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EVALUATION OF ANTIHISTAMINIC ACTIVITY OF *SIDA RHOMBIFOLIA* LINN IN MANAGEMENT OF ALLERGIC ASTHMA

Anupama A. Suralkar*, Kuldeep K. Gaikwad, Asha S. Jadhav, Gayatri S. Vaidya

Center for Innovation in Pharmaceutical Education, Research and Development (CIPERD), Padm. Dr. D.Y. Patil, Institute of Pharmaceutical Sciences & Research, Pimpri, Pune-411018.

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For Correspondence:

Anupama A. Suralkar

Center for Innovation in Pharmaceutical Education, Research and Development (CIPERD), Padm. Dr. D.Y. Patil, Institute of Pharmaceutical Sciences & Research, Pimpri, Pune-411018

E-mail:

anupamaas@rediffmail.com

ABSTRACT

Traditionally, *Sida rhombifolia* Linn is use in treatment of allergic inflammatory diseases such as a bronchial asthma, bronchitis, wheezing, cough, short windedness, swelling, wounds, burns, itch, sores, eczema and skin diseases. Leaves are used in all kinds of inflammations. Leaves are reported to contain flavonoids which display several pharmacological properties in treating the allergic inflammations. Hence, the present study was undertaken to investigate the effect of ethanolic extract of *Sida rhombifolia* (SR) (Linn.) leaves on histamine induced smooth muscle contraction of isolated goat tracheal chain preparation and histamine induced bronchoconstriction in guinea pigs. SR showed significant decrease in contraction of isolated goat tracheal chain preparation ($p<0.01$) while in histamine induced bronchoconstriction in guinea pigs SR showed significant prolongation of preconvulsive dyspnea ($p<0.01$) and also showed dose dependent increase in percent protection of histamine induced bronchoconstriction in guinea pigs. These results suggest that SR may prove to be potential therapeutic drug for treating allergic inflammatory diseases such as allergic asthma and effect may be due to its antihistaminic activity and bronchodilating activity.

INTRODUCTION

Histamine is an important mediator of immediate allergic (type-1) and inflammatory reactions [1]. Asthma is a chronic inflammatory lung disease that is characterized by airway hyper-responsiveness to allergens, airway edema and increased mucus secretion [2]. Allergic asthma, which affects an estimated 100 million individuals worldwide is caused by chronic airway inflammation associated with IgE- synthesis and subsequent Th2 (T-helper type-2 cell)-responses. Asthma is characterized by airway inflammation and airway hyper responsiveness to the spasmogens such as histamine, acetylcholine and 5-hydroxytryptamine (5-HT).The pathophysiological hallmark of asthma is the infiltration of inflammatory cells, including eosinophils, neutrophils, lymphocytes and macrophages. These cells release various inflammatory mediators, including histamine and cytokines [3]. According to World Health Organization (WHO) statistics it is estimated that 300 million have asthma, markedly affecting the quality of life of these individuals and their families and negatively impacting the socio-economic welfare of society and 250,000 avoidable asthma deaths occur in the world each year [4].

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 [5]. The use of traditional medicine is expanding to newer horizons and plants still remain as the novel source of structurally important compounds that lead to the development of innovative drugs. Naturally occurring compounds from plants are still used in pharmaceutical preparations in pure or extracted forms [6].

Sida rhombifolia Linn is a short-lived perennial subshrub (woody stem and herbaceous branches) commonly growing to 60 cm, but sometimes reaching 1.5 m in height. The alternate leaves are variable in both shape and size and grows today in over 70 countries throughout the tropical, subtropical and warm temperate regions [7]. It is commonly known as Huang hua mu [China], Country mallow [English], Bala, Mahabala [India], Chikna, Sahadevi [Marathi], Bariara, Swetbarela [Hindi], Baladana [Gujarati] and Sittamutti [Madras] [8, 9].

Leaves contains chemical constituents such as ascorbic-acid, beta-carotene, beta-phenethylamine, calcium, carbohydrates, fat, fiber, gums, riboflavin, zinc, iron, niacin, thiamin and hipaphorine while phytochemical constituents such as flavonoids (& their glycosides), alkaloids (pseudoephedrine, vascin, vasicine.), phenolic compounds, saponins, steroids (& their glycosides), tannins, triterpenoids (& their glycosides) [8, 10]. Roots contain

alkaloids, choline, cobalt, copper, cryptolepine, and ephedrine and stems contain magnesium, mucilage [8]. Fruits contain tannins, phenolics, alkaloids, flavonoids while seeds contain ecdysterone [11, 12]. Plant is reported to possess activities such as antinociceptive and anti-inflammatory activity [10], *In-Vitro* antioxidant [13], analgesic activity [14], anti-inflammatory and hepatoprotective activity [15], Free radical scavanging activity [16], antiproliferative activity [17], antigout activity [18], antidirrhoeal activity [19], antiarthritic activity [20], nephroprotective activity [21], hypoglycemic and hypolipidemic activity [22] etc.

Flavonoids display several pharmacological properties in treating the allergic inflammations, acting as anti-inflammatory and antioxidant agents [23]. Saponins [24], steroids [3], tannins [25] and their related compounds are reported to possess anti-inflammatory activity.

Traditionally, SR commonly known as a "Mahabala" is use in treatment of allergic diseases such as a bronchial asthma, bronchitis, wheezing, cough, short windedness, swelling, wounds, burns, itch, sores, eczema, skin diseases, dermatosis, tonsilitis and respiratory inflammations [8]. Traditionally leaves are used in all kinds of inflammations and removes "Tridosha" [9].

Hence, taking into consideration the traditional claims, phytochemical constituents and reported activities, study was planned to evaluate the antihistaminic activity of *Sida rhombifolia* Linn Linn in management of allergic asthma.

MATERIAL AND METHODS

Experimental animals

Dunkin-Hartley Guinea pigs weighing 350-400 gm were housed in standard cages at room temperature 25 ± 2 °C and 50±5% relative humidity, under a light/dark cycle of 12/12 h, for 1 week before the experiments. Guinea pigs were provided with standard fresh vegetables and water *ad libitum*. Goat trachea was obtained from the slaughterhouse and kept in Kreb's solution. Laboratory animal handling and experimental procedures were performed in accordance with the guidelines of CPCSEA and experimental protocol was approved by Institutional Animal Ethics Committee. **(CPCSEA Approval No: 198/99).**

Selection, procurement of plant material and preparation of extract

The Fresh leaves of SR (family: malvaceae) were selected for study [15, 26]. Fresh leaves of SR were collected from local areas of Tiruvanantpuram, Kerala, India. The specimen was authenticated at Botanical Survey of India (BSI), Pune, where a sample specimen (Voucher number: KUGSID 1) has been deposited. The extractions of leaves of *Sida rhombifolia* were carried by soxhlation method [13]. In this method 1000 gm of leaf powder was extracted with

95% ethanol. It was then filtered and concentrated to obtain the ethanolic extract of *Sida rhombifolia* (SR). The % yield obtained from leaves was 25% w/w.

EXPERIMENTAL DESIGN

Acute oral toxicity study and selection of doses

Dose was selected by using acute oral toxicity study [28]. The toxicity study for ethanolic extract of leaves of was performed using rats. The animals were fasted overnight prior to the experiment and maintained under standard conditions. To find the LD50 of ethanolic extract of leaves of SR, six groups of rats, containing six in each group, were given SR in the doses of 500, 1000and 2000 mg/kg orally. The animals were observed for 5 min every 30 min till 2 h and then at 4, 8 and 24 h after treatment for any behavioral changes/mortality. They were further observed daily for 7 days for mortality. No mortality up to 7 days after treatment was observed with the ethanolic extract of leaves of SR and therefore was found safe up to dose of 2000 mg/kg. Accordingly 100,200 and 400 mg/kg p.o. doses were selected for guinea pigs.

STATISTICAL ANALYSIS

The results were expressed as mean ± SEM from 5 animals (n=5). Statistical analysis done by using Student's't'-test and one way ANOVA followed by Dunnett's test [29]. $p<0.05$*, $p<0.01$** were considered significant.

METHODS

Isolated goat tracheal chain preparation.

The goat tracheal tissue was obtained immediately after slaughter of animals. Pieces of trachea were collected in freshly prepared ice-cold oxygenated Kreb's solution (Composition (mM): NaCl, 115; KCl, 4.7; CaCl2, 2; NaHCO3, 25; KH2PO4, 1.2; MgCl2, 1.2; glucose, 11.5). Goat trachea was then cut into individual rings and tied together in series to form a chain. It was suspended in bath containing Kreb's solution and maintained at 37 ± 1ºC, a stream of air was bubbled through the organ tube (1 bubble/sec). One end of the tracheal muscle was attached to S-shaped aerator and the other attached to isotonic frontal writing lever to a drum. The tissue was allowed to equilibrate for 45 min under a load of 400 g. A dose response curve for histamine was recorded at variant molar concentrations by maintaining 15 min time cycle. After obtaining dose response curve of histamine (30ug/ml) on trachea, the SR (100 µg/ml) was added to reservoir and same doses of histamine were repeated. Graph of percentage of maximum contractile response on ordinate and negative log of molar concentration of histamine on abscissa was plotted to record dose response curve of histamine,in absence-presence of ER,standard drug Chlorpheniramine maleate(1µg/ml)[1,30].

Histamine induced bronchconstriction in guinea pigs.

Overnight fasted guinea pigs were randomly divided into five groups (n=5). Prior to drug treatment, each animal was placed in the histamine chamber and exposed to 0.2 % histamine aerosol. The Preconvulsion dyspnoea time (PCD) was noted for each animal. The Preconvulsion dyspnoea time is the time of aerosol exposure to the onset of dyspnoea leading to the appearance of convulsion. As soon as preconvulsive dyspnoea commenced, animals were removed from the chamber and placed in fresh air to recover from dyspnoea for 24 hours. This time for preconvulsive dyspnoea was recorded as basal value. After 24 hours, animals belonging to group I served as control and were administered with phosphate buffer (1ml/kg, p.o.); Animals belonging to group II were administered with Chlorpheniramine maleate (2 mg/kg, i.p.) while group III to V were received respective doses of SR. These animals were again subjected to histamine aerosol later at an interval of 1 hr, 4 hr and 24 hr and to determine Preconvulsion dyspnoea time (PCD). The protection offered by the treatment was calculated by using the following formula:

$$\% \text{ Protection} = (1 - T1/T2) \times 100$$

Where, T1 = the mean of PCT before administration of test drugs, T2 = the mean of PCT after administration of test drugs at 1hr, 4hr and 24 hr [31,32].

STATISTICAL ANALYSIS

The results have been indicated in terms of mean ± SEM, (n=5). Difference between the groups was statistically determined by One way ANOVA with Dunnett's test. The level of significance was set at $^{**}p<0.01$.

RESULTS

Isolated goat tracheal chain preparation.

Histamine produced dose dependant contraction in goat tracheal chain preparation at the concentration 30 µg/ml. This was significantly inhibited by modified PSS into which the preparation was incubated with ethanolic extract of leaves of SR at 100 µg/ml. This indicates that there was competitive antagonism between histamine and SR for H_1 receptors present on the smooth muscle.

Table 1: Effect of ethanolic extract of leaves of *Sida rhombifolia* on histamine induced contraction of smooth muscle using isolated goat tracheal chain preparation.

Dose of histamine (30µg/ml) (ml)	- Ve log molar concentration of histamine	% Maximum contraction	
		Control	SR 100
0.1	6.38	30.95 ±1.68	17.36±1.34**
0.2	5.91	36.66±1.21	18.42±1.86**
0.4	5.54	41.42±1.21	26.84±1.93**
0.8	5.21	52.38±1.68	37.89±1.34**
1.6	4.89	81.42±2.54	52.63±1.86**
3.2	4.59	94.76±1.75	65.65±0.54**

Group- I (Control) = D.R.C. of histamine (30 µg/ml) in absence of test drug; Group- II (SR 100) = D.R.C. of histamine (30 µg/ml) in presence ethanolic extract of SR (100µg/ml).

Histamine induced bronchoconstriction in guinea pigs.

The guinea pigs when exposed to 0.2 % w/v histamine aerosol showed signs of progressive dyspnoea leading to convulsions. Chlorpheniramine maleate (2 mg/kg, i.p) prolonged the preconvulsive dyspnea in 1st,4th hr and 24th hr compared to control. The ethanolic extract of leaves of SR at doses of 100,200,400 mg/kg p.o. prolonged the preconvulsive dyspnea at 1st, 4th hr and 24th hr as compared to control while SR at doses of 100,200,400 mg/kg p.o. shows the increase in percent protection but percent protection was found to be less than Chlorpheniramine maleate (2 mg/kg, i.p) except for dose of 400mg/kg p.o. at 24th hr.

Table 2: Effect of ethanolic extract of leaves of *Sida rhombifolia* on preconvulsive dyspnea and percent protection in histamine induced bronchoconstriction in guinea pigs.

Groups (n = 5)	Preconvulsive dyspnea (in Sec) (Mean ± SEM) at				Percent protection (%)		
	Before treatment	After treatment					
		1 hr	4hr	24hr	1 hr	4hr	24hr
I (Control)	26.2 ± 0.734	18.2 ± 0.583	15.2 ± 0.583	25.2 ± 0.860	---	----	---
II (Std)	25.6 ± 0.509	77 ± 0.707**	87.8 ± 0.583**	28.8± 0.969*	66.74	70.8	8.07
III (SR 100)	27.6 ± 0.871	34.6 ± 0.927**	63.8 ± 0.734**	27.8± 0.583	19.84	56.7	4.14
IV (SR 200)	27.2 ± 1.15	57.6 ± 0.748**	72.4 ± 0.927**	28.4± 0.818*	52.84	62.33	7.42
V (SR 400)	26.6 ± 0.748	67 ± 1.140**	78.8 ± 1.393**	29.8± 0.583**	60.28	66.27	10.68

Group- I (Control) = Aerosolized Histamine (0.2 % w/v); Group-II (Std) = Aerosolized Histamine (0.2 % w/v) + Chlorpheniramine maleate (2 mg/kg, i.p.); Group-III (SR 100) = Aerosolized Histamine (0.2 % w/v) + Ethanolic extract of leaves of SR (100mg/kg p.o.); Group- IV (SR 200) = Aerosolized Histamine (0.2 % w/v) + Ethanolic extract of leaves of SR (200mg/kg, p.o.); Group- V (SR 400) = Aerosolized Histamine (0.2 % w/v) + Ethanolic extract of leaves of SR (400mg/kg, p.o.)

DISCUSSION

Histamine is an important mediator of immediate allergic (type-1) and inflammatory reactions. It causes bronchoconstriction by activating H_1–receptors. The trachea is used for the experimental purpose rather than the bronchi since it is easier to dissect and has the same reactions to spasmogenic and spasmolytic drugs. The goat tracheal chain is easier to handle and to prepare; it is also much more sensitive than guinea pig tracheal chain. Although, the method is known for its suitability in the study of antispasmodic drugs in general, emphasis is given on its use in the testing of bronchodilators. This is because of the close anatomical and physiological association, which exists between tracheal and bronchial musculature. Therefore, the dose relative contractile responses of different agonists like ACh, Histamine, 5- Hydroxytryptamine and Bradykinin can be observed in isolated goat trachea. 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 [30].

Histamine produces highly variable effects on the airway smooth muscle of mammalian species.Histamine antagonism modulated by the relaxing factors involved and may be due to the suppression of histamine H_1-receptor [1].

SR has inhibitory effects on release of mediators of inflammation such as histamine, 5-hydroxytryptamine and bradykinin etc [15, 26].

In the present study, there was competitive antagonism between histamine and SR for H_1 receptors present on the smooth muscle. Histamine showed maximum contraction while SR significantly inhibited the histamine induced contraction of isolated goat tracheal chain preparation. This effect may be due to its antihistaminic, antispasmodic or bronchodilating activity and which further may contribute in the management of allergic asthma.

Histamine induced bronchoconstriction is the traditional immunological model of antigen induced airway obstruction. Histamine when inhaled causes hypoxia and leads to convulsion in Guinea pigs and causes very strong smooth muscle contraction, profound hypotension, and

capillary dilation in cardiovascular system. A prominent effect caused by histamine leads to severe bronchoconstriction in the guinea pigs that causes asphyxia and death. Bronchodilators can delay the occurrence of these symptoms [32].

The role of histamine in asthma is well established. The close resemblance of pulmonary responses to histamine challenge in both guinea pigs and humans, as well as the anaphylactic sensitization made this species the model of choice. In the present study, guinea pigs were used because of the extreme sensitivity of their airways to the primary mediators of bronchoconstriction, including histamine and leukotrienes, and their ability to be sensitized to foreign proteins. Although there are various model of asthma, guinea pig airways react to histamine, acetylcholine, leukotrienes, and other bronchoconstrictors in a manner similar to that seen in humans. Another similarity between the guinea pig model and asthmatic patients is that enhanced bronchoconstriction occurs in both species following sensitization, in response to β- adrenergic antagonists. Thus, the guinea pig model resembles the human allergic pathology in several aspects, especially in terms of mediator release. Histamine antagonists can be conveniently recognized and assayed by their ability to protect guinea pigs against lethal effects of histamine-induced bronchospasm [34].

Histamine when inhaled has been shown to induce bronchoconstriction by direct H_1 receptor activation and also by a neutrally mediated bronchoconstrictor effect via vagal reflexes. Histamine has shown to activate action potentials in the intrapulmonary vagal afferents [35]. The ability to afford protection against histamine induced bronchospasm in guinea pigs shows antihistamine like action. Histamine is a central mediator in the pathogenesis of allergic and inflammatory disorders [29].

In the present study, histamine showed less prolongation in the latent period of convulsions while SR extract also prolonged the latent period of convulsions. But prolongation in the latent period of convulsions was found to be less as compared to Chlorpheniramine maleate. This effect may be due to its antihistaminic, antispasmodic or bronchodilating activity and which further may contribute in the management of allergic asthma.

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