

Antidiarrhoeal Activity of *Aerva lanata* in Experimentally Induced Diarrhoea in Rats

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Summary

Aerva Lanata belongs to family Borogenaseae has been widely used in Indian folk medicine for treatment of urinary disorder, boils, cephalalgia, cough, strangury and lithiasis. This study was undertaken to evaluate the antidiarrhoeal effect of alcoholic extract of *Aerva lanata* by using different experimental models. It was found that both doses of extract showed significant protection against PGE₂ induced enteropooling, which might be due to the inhibition of synthesis of prostaglandins that is responsible for diarrhoea. Antidiarrhoeal effect was accessed by castor oil induced diarrhoea, charcoal meal test and PGE₂ induced diarrhoea. Loperamide (2 mg/kg) and atropine (0.1mg/kg) were used as standard drugs. Alcoholic extract was used in 400 and 800 mg/kg dose p.o.

Key words: *Aerva lanata*; castor oil diarrhea; charcoal meal; PGE₂; methanol extract.

Introduction

Aerva lanata is also called as Pasanabheda, Chaya belongs to the family Amaranthaceae. It is distributed throughout India, in waste land. The *Aerva lanata* plant was reported to contain β -sitosterol^(1,2,3,4), amyirin^(2,4), betulin, hentriacontane⁽⁴⁾, Campesterol^(3,4), stigmasterol, stigmasterol acetate⁽³⁾, daucosterol⁽⁵⁾, β -sitosterol palmitate⁽²⁾, ergosterol, lupeol⁽³⁾, β -amyirin⁽⁴⁾, olean-12-en-28-oic acid-3, 16-dioxymethyl ester⁽³⁾, Kaempferol, kaempferol-3-galactoside, kaempferol-3-rahmno galactoside⁽¹¹⁾, starch⁽¹¹⁾, Free sugars: fructose, galactose, rhamnose, sucrose⁽¹⁾.

β -carboline-1 propionic acid, 10-methoxy-canthin-6-one, 10-hydroxy-canthin-6-one, 10- β -D-glucopyranosyloxy canthin-one, 6-methoxy- β -carboline-1-propionic acid⁽⁶⁾, aervoside^(7,8), aervolanine⁽⁸⁾, alkaloids were isolated from *Aerva lanata*. Four new alkaloids viz., aervine, methylaervine, aervoside and aervolanine⁽⁸⁾, were isolated and their structures established and confirmed by ¹³C-NMR and NOE data:

The flowering and fruiting parts of the *Aerva lanata* plant contain hemicellulose, starch, an acid-soluble polysaccharide and water-soluble polysaccharide; monosaccharide contains of polysaccharides determined⁽⁹⁾. Ethyl acetate and alcoholic extracts of *Aerva lanata* whole plant showed antimicrobial activities while petroleum ether, ethylacetate and alcoholic extracts, showed significant cytotoxic activity⁽¹⁰⁾. *Aerva lanata* was screened for its diuretic and hepatoprotective activity. The alcoholic extracts were prepared from leaves, stem and roots for screening. All the extracts were found to have significant diuretic activity, while hepatoprotective activity was found in case of leaf and root extracts only⁽¹¹⁾. Alcoholic extract of *Aerva lanata* was tested for diuretic activity, while the effect of benzene and alcoholic extracts of *Aerva lanata* were investigated in the rat to evaluate the anti-inflammatory activity. Carrageenan –induced rat hind paw edema method was employed to test anti-inflammatory activity. Alcoholic extract (800mg/kg) produced inhibition of carrageenan-induced rat paw edema (P<0.05). The parameters measured for diuretic activity were total urine volume, sodium, potassium and chloride content. The results indicate that the alcoholic extract at a dose of 800mg/kg act as diuretic, with respect to control⁽¹²⁾. The plant *Aerva lanata* showed its effect on cisplatin and gentamycin model of acute renal failure⁽¹³⁾ and also reported for antidiabetic.⁽¹⁴⁾

However, detailed investigations of antidiarrheal activity of *Aerva lanata* had not been carried out so far. Hence, this leads us to study for antidiarrheal activity of *Aerva lanata* in different diarrhoeal models.

