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Antiasthmatic Activity of *Hemidesmus Indicus* Roots

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ABSTRACT

The present investigation was aimed to investigate the possible anti-asthmatic activity of methanolic extract of roots of *Hemidesmus indicus(MEHI). Hemidesmus indicus* extract is evaluated for its anti-asthmatic activity in Guinea pig ileum, tracheal chain and rat ileum preparation, compound 48/80 induced mast cell degranulation. Extract exhibited a significant (P<0.05, P<0.01) anti-asthmatic activity with the doses of 100, 200 and 300 mg/kg b.w. in rats and significant (P<0.05, P<0.01) inhibition in histamine and acetylcholine induced contraction of smooth muscle preparations. From results of this study we concluded that methanolic extract of *Hemidesmus indicus* roots has potential anti-asthmatic activity in various animal models and suggestive potential in prophylaxis and management of asthma.

Keywords: *Hemidesmus indicus*, Methanolic extract, Asthma.

I. INTRODUCTION

Bronchial asthma is a chronic respiratory disorder affecting a large proportion of population throughout the world. The currently used drugs for the treatment of this dreadful disease in modern medicine are far from satisfactory as they provide only symptomatic relief, produce several adverse effects and may lose effectiveness on continued use. Hence numbers of drugs from indigenous plant sources have been explored for their anti-asthmatic and bronchodilator activities during the last three decades. *Hemidesmus indicus* belonging to family Asclepiadaceae, known as *Indian Sarsaparilla* or *Anantmul* used for medicinal purpose in different parts of the world. The plant is distributed throughout India and many parts of the world in plains and low hills (Anup Austin 2008). The root is sweet bitter, cooling, aphrodisiac, antipyretic and cures leprosy, leucoderma, asthma, bronchitis and general debility(Nadkarni 1954). Traditionally it is used as blood purifier, diuretic antirheumatic and antidote for snake bite (Satyavati 1987). Roots are reported to have antimicrobial (Sivarajan VV et al, 2008) and anti-inflammatory(Datta MK et al 1982, and Alam MI 1998) properties. Studies with methanolic extract of bark of *H. indicus* have shown protection against rifampicin and isoniazide induced hepatic damage (Prabakan M et al, 2000). Root bark has been reported to possess antioxidant activity (Ravishankara MN et al 2002).

II. MATERIALS AND METHOD

2.1.Plant material

The roots of *Hemidesmus indicus* were collected from Nagpur region Maharashtra during July 2016 and authenticated by Botanist, Dr. Bhuskute, at College of Science Amgaon, India and voucher specimen was deposited to the same (voucher KNCP2016/7).

2.2 Preparation of extract

The shade dried roots *Hemidesmus indicus*. Powdered (40 size mesh) and around 500 gm of powder was subjected to extraction (soxhlet) with petroleum ether to defatt the powder. Each time before extracting with next solvent the powdered material was dried at room temperature, and the defatted powder was macerated with methanol. After the effective extraction, solvent were concentrated under rotary vacuum evaporator and extract was then weighed (yield 5.83% w/w). The obtained extracts were subjected to phyochemical investigation and pharmacological screening for its anti-asthmatic activity.

2.4 Drugs and Chemicals

All chemicals used in the present study were analytical grade and purchased from Merck specialties pvt. Ltd. Mumbai, India. Compound 48/80 and Egg albumin purchased from Sigma (USA) and Glaxo Laboratories, Mumbai, respectively. Dexamethasone purchased from Cadila Healthcare Ltd., India. Sodium cromoglycate purchased from Cipla Ltd Goa. India. ß-sitosterol was purchased from Sigma Chemicals USA.

2.5 Animals

Male wistar albino rats (180-200 g), Swiss albino mice (20-25 g) and Guinea pigs (350-400g) were obtained from the lacsmi biofarms animal Centre, Pune and kept in standard environmental conditions. They were fed with standard pellet diet and water *ad libitum*. Experiments were carried out in accordance with CPCSEA guidelines and the study was approved by Institutional animal ethical committee (KNCP/c/17/08/CPCSEA).

2.6 Acute toxicity study

Acute oral toxicity study was carried out as per guidelines set by Organization for Economic Co-operation and Development (OECD) revised draft guidelines 425 received from CPCSEA. In each steps six animals were used, fasted overnight and administered (p.o) with single dose of drug and starting dose 300 mg/kg b.w and then 500, 1000, 1500, 2000, 2500, 3000, 3500, 4000 and 5000 mg/kg b.w. respectively. After administration of the test compounds, animals were observed individually and continuously for 30 min, 2 hr and 24 hr to detect changes in the autonomic or behavioral responses and also for tremors, convulsion, salivation, diarrhea, lethargy, sleep and coma and then monitored for any mortality for the following 14 days (Ecobichon, 1997) .

2.7. Anti-asthmatic activity

2.7.1 Isolated tissue experiments:

2.7.1.1 Guinea pig ileum and tracheal chain preparation

Guinea pigs were fasted for 24 hr and later were sacrificed and pieces of ileum as well as trachea were isolated. The tissues were quickly transferred to petridishes containing Tyrode solution and Kreb's solution respectively. The tracheal chain was prepared and mounted in an organ bath maintained at 37^0C and containing Kreb's solution according to the method described by (Sheth *et al*. 1972).

2.7.1.2 Rat ileum preparation

Albino rats were fasted overnight. The next day the animals were sacrificed and a small piece of ileum was isolated and mounted in an organ bath containing Tyrode solution maintained at 37^0C. A basal tension of 500 mg was applied and the tissue was stabilized for 30 min. The tissue was then exposed to graded doses of acetylcholine and contractions were recorded as described by (Sheth *et al*. 1972).

2.7.2 Compound 48/80 induced rat mesenteric mast cell degranulation

Nortan *et al*, 1954 and Shah *et al*, 2003)

2.7.3 Homologous Passive cutaneous anaphylaxis in rats

The homologous antiserum was prepared according to the method described by (Gupta *et al*, 1989).

2.7.5 Statistical analysis:

The results were expressed as mean ± S.E.M, and statistical significance between treated and control groups was analyzed using of One way analysis of variance (ANOVA), followed by Dunnett's t-test and tested for significance using paired Student's t-test where $P<0.05$ was considered statistically significant.

III. RESULTS

3.1 Phytochemical screening:

Phytochemical screening of the plant extract revealed the presence of flavonoids, tannins, saponins, steroids. carbohydrates, and glycosides.

3.2 Acute toxicity study:

Single dose (250, 500, 2000 and 5000 mg kg^{-1}) of *H indicus* alcoholic extract (MEHI)administered to albino mice showed no death up to 14 days study period. Hydro alcoholic extract of *H indicus (MEHI)* was found to be safe upto 5000 mg/kg p.o. given to mice. No any sign for behavioural as well as any physical changes were found.

3.3 Anti-asthmatic activity

3.3.1 Isolated tissue experiments:

3.3.1.1 Guinea pig ileum preparation

Histamine (10µg/ml) produced dose dependent contraction of guinea pig ileum. Pretreatment with hydro alcoholic extract of *Leptadenia reticulata* (0.8 mg/ml) significantly inhibited ($p<0.01$) the contractile effect of histamine.(Table1.)

3.3.1.2 Guinea pig tracheal chain preparation

Histamine (10µg/ml) produced dose dependent contraction of guinea pig ileum. Pretreatment with extract of *H. indicus* (1.2 mg/ml) significantly inhibited ($p<0.01$) the contractile effect of histamine(Table 2).

3.3.1.3 Rat ileum preparation

Acetylcholine (10µg/ml) produced dose dependent contraction of rat ileum. Pretreatment with hydro alcoholic extract of *H. indicus* (1 mg/ml) significantly inhibited ($p<0.01$) the contractile effect acetylcholine (Table 3).

3.3.2 Compound 48/80 induced rat mesenteric mast cell degranulation

Compound 48/80 produced significant disruption of mast cells which was significantly inhibited in a dose dependant manner by pretreatment with the *Hemidesmus indicus* in concentrations of 300µg/ml, 500µg/ml and700µg/ml resulted in significant reduction ($p<0.01$) in degranulation of mast cells when challenged with compound 48/80. The protection was comparable to the standard drug Disodium cromoglycate (10µg/ml) (Table 4)

IV. DISCUSSION

The development of bronchial asthma is related to immediate hypersensitivity reaction. With the ever growing interest interest in natural medicine many herbal plants has been reported to be used in treatment of patients with asthma (Jawla et al 2010) due to their less adverse reaction compared with synthetic drugs. The present study was undertaken for the evaluation of antiasthmatic activity of hydro alcoholic extract of *Hemidesmus indicus*. It seems to be promising plant for the treatment of bronchial asthma because of its reported hepatoprotective (Amitkumar *et al,* 2011) and anticancer (Sathiyanarayan *et al* 2007) activity. The result of the

present study reveals significant protection against mast cell degranulation, anti anaphylactic and antispasmodic activity on various invivo and invitro experimental models.

Mast cell disruption is mediated by activation of IgE antibodies. Stabilization of mast cell membrane could be one of the possible mechanism of LRLHE responsible for their effectiveness, probably by raising the cyclic Amp levels due to inhibition of the phosphodiesterase enzyme (Geeta et al.1981). On isolated tissue like guinea pig ileum and tracheal chain preparation substantiate the H1 antihistaminic and antimuscarinic activity of LRLHE. Histamine is the most implicated mediator in bronchoconstriction that accompany asthma (Rang and Dale, 1987). The result of this study indicates a similar rightward shift in dose response curve of histamine in presence of extract indicating antihistaminic activity (Shamsa. Ahmadiani et al.1999). The antiasthmatic activity of *Hemidesmus indicus*. can be attributed to its antihistaminic (H_1-antagonist), antiallergic, mast cell stabilizing, anti-inflammatory activity suggestive of its potential in prophylaxis and management of asthma.

V. CONCLUSION

From the results of this study we concluded that *Hemidesmus indicus* has potential antiasthmatic activity in animal models and supports its traditional claim. The antiasthmatic activity is probably may be due to presence of flavonoids, saponins, tannins, further detail study needs to be conducted to isolate and characterize the chemical compounds in the plant which are responsible for biological activity.

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Tables :

Table 1. Effect of MEHI (1 mg/ml) on histamine induced contraction of guinea pig ileum preparation.

		Maximum contraction (%)	
Histamine (10 µg/ml) Dose (ml)	-ve log molar conc. of Histamine	Control	MEHI (0.8 mg/ml)
0.1	5.92	29.79 ± 1.580	23.03± 1.869**
0.2	5.70	44.68 ± 4.240	33.37± 2.295**
0.4	5.38	59.89 ± 3.968	44.11± 3.898**
0.8	5.14	77.84 ± 2.143	64.30± 1.675**
1.6	4.84	88.35 ± 1.638	73.11 ± 1.067**
3.2	4.54	100 ± 2.450	79.17 ±4.870*

n=6, Values are in Mean ±S.E.M. Statistical analysis done by using Student's t-test, *p<0.05, **p<0.01 significantly different from control.

Table 2 Effect of MEHI (1.2 mg/ml) on histamine induced contraction of guinea pig tracheal chain preparation.

		Maximum contraction (%)	
Histamine (10 µg/ml) Dose (ml)	-ve log molar conc. of Histamine	Control	LRLHE (1.2 mg/ml)
0.1	6.04	43.68 ± 2.711	29.38 ± 2.544**
0.2	5.74	49.85 ± 2.358	33.63 ± 2.432**
0.4	5.44	64.19 ± 3.156	44.52 ± 3.3115**
0.8	5.14	82.00 ± 3.507	54.89 ± 3.480**
1.6	4.84	100 ± 2.320	75.48 ± 2.810**

n=6, Values are in Mean ±S.E.M. Statistical analysis done by using Student's t-test, *p<0.05, **p<0.01 significantly different from control.

Table 3. Effect of MEHI (1 mg/ml) on acetylcholine induced contraction of rat ileum preparation.

		Maximum contraction (%)	
Acetylcholine (10 µg/ml) Dose (ml)	-ve log molar Conc. of Acetylcholine	Control	LRLHE (1 mg/ml)
0.1	6.15	38.14 ± 2.226	23.49 ± 1.631*
0.2	5.85	43.92 ± 1.914	28.73± 1.291**
0.4	5.55	55.98 ± 1.996	33.97± 1.727**
0.8	5.25	76.74 ± 1.730	44.77± 2.629**
1.6	4.95	100 ± 2.420	72.43 ±1.861**

n=6, Values are in Mean ±S.E.M. Statistical analysis done by using Student's t-test, *p<0.05, **p<0.01 significantly different from control.

Table 4. Effect of MEHI on Compound 48/80 induced rat mesentric mast cell degranulation

Groups	**Mast cells %**		**Percent Protection**
Dose (µg /ml)	**Intact**	**Disrupted**	
Control	27.20 ±1.02	72.80 ±1.02	-
Standard	71.40 ± 1.36**	28.60 ± 1.36**	60.71
MEHI 300	52.60 ± 1.03**	47.40 ± 1.03**	34.89
MEHI 500	56.40 ± 1.12**	43.60 ±1.12 **	40.38
MEHI 700	64.60 ± 1.80**	35.40 ± 1.80**	51.37

n=6, values are expressed in Mean ±S.E.M Statistical analysis done by ANOVA followed by Dunnett's test *p<0.05,**p<0.01 compared to control group