

**ANTIASTHMATIC ACTIVITY OF ETHANOLIC EXTRACT OF *Aerva lanata* Linn.**

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**Summary**

Now days, herbal 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 assessment has become the criteria for acceptance of traditional health claims. *Aerva lanata* (Amaranthaceae), commonly known as Kapurijadi (Hin.), Buikallan (Pb.) is used in the traditional system of medicine as an antiasthmatic activity. We examined the effect of ethanolic extract of aerial parts of *Aerva lanata* at 100 µg/ml in-vitro in the isolated goat tracheal chain preparation model and 30 & 60mg/kg doses orally in-vivo model using clonidine-induced catalepsy, mast cell degranulation in mice. The extract showed significant dose-dependent antiasthmatic activity.

**Keywords:** Antiasthmatic activity, *Aerva lanata*, Mast cell degranulation.

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### **Introduction**

*Aerva lanta* Linn. is a erect or prostrate herbaceous common wayside weed which is recognized by its white axillary bunches of small woolly flowers. It is abundant on the plains in the warmer parts of India<sup>1</sup> and almost all over the plains upto an altitude of 3000 m. It is commonly known as Kapurijadi in Hindi and Buikallan in Punjabi.

*Aerva lanata* contains  $\beta$ -sitosteryl palmitate,  $\alpha$ - amyirin and  $\beta$ -sitosterol and tannin. *Aerva lanata* is endowed with chemical compounds such as steroids, flavonoids, alkaloids, polysaccharides and saponin<sup>2</sup>. *Aerva lanata* also reported for six alkaloids like Canthin - 6-one and  $\beta$ -Carboline, Hentriacontane,  $\beta$ -sitosterol and its D-glucoside,  $\alpha$ -amyirin and betulin on dry plant material basis<sup>3</sup>. Four flavonoid glycosides, betulin, campesterol, Chrysin etc. were reported to present in *Aerva lanata*<sup>4</sup>.

Traditionally *Aerva lanata* is used as diuretic, demulcent, in headache and lithiasis. According to Ayurveda, it is also useful in strangury. In ethano-medicine *Aerva lanata* is regarded as a valuable medicine for cough, sore throat, indigestion, wounds and as a vermifuge for children. It is also used for diabetics<sup>5</sup>. The flowers are used for the removal of kidney stones and in gonorrhea<sup>6</sup>. The herb has been used for its therapeutic effects in renal diseases by some Unani physicians and to treat urinary calculi<sup>7</sup>. *Aerva lanata* has been documented for its urolithiasis effects<sup>8</sup>, antimicrobial<sup>9</sup>, cytotoxicity<sup>10</sup>, antidiabetic<sup>11</sup>, nephroprotective<sup>12</sup>, immunomodulatory<sup>13</sup>, diuretic and its anti-inflammatory activities<sup>14</sup>. Traditional claims inspired us to evaluate the *Aerva lanata* for its Antiasthmatic activity.

### **Materials and Methods**

#### **Collection of Plant material**

Aerial parts of *Aerva lanata* were collected from wild source from the state of Punjab (India) and were authenticated by Dr. Sumer Chandra, Scientist D, F.R.I. Deemed University, I.P.E., Kaulagarh Road, Dehradun-248195 (India) as Specimen voucher no. SCP-301.

#### **Extraction of plant materials**

The aerial parts were shade dried, powdered and passed through 80 mesh sieve. The powdered material was extracted with ethanol using Soxhlet's apparatus. The extract obtained was dried in rotary vacuum evaporator at 40<sup>0</sup> C.yielding a yellowish green colored viscous mass (1.16%). The phytochemical test of ethanolic extract showed the presence of alkaloids, saponin flavonoid and tannins.

#### **Animals**

Isolated adult goat tracheal tissue and adult albino mice of either sex weighing 25-30 g were used for studies. Isolated adult goat trachea tissue was obtained immediately after slaughter of the animal from slaughter house. Pieces of the trachea were collected in the ice cold oxygenated Kreb's solution. The albino mice were obtained from animal house of Institute of Microbial Technology (IMTECH), Sector 39-A, Chandigarh, 160036. They were housed in polypropylene cages with standard pellet and water ad libitum. In all experimental sets 5 mice were used for each treatment. The Institutional Animal Ethical committee (IAEC) has approved the protocol of the study.

**Antiasthmatic activity****1) Isolated goat trachea chain preparation**

Isolated adult goat tracheal tissue was obtained immediately from slaughter house of the animals. Trachea was cut into individual rings and tied together in series to form a chain. Trachea was suspended in bath of Kreb's solution and was continuously aerator at  $37 \pm 0.5^\circ\text{C}$ . DRC of histamine in plane Kreb's solution and in 100  $\mu\text{g/ml}$  *Aerva lanata* 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>15-16</sup>.

**2) Clonidine induced catalepsy in mice**

Albino mice were divided into five groups (n = 5). Control group received distilled water (10ml/kg) and Standard group received Chlorpheniramine maleate (10 mg/kg, i.p.) and group III, and IV received single dose of ethanolic extract (30,60mg/kg p.o. body weight) respectively. 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>17-18</sup>.

**3) Mast Cell degranulation**<sup>19</sup>

Mice were divided in four groups, (n=5).The three days drug treatment schedule was followed. Control group received distilled water (10 ml/kg, p.o.) and Standard group was treated with disodium cromoglycate (0.5mg/kg, i.p.).Group-III and IV was treated with ethanolic extract of *Aerva lanata* 30 and 60 mg/kg, p.o. respectively. On 4<sup>th</sup> day, each animal were injected with 4ml/kg 0.9% NaCl solution into peritoneal cavity. The abdomen was gently massaged for few minutes. The peritoneal cavity was carefully opened and the fluid containing mast cells was aspirated and collected in test-tube containing 8 ml of animal cell culture media RPMI-1640 buffer solution (7.2-7.4).The mast cells were then washed with same buffer solution by centrifugation at a speed of 400-500 rpm and the pallet of mast cells was collected. Then 0.5 $\mu\text{g/ml}$  Clonidine solution was added to the mast cell suspension and incubated at  $37^\circ\text{C}$  in a water bath for 10 min. Later they were stained with 1% toluidine blue dye and observed under high power microscope (400X). Total 100 cells were counted from different visual areas and percent protection against clonidine induced mast cell degranulation was calculated<sup>20</sup>.

$$\% \text{ Protection} = \frac{T_2 - T_1}{T_2} \times 100.$$

T<sub>1</sub> = Control group.

T<sub>2</sub> = Test group.

**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

The ethanolic extract of aerial parts of *Aerva lanata* was evaluated for their antiasthmatic activity.

#### 1) Isolated goat trachea chain preparation

In the present study, it was observed that *Aerva lanata* ethanolic extract inhibits contraction produced by histamine in these tissue preparations. Histamine (30µg/ml) was taken in different dose level and DRC was plotted. Study showed that *Aerva lanata* ethanolic extract exhibits significant (\*\*\*)p<0.01) percentage decreased contraction at concentration 100µg/ml in goat tracheal chain preparation. Dose dependent response relationship was seen. (Table-1)

**Table-1) Effect of *Aerva lanata* ethanolic extract on histamine induced contraction on isolated goat tracheal chain preparation.**

Sr. No	Dose of histamine (30µg/ml)	Control group % maximum contraction	Test group % maximum contraction
1	0.1	45.16± 2.19	19.36±0.988*
2	0.2	65.16±3.06	32.14±1.687*
3	0.4	69.91±2.319	34.27±1.138**
4	0.8	75.12±2.626	39.15±1.308**
5	1.6	84.6±1.721	44.06±0.8851***
6	3.2	100±1.721	50.36±0.9309***

n = 5,

Values are in Mean ± SEM.

Control = D.R.C. of Histamine in absence of ethanolic *Aerva lanata* extract.

Test (A.I) = D.R.C. of Histamine in presence of ethanolic *Aerva lanata* extract (100µg/ml).

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

\* p<0.05, \*\* p< 0.01, \*\*\*p<0.001, significantly different from control.

#### 2) Clonidine induced catalepsy in mice

Clonidine (1mg/kg, s.c.) produced catalepsy in mice, which remained maximum for 2 hours. The vehicle treated group has shown maximum duration of catalepsy (96.6 ± 1.6sec.) at 120 minute after the administration of clonidine. There was significant inhibition (\*\*p<0.01) of Clonidine induced catalepsy in the animal pretreated with *Aerva lanata* extract (30 & 60 mg/kg, p.o.) and the duration of catalepsy was found to be 79.6 ± 1.20 and 71.2 ± 2.47 seconds respectively. Chlorpheniramine maleate, (10 mg/kg, i.p.) treated group significantly reversed (\*\*p<0.01) the Clonidine induced catalepsy in mice. (Table-2)