Materials and Methods

Plant Material Collection and Extraction:

Whole plant of *Aerva lanata* was collected and authenticated by Dr. S. N. Dwivedi, Head of the department of botany Janata Post Graduate College A. P. S. University Rewa-486002 M. P. India. The whole plant is then dried, powdered and stored in airtight containers for further use. The powdered material was subjected to soxhlet extraction with various solvents ranging from non-polar to polar. The solvents used were Petroleum ether, benzene, chloroform, alcohol and water. Each time before extraction with next solvents the marc was air-dried. All the extracts were concentrated by distilling the solvent at low temperature. They were then weighed and percentages of different extractive values were calculated with respect to air-dried substance. Alcoholic extract was selected for antidiarrheal activity on the basis of phytochemical screening and TLC pattern.

Experimental animals

Albino Wister rats of both sex weighing between 150-240 g were used. Institutional Animal Ethics Committee approved the experimental protocol; animals were housed under standard conditions of temperature ($24 \pm 2^\circ\text{C}$) and relative humidity (30-70%) with a 12:12 light: dark cycle. Animal handling was performed according to Good Laboratory Practice (GLP). The animals were given standard diet and water *ad libitum*.

Evaluation of Antidiarrhoeal activity

Castor oil induced diarrhea⁽¹⁵⁾

Rats of either sex (150-200gm) fasted for 18 hours were divided into 5 groups (Group I – Group V) of 6 animals each. The groups received the following treatment by oral gavage: Group I: Distilled water (0.2 ml), Group II: received standard drug, Loperamide (2 mg/kg) orally as suspension, Group III: *Aerva lanata* alcoholic extract 400 mg/kg body weight, Group IV: *Aerva lanata* alcoholic extract 800 mg/kg body weight and Group V: Distilled water (0.2 ml). (In Groups III and IV, the extract was dissolved in 0.2 ml of distilled water). Thirty minutes after the treatments, castor oil (0.2 ml) was administered by gavage to groups II, III, IV and V.

Following the administration of castor oil, the animals were placed in separate wired cages for observation. The total number of faeces and the number of wet faeces passed was recorded over a

period of 4 hours after the administration of castor oil. Weight of paper before and after defecation was noted.

Charcoal meal test⁽¹⁶⁾

Rats of either sex (150-225 g) were fasted for 18 h. They were divided into four groups (n=6). The first group which served as control was administered with aqueous 1% tragacanth suspension. The second group receives standard drug atropine (0.1 mg/kg) subcutaneously. The alcoholic extract was administered orally at 400 mg/kg to third group and 800 mg/kg to fourth group as suspension. The animals were given 1ml of 10% activated charcoal suspended in 10% aqueous tragacanth powder p.o., 30 min after treatment. Animals were euthanized 30 min after charcoal meal administration by ether anesthesia. The abdomen was cut off and the small intestine carefully removed. The distance travelled by charcoal plug from pylorus to caecum was measured, and expressed as percentage of the distance traveled by charcoal plug for each of animal.

PGE₂ induced enteropooling⁽¹⁶⁾

Rats of either sex (150-235 g) were fasted for 18 h. They were then divided into four groups (n=6). A solution of PGE₂ was made in the 5%v/v alcohol in the normal saline. The first group, which served as control, was administered with PGE₂ (100 µg/kg p.o.) only. The second group, which served as vehicle control was administered with aqueous 1% tragacanth suspension by oral route. The extract was administered orally at 400 mg/kg to third group and 800 mg/kg to fourth group as suspension. Immediately after extract administration PGE₂ was administered. After 30 min following administration of PGE₂ each rat was sacrificed and whole length of the intestine from pylorus to caecum was dissected out, its content collected in measuring cylinder and volume measured.

Statistical analysis

The data are represented as mean ± S.E.M. and statistical significance between treatment and control groups was analyzed using of one-way ANOVA, followed by Dunnett's test where P<0.05 was considered statistically significant.

Results and Discussion

Castor oil brings about changes in electrolyte and water transport and increases peristaltic activity. These changes are associated with prostaglandins that contribute to the patho-physiological functions in the gastro intestinal tract. Release of prostaglandins is also a major cause of arachidonic acid-induced diarrhea. This is characterized by an increase in the secretion of water and electrolytes, an increase in intestinal transit time and an increase in wet faeces. Evaluation of antidiarrhoeal activity of alcoholic extract of *Aerva lanata* by castor oil induced diarrhoea is given in **Table 01**.

Table No. 01

Evaluation of antidiarrhoeal activity of alcoholic extract of *Aerva lanata* by castor oil induced diarrhoea

Treatment	Mean wet defecation	Mean increase in weight of paper (gm)	Delay in defecation time (min)
Control	8.807±0.97	2.923±0.39	33.166±8.005
Loperamide(2 mