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In vitro H_1-receptor antagonist activity of methanolic extract of tuber of *Stephania glabra*

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Abstract

In the present study, methanolic extract of tuber of *Stephania glabra* was evaluated for H_1-bloker activity by employing *in vitro* screening models of guinea pig ileum and goat tracheal chain preparation. Goat isolated trachea and guinea pig ileum contracted to histamine in a dose-dependent manner while chlorpheniramine blocked this effect. The methanolic extract produced significant dose-dependent H_1-receptor antagonist activity by blocking histamine-induced contraction.

Introduction

Herbal medicines are being increasingly utilized to treat a wide variety of diseases, though the knowledge about their mode of action is relatively scanty. So there is a growing interest regarding the pharmacological evaluation of various plants used in traditional system of medicine (Biswas et al., 2009). The genus Stephania belongs to family Menispermaceae, a large family of about 65 genera and 350 species, distributed in warmer parts of the world. Over 150 alkaloids together with flavonoids, lignans, steroids, terpenoids and coumarins have been identified in the genus, and many of these have been evaluated for biological activity. *Stephania glabra* Vern. is a large, climbing shrub, indigenous to lower Himalaya of India. The tubers of the plant used for the treatment of variety of disorders, including asthma, tuberculosis, dysentery and fever. It is used as psycomedicine by natives in India (James, 1992; CSIR, 1989). More than thirty alkaloids of different classes have been isolated from the plant (Semwal and Rawat, 2009). The present study is designed to investigate the H_1-blocker activity.

Materials and Methods

Plant material

The plant was collected from Dhamrol of Hamirpur district, Hondra Prodesh, India and was authenticated from the Regional Research Institute as voucher specimen No. 649 by Dr. Rajesh Dabur.

Preparation of extracts

The tuber was dried under shade, coarsely powdered and passed through 40 mesh sieves. The powdered material (500 g) was extracted with methanol using Soxhlet apparatus. The extract obtained was dried in rotary vacuum evaporator at 40°C, yielding a dark brown color viscous mass 25 g (5.0%).

Animals

Guinea pigs (300-400 g) of either sex were procured from National toxicological centre, Pune, India. The animals were housed for 2 weeks prior to the experiment for acclimatization in the animal house of Institute. Animals were maintained under controlled condi-

Table I

Effect of methanol extract of the tuber of *Stephania glabra* on histamine-induced contraction in isolated guinea pig ileum

Histamine concentration (µg/mL)	%Contraction of isolated guinea pig ileum		
	None	Methanol extract (100 µg/mL)	Chlorpheniramine (10 g/mL)
1	45.1 ± 1.2	20.2 ± 1.4[b]	18.3 ± 1.0[b]
2	65.4 ± 2.1	30.4 ± 1.7[b]	30.1 ± 1.7[b]
4	68.9 ± 2.3	42.8 ± 2.8[b]	34.3 ± 1.1[b]
8	84.4 ± 1.6	48.4 ± 2.4[b]	38.2 ± 1.3[b]
16	86.6 ± 1.7	59.2 ± 2.0[b]	46.1 ± 0.9[b]
32	100.0 ± 1.7	64.4 ± 2.2[b]	51.4 ± 0.9[b]

Statistical analysis done by using Student's t-test and ANOVA followed by Dunnet's test. [a]p<0.05, [b]p< 0.01, [c]p< 0.001 significantly different from control

tions of temperature 26 ± 2°C, relative humidity 44-56%, and photo schedule (12 hours light/dark). Animals were provided with standard diet (Amrut feeds, Mumbai, India) and water *ad libitum*. The food was withdrawn 18 hours, before the start of the experiment.

Effect of test drug on isolated guinea pig ileum preparation

Overnight fasted guinea pig was sacrificed and ileum was mounted in an organ bath containing Tyrode solution. The Tyrode solution was continuously aerated and maintained at 37 ± 0.5°C. The tissue was allowed to equilibrate for 30 min. under a load of 500 mg, contact time of 30 sec. and the response of Histamine was recorded by 5 min time cycle. After obtaining a dose response curve of histamine (10 µg/mL) on ileum, Methanolic extract of tuber of *S. glabra* (100 µg/mL) was added to the reservoir and same doses of histamine were repeated in presence of extract. Same procedure was repeated for standard drug (chlorpheniramine 10 µg/mL) as methanolic extract. Graph of percentage of maximum contractile response on ordinate and negative logarithm of molar concentration of histamine on abscissa was plotted to record dose response curve of histamine, in absence and presence of extract and standard drug (Kumar et al., 2010a).

Effect of test drug on isolated goat trachea chain preparation

Isolated adult goat tracheal tissue was obtained immediately after slaughter of the animals (Kumar et al., 2010b). Trachea was cut into individual rings and tied together in series to form a chain. Trachea was suspended in bath of Kreb's solution of the composition: NaCl 6.9, KCl 0.35, $CaCl_2$ 0.28, $MgSO_4$ 0.28, $NaHCO_3$ 2.1, KH_2PO_4 0.16 and glucose 2.0 g/L, which was continuously aerated and maintained at 37 ± 0.5°C. One end of the tracheal chain was attached to an S-shaped aerator tube and other attached to an isotonic frontal writing lever to smoked drum (magnification 10 -12-fold). Tissue was allowed to equilibrate for 45 min. Under a load of 400 mg (Nag and Lahiri, 1974). A dose response curve for histamine was taken by maintaining 15 min time cycle. After obtaining a dose response curve of histamine on trachea, the methanol extract/ chlorpheniramine were added to the respective reservoir and same doses of histamine were administered repeatedly. Graph of percentage of maximum contractile response on ordinate and negative logarithm of molar concentration of histamine on abscissa were plotted to record dose response curve of histamine, in absence and in presence methanol extract/chlorpheniramine.

Statistical analysis

The statistical analysis was performed by using Student's t-test, one-way analysis of variance (ANOVA) Followed by Dunnett's test for individual comparison of groups with control.

Results

Histamine showed dose-dependent contraction of smooth muscles. The methanol extract (100 µg/mL) inhibited histamine-induced contraction of isolated guinea pig ileum (Table I) and goat tracheal chain preparation (Table II) significantly ([a]p<0.05, [b]p<0.01).

Discussion

Histamine is a chemical mediator in the body, excessive release of which produces the variable effects on the airway smooth muscle of mammalian species. It has role in various diseases like asthma, bronchitis, cough, inflammation and allergic disorders. Histamine contracts the tracheo-bronchial muscle of guinea pig, goat, horse, dog and man. Goat tracheal chain is easier to handle and to prepare; it is also much more sensitive than guinea pig tracheal chain. In the present study of effect of test drug on the isolated goat tracheal chain preparation; there is right side shift of dose response curve of histamine in the presence of methyl extract and

Table II

Effect of methanol extract of *Stephania glabra* on histamine-induced contraction in isolated goat tracheal chain preparation

Histamine concentration (µg/mL)	%Contraction of isolated goat tracheal chain preparation		
	None	Methanol extract (100 µg/mL)	Chlorpheniramine (10 µg/mL)
1	20.9 ± 1.2	14.6 ± 0.8	15.3 ± 0.7
2	35.4 ± 1.3	19.9 ± 0.9[a]	22.3 ± 0.8[b]
4	45.0 ± 1.3	28.5 ± 1.9[a]	28.7 ± 1.1[b]
8	64.6 ± 1.7	35.1 ± 1.0[a]	38.0 ± 1.1[b]
16	74.1 ± 3.5	39.4 ± 1.6[a]	40.3 ± 2.1[b]
32	89.1 ± 2.5	48.7 ± 1.1[b]	45.5 ± 2.1[b]
64	100.0 ± 3.4	60.3 ± 1.2[c]	54.5 ± 2.4[b]

Statistical analysis done by using Student's t-test and ANOVA followed by Dunnet's test. [a]p<0.05, [b]p<0.01, [c]p<0.001 significantly different from control

chlorpheniramine indicating antihistamine action (Bhujbal et al., 2009). Guinea pig ileum is also used for screening of antihistaminic activity. The stimulation of H_1-receptors produces graded dose-related contraction of isolated guinea pig ileum. The extract (100 µg/mL) significantly inhibited the histamine induced contraction of isolated guinea pig ileum and goat tracheal chain preparations, indicating its H_1-receptor antagonistic activity. The antihistamine activity showed by the plant may be because of the chemical moieties (Saraf and Patwardhan, 1998; Bouic and Lamprecht, 1999). However this claim demands for further research and the studies are infect underway to isolate and characterize the active principles responsible for the activity.

Ethical Issue

Institutional Animal Ethics Committee approved the experimental protocol (198/99/ CPCSEA/17). The pharmacological work was carried out as per norms of CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals).

Conflict of Interest

Authors declare conflict no conflict of interest.

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