressed as mean±SEM. Results were analyzed using one and two way analysis of variance (ANOVA). The data were presented as mean±SEM. The statistical significance between the groups has been tested by ANOVA followed by Dunnett's test. A probability value less than 0.05 were considered as significant.

Table 1. Effect of *Actinopterus radiata* ethanol extract (AREE) on isolated rat ileum preparation (Histamine)

Sr. No	Log dose Histamine#	Percent of maximum response				
		Control Histamine	Vehicle PEG-400, 5% /ml	AREE (2 mg/ml)	AREE (4 mg/ml)	AREE (10 mg/ml)
1	1.0	7.15±0.7	8.10±0.69	7.10±1.410	5.20±0.523	6.00±0.256
2	0.69	14.16±0.947	13.45±0.859	10.12±1.862	9.48±0.527	9.90±0.547
3	0.39	26.66±2.12	25.39±2.687	18.60±0.746	17.90±1.876	16.84±0.587
4	0.09	47.42±2.58	45.21±2.987	36.80±1.832	32.43±2.680	30.54±0.458
5	0.20	92.80±3.76	88.24±3.874	68.21±3.872*	52.80±3.142*	46.52±1.24*

Histamine dose, 1ml, 2 ml, 4ml, 8ml, 1.6ml (0.1 µg/ml)

n=4, values are expressed in mean±SEM,

*p< 0.05 compared with histamine induced contraction (85 mm as 100 %)

Table 2. Effect of *Actinopterus radiata* ethanol extract (AREE) on isolated rat ileum preparation (Ach)

Sr. No	Log dose Ach#	Percent of maximum response				
		Control Ach	Vehicle PEG-400, 5% /ml	AREE (2 mg/ml)	AREE (4 mg/ml)	AREE (10 mg/ml)
1	1.0	10.25±0.984	9.83±0.982	7.89±0.789	6.87±0.825	6.10±0.498
2	0.69	18.64±1.453	16.54±1.482	14.01±1.035	13.58±1.698	12.58±1.954
3	0.39	30.48±2.040	29.51±2.457	29.57±1.589	26.56±1.458	24.80±2.478
4	0.09	54.32±2.710	49.57±2.410	42.54±3.012	41.20±2.369	38.90±1.256
5	0.20	94.31±2.140	90.24±2.991	64.22±1.865*	56.98±2.881*	42.40±1.254*

Ach dose, 1ml, 2 ml, 4ml, 8ml, 1.6ml (0.1 µg/ml)

n=4, values are expressed in mean±SEM

*p< 0.05 compared with Ach induced contraction (92 mm as 100 %)

Results

AREE exerted antagonistic effect on histamine and acetylcholine induced contraction ($P < 0.005$). Significance seen at dose of 2, 4, 10 mg/ml for histamine Table 1 (68.21 ± 3.872 , 52.80 ± 3.142 , 46.52 ± 1.24) similarly the ethanol extract at 2, 4, 10 mg/ml relaxes, tissue at (64.22 ± 1.865 , 56.98 ± 2.881 , 42.40 ± 1.254) which is precontracted by acetylcholine Table 2.

Discussion

Asthma is multifactorial disease, in which many cells and cellular elements play a role in particularly mast

cell and its mediators, eosinophils, T lymphocytes, macrophages, neutrophils and epithelial cells, also the different allergens, environmental conditions, drugs play an important role in asthma induction. Greater than 50 different mediators have been implicated in asthma, among these histamine and acetylcholine are the major targeting mediator in treatment of asthma. The bioactive crude extract (AREE) inhibiting the histamine and Ach induce contraction on isolated rat ileum preparation, so extract affirm the antihistaminic and anticholinergic effect in presence of agonist like histamine and acetylcholine which justify the H1 antagonistic and anticholinergic claim of plant.

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