

**ANTIASTHMATIC ACTIVITY OF ROOTS OF  
*Hemidesmus indicus* R. Br.**

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**Summary**

Over the past decade, herbal and ayurvedic drugs have become a subject of world importance, with both medicinal and economical implications. A regular and widespread use of herbs throughout the world has increased serious concerns over their quality, safety and efficacy. Thus, a proper scientific evidence or assessment has become the criteria for acceptance of herbal health claims. *Hemidesmus indicus* R.Br. (Asclepiadaceae), commonly known as Anantamul, is widely used in the traditional system of medicine as an antiasthmatic activity. We examined the effect of alcohol extract of roots of *Hemidesmus indicus* at 25, 50,100mg/kg doses orally in the isolated goat tracheal chain preparation, passive paw anaphylaxis in rat, clonidine-induced catalepsy in mice. The extract showed significant dose-dependent antiasthmatic activity in all these models.

**Keywords:** Antiasthmatic activity, *Hemidesmus indicus* , Asclepidaceae.

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## Introduction

*Hemidesmus indicus* R. Br. (Asclepiadaceae) is a twining shrub commonly found in India. The main parts used in traditional medicine to be developed are the roots and root bark. The roots of the plant are woody and have a sweet taste and used in the inflammation, respiratory disorder, skin diseases, fever, asthma, eye diseases, kidney and urinary disorder, rheumatism, diarrhoea and bronchitis<sup>1</sup>. It has also been used in combination with other drug for snake bite<sup>2</sup>

Anantmul is reported to contain pregnane glycosides, coumarino-lignoid, terpenoids and sterols<sup>3-5</sup>. It also contains tannins, fatty acids, saponins, resin acid<sup>6</sup>. Anantmul is reported to have anti-inflammatory and antipyretic activities, antidiarrhoeal effect, antinociceptive activity, antioxidant and antithrombotic activity, antitumor activity and hepatoprotective activity<sup>7-10</sup>.

Even though *Hemidesmus indicus* was reported to be useful in a many ailments, scientific evaluation of the plant was not reported for its antiasthmatic activity. Hence, in the present study, the antiasthmatic activity of extract of roots of *Hemidesmus indicus* was studied using different in vivo and in vitro animal models.

## Materials and Methods

### Plant Material and Extraction

Dried roots of *Hemidesmus indicus* were purchased from local markets of Pune district and were authenticated at Botanical Survey of India, Pune by Dr. P.G. Diwaker (Scientist D). The roots were dried under shade, powdered and passed through 40 mesh sieve. The powdered material (500 g) was extracted with ethanol using Soxhlet apparatus. The extract obtained was dried in rotary vacuum evaporator at 40<sup>0</sup> C., yielding a dark brown coloured viscous mass 30 g (6.0% ).

### Animals

Isolated adult goat tracheal tissue, Albino mice and Albino rates (Wistar Strain) of either sex weighing 20-25 g and 150-200 g respectively were used for studies. Isolated adult goat trachea tissue was obtained immediately after slaughter of the animal. Pieces of the trachea were collected in the ice cold oxygenated Krebs solution. The albino mice and albino rats were obtained from animal house of National Chemical Laboratory, Pune. They were housed in polypropylene cages with standard pellet chow and water ad libitum. In all experimental sets, 5 rats and 5 mice were used for each treatment.

### Antiasthmatic Activity

#### 1) Isolated goat trachea chain preparation

Isolated adult goat tracheal tissue was obtained immediately after slaughterhouse of the animals. Trachea was cut into individual rings and tied together in series to form a chain. Trachea was suspended in bath of Krebs solution and was continuously aerator at 37 ± 0.5°C. DRC of histamine in plane Krebs solution and in 80 µg/ml *Hemidesmus* extract in Krebs solution was

taken. Graph of percentage of maximum contractile response on ordinate and concentration of histamine on abscissa was plotted to record dose response curve of histamine, in absence and in presence of drug extract<sup>11-12</sup>.

## 2) Passive paw anaphylaxis in rats

Rats (Wistar) were given (s.c.) three doses of 100 µg of egg albumin adsorbed on 12 mg of aluminum hydroxide gel prepared in 0.5 ml of saline on 1<sup>st</sup>, 3<sup>rd</sup>, 5<sup>th</sup> day. On 10<sup>th</sup> day of sensitization blood was collected from the retro orbital plex and collected blood was allowed to clot and the serum was separated by centrifugation at 1500rpm. Animals were divided into five groups (n = 5). Animals belonging to group I served as control and were administered only the vehicle (10ml/kg p.o.). Animals belonging to groups II, III, IV received three doses (25, 50, 100mg/kg p.o.) respectively of *Hemidesmus* extract. Animals of group V, as positive control group received Dexamethasone (0.27mg/kg p.o.). The animals were passively sensitized with 0.1ml of the undiluted serum into the left hind paw of animals. The contra lateral paw received an equal volume of saline. Drug treatment was given 24 hr after sensitization. Animals were challenged in the left hind paw with 10µg of egg albumin in 0.1ml of saline, and the paw inflammation was measured using a Plethysmometer. The difference in the reading prior to, and after antigen challenge represented the edema volume and the percent inhibition of volume was calculated by using the following formula.

$$\text{Percent Inhibition} = 1 - (V_t / V_c) \times 100$$

$V_t$  = Mean relative change in paw volume in test group

$V_c$  = Mean relative change in paw volume in control group.

Prior drug treatment animals were sensitized with serum. Next 24 hours, after drug treatment animals again challenged for 10 µg egg albumin and edema inhibition was calculated.<sup>13-14</sup>

## 3) Clonidine induced catalepsy in mice

Albino mice were divided into five groups (n = 5). Control group received saline (10ml/kg) and other groups received single dose of extract (25, 50, 100 mg/kg p.o. Body weight) respectively. Chlorpheniramine maleate (10 mg/kg, i.p.) was used as standard. All the groups were received clonidine (1 mg/kg s.c.) one hour after the drug administration and the duration of catalepsy was measured at 15, 30, 60, 90, 120, 150 and 180 min<sup>15-16</sup>.

## Statistical Analysis

The statistical analysis was performed by using one-way analysis-of-variance (ANOVA) Followed by Dunnett's test for individual comparison of groups with control.

## Results

## 1) Isolated goat trachea chain preparation

It was observed that *Hemidesmus* extract inhibits contraction produced by histamine in these tissue preparations. Histamine (50µg/ml) was taken in different dose level and DRC was plotted. Study revealed that *Hemidesmus indicus* extract exhibits significant ( $p<0.01$ ) percentage decreased contraction at concentration 80 µg /ml in goat tracheal chain preparation Dose dependent response relationship was seen. (Table-1)

**Table 1) Effect of *Hemidesmus indicus* extract on histamine induced contraction on isolated goat tracheal chain preparation.**

Groups	Dose of histamine ( 50 µg/ml) (ml)	Control group % maximum contraction (Mean ± SEM)	Test group % maximum contraction (Mean ± SEM)
1	0.1	21.46 ± 1.95	9.62 ± 0.93**
2	0.2	25.91 ± 1.95	12.22 ± 1.37**
3	0.4	43.33 ± 1.62	21.11 ± 1.24**
4	0.8	55.17 ± 2.10	27.77 ± 1.69**
5	1.6	81.46 ± 1.95	39.24 ± 1.09**
6	3.2	94.06 ± 1.87	45.51 ± 1.48**

n = 6

Values are in Mean ± SEM.

Control = D.R.C. of Histamine in absence of *Hemidesmus indicus* extract.

Test = D.R.C. of Histamine in presence of *Hemidesmus indicus* extract.(80µg/ml)

Statistical analysis done by using Student's 't'-test.

\*\* $p<0.01$ , significantly different from control.

## 2) Passive paw anaphylaxis in rats

There was significant inhibition in rat paw edema at the dose 50mg/kg of *Himidiscus* extract, in all time intervals when percentage inhibition was calculated but more specific effect was seen at 3hour interval time. It was 39.07% and 57.82% for 50mg/kg and dexamethasone respectively. Paw edema volume also significantly ( $p<0.01$ ) decreased in all time intervals at this dose only. Control group showed (0.64 ± 0.0318) paw edema volume and that of for 50 mg/kg dose and dexamethasone was (0.39 ± 0.0386) and (0.27 ± 0.0227) at 3 hour interval. Results are comparable with that of standard dexamethasone. It was seen that further increase in dose showed decrease in activity. (Table-2)

**Table 2) Effect of *Hemidesmus indicus* extract on passive paw anaphylaxis in rats**

Groups		Paw Edema Volume (ml)				
		Mean $\pm$ SEM				
Sr.no.	Dose	1/2hr	1hr	2hr	3hr	4hr
1	Control	0.73 $\pm$ 0.0467	0.68 $\pm$ 0.0263	0.65 $\pm$ 0.0183	0.64 $\pm$ 0.0318	0.61 $\pm$ 0.0297
2	Dexamethasone	0.30 $\pm$ 0.0205**	0.26 $\pm$ 0.0264**	0.27 $\pm$ 0.0116**	0.27 $\pm$ 0.0227**	0.24 $\pm$ 0.0217**
3	25	0.47 $\pm$ 0.0292**	0.45 $\pm$ 0.0346**	0.42 $\pm$ 0.0386**	0.41 $\pm$ 0.0380**	0.41 $\pm$ 0.0354**
4	50	0.45 $\pm$ 0.0336**	0.44 $\pm$ 0.0491**	0.40 $\pm$ 0.0285**	0.39 $\pm$ 0.0386**	0.38 $\pm$ 0.0330**
5	100	0.48 $\pm$ 0.0208**	0.47 $\pm$ 0.0303**	0.43 $\pm$ 0.0242**	0.42 $\pm$ 0.0367**	0.39 $\pm$ 0.0269**

n = 5; \*p<0.05, \*\*p<0.01, compared with control group (ANOVA followed by Dunnett's test)

**Table 2.1) Table showing percentage inhibition of paw edema volume**

Groups		Percentage inhibition of Paw Edema Volume (%)				
Sr. no.	Dose	1/2hr	1hr	2hr	3hr	4hr
1	25	32.62	33.83	35.39	35.94	32.79
2	50	38.36	35.30	38.47	39.07	37.71
3	100	34.25	30.89	33.85	34.38	36.07
4	Dexamethasone	58.97	61.68	58.47	57.82	60.66

### 3) Clonidine induced catalepsy in mice

Clonidine (1mg/kg, s.c.) produced catalepsy in mice, which remained for 3 hours. The vehicle treated group has shown maximum duration of catalepsy ( $120 \pm 3.5$ sec.) at 180 minute after the administration of clonidine. There was significant inhibition ( $p < 0.05$ ,  $p < 0.01$ ) of Clonidine induced catalepsy in the animal pretreated with *Hemidesmus indicus* extract (25, 50, 100 mg/kg, p.o.) and the duration of catalepsy was found to be  $99.4 \pm 8.17$ ,  $91.0 \pm 4.42$  and  $107 \pm 6.15$  seconds respectively. Chlorpheniramine maleate, (10 mg/kg, i.p.) treated group significantly reversed ( $p < 0.01$ ) the Clonidine induced catalepsy in mice. (Table-3,)

**Table-3) Effect of *Hemidesmus indicus* on clonidine induced catalepsy in mice.**

Group	Duration of catalepsy (sec) at						
	Mean + SEM						
	15 min	30 min	60 min	90 min	120 min	150 min	180 min
I	68.4 ± 2.63	79.4 ± 2.85	79.6 ± 2.01	79.8 ± 3.90	82.2 ± 2.95	83.2 ± 4.15	120 ± 3.53
II	25.6 ± 2.82**	27.0 ± 2.09**	23.4 ± 1.77**	20.0 ± 2.84**	28.8 ± 2.65**	28.2 ± 2.39**	54.0 ± 3.06**
III	49.6 ± 7.18*	57.8 ± 2.13**	67.8 ± 2.08*	61.0 ± 4.38**	62.4 ± 5.40**	65.0 ± 3.53*	99.4 ± 8.17*
IV	44.6 ± 4.91**	45.4 ± 1.83**	47.8 ± 2.92**	58.0 ± 2.16**	56.0 ± 3.52**	49.6 ± 1.93**	91.0 ± 4.42**
V	47.0 ± 3.03*	50.8 ± 2.93**	60.2 ± 4.65**	66.0 ± 2.84*	67.8 ± 1.85*	63.2 ± 2.45**	107 ± 6.15

Group-I = Vehicle (10ml/kg, i.p.)

Group-II = Chlorpheniramine maleate (10 mg/kg, i.p.)

Group-III = *Hemidesmus indicus* extract (25 mg/kg, p.o.)

Group-IV = *Hemidesmus indicus* extract (50 mg/kg, p.o.)

Group-V = *Hemidesmus indicus* extract (100 mg/kg, p.o.)

Statistical analysis done by ANOVA followed by Dunnett's test.

\* $p < 0.05$ , \*\* $p < 0.01$ , compared to control group

### Discussion

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 the isolated goat tracheal chain preparation; there is right side shift of Dose Response Curve (DRC) of histamine in the presence of *Hemidesmus indicus* R.Br ethanolic extract indicating antiasthmatic action. In passive paw edema models, extract showed the dose dependent responses. Thus *Hemidesmus indicus* R.Br can prevent the release of inflammatory mediators or inflammation in asthma.. The extract also significantly inhibited the clonidine induced catalepsy. The inhibition of clonidine induced catalepsy by *Hemidesmus indicus* may be due to the potential to antagonize H<sub>1</sub> receptor or inhibition of mast cell degranulation induced by clonidine.

In conclusion the present study confirmed that the ethanolic extract of *Hemidesmus indicus* R.Br roots exhibits significant dose dependent antiasthmatic activity in various in-vitro and in-vivo animal models and further supports the traditional claim of plant in the treatment of asthma. Further studies are infact underway to isolate and characterize the active principle responsible for the antiasthmatic activity.

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