**Table No. 2) Effect of ethanolic extract of *A. l.* on Clonidine induced catalepsy in mice.**

Group	Duration of catalepsy (sec) at Mean $\pm$ SEM						
	15 min	30 min	60 min	90 min	120 min	150 min	180 min
Control (10ml/kg.)	24.6 $\pm$ 1.28	54 $\pm$ 1.70	68 $\pm$ 1.58	82.2 $\pm$ 1.06	96.6 $\pm$ 1.6	82.8 $\pm$ 1.35	80.8 $\pm$ 1.56
Std (10 mg/kg.)	19.4 $\pm$ 0.93**	24.2 $\pm$ 1.06**	27.4 $\pm$ 1.20**	27.2 $\pm$ 1.06**	26.6 $\pm$ 1.50**	27.8 $\pm$ 1.31**	53.8 $\pm$ 1.56**
A.l. <sub>1</sub> (30mg/kg.)	51.4 $\pm$ 0.92**	53.2 $\pm$ 2.0	62.8 $\pm$ 1.28	64.4 $\pm$ 2.37**	79.6 $\pm$ 1.20**	66.4 $\pm$ 1.63**	63.4 $\pm$ 1.50**
A.l. <sub>2</sub> (60mg/kg.)	42.4 $\pm$ 1.07**	43.6 $\pm$ 1.72**	61.2 $\pm$ 1.85**	47 $\pm$ 1.84**	71.2 $\pm$ 2.47**	46.4 $\pm$ 1.56**	55.6 $\pm$ 1.20**

n = 5, Values are in Mean  $\pm$  SEM. Control = Vehicle, d.w. (10 ml/kg, p.o.). Std. = Chlorpheniramine maleate (10 mg/kg, p.o.). A.l.<sub>1</sub> = Ethanolic extract of *Aerva lanata* (30 mg/kg, p.o.). A.l.<sub>2</sub> = Ethanolic extract of *Aerva lanata* (60 mg/kg, p.o.). Statistical analysis done by using Student's 't'-test. & ANOVA followed by Dunnett's test. \*\*P < 0.01 considered significant compared to control group.

### 3) Mast cell degranulation:

Clonidine challenge resulted in significant degranulation of mast cell. Pretreatment of sensitized animal with standard drug Disodium cromoglycate showed protection 70.6% and *Aerva lanata* at a dose of (60mg/kg i.p) showed percentage protection of 68.9%. (Table: 3)

**Table 3) Effect of Ethanolic extract of *Aerva lanata* on mast cell degranulation in mice.**

Group.	Treatment	Intact mast cell	Disrupted mast cell	%Protection
I	Control	20.4 $\pm$ 1.07	79.6 $\pm$ 1.07	-
II	Disodium cromoglycate	69.4 $\pm$ 0.92	30.6 $\pm$ 0.92**	70.6%.
III	A.l. <sub>1</sub> (30mg/kg.)	57.0 $\pm$ 1.70	43.0 $\pm$ 1.70**	64.2%
IV	A.l. <sub>2</sub> (60mg/kg.)	65.6 $\pm$ 1.72	34.4 $\pm$ 1.72**	68.9%

n = 5, Values are in Mean  $\pm$  SEM. Control = Vehicle, d.w. (10 ml/kg, p.o.). Std. = Chlorpheniramine maleate (10 mg/kg, p.o.). A.l.<sub>1</sub> = Ethanolic extract of *Aerva lanata* (30 mg/kg, p.o.). A.l.<sub>2</sub> = Ethanolic extract of *Aerva lanata* (60 mg/kg, p.o.). Statistical analysis done by using Student's 't'-test. & ANOVA followed by Dunnett's test. \*\*P < 0.01 considered significant compared to control group

### Discussion

Histamine contracts the trachea-bronchial muscle of guinea pig, goat, horse, dog and man<sup>21</sup>. Goat tracheal chain is much more sensitive than guinea pig and easier to handle. 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 ethanolic extract of *Aerva lanata* Linn. indicating antiasthmatic activity. The ethanolic extract significantly inhibited the clonidine induced catalepsy. The inhibition of clonidine induced catalepsy by *Aerva lanata* may be due to the potential to antagonize H<sub>1</sub> receptor or inhibition of mast cell degranulation induced by clonidine. Uvnas studied the mast cell degranulation and its correlation with the release of histamine after administration of compound 48/80, the mast cell degranulating agent<sup>22</sup>. Both clonidine and compound 48/80 act through the dynamic expulsion of granules without causing any damage to the cell wall<sup>23</sup>. Lakadawala<sup>20</sup> have shown that Clonidine releases histamine from mast cells in a similar manner to a selective liberator like compound 48/80. It is known that disodium cromoglycate a standard mast cell stabilizer prevents degranulation of mast cells by raising the cyclic adenosine monophosphate (AMP)<sup>24</sup>. It has been known that all pharmacological agents that increase intracellular levels of AMP relax airway smooth muscle and inhibit the release of autocooids from the tissue and basophil. Present study showed dose dependent statistically significant stabilization of mast cell by ethanolic extract of *Aerva lanata*

In conclusion the present study confirmed that ethanolic extract of aerial parts of *Aerva lanata* exhibits significant dose dependent antiasthmatic activity in-vitro and in-vivo animal models and further supports the traditional claim of herb in the treatment of asthma. Further studies are required to isolate and characterize the active principle responsible for the antiasthamatic activity.

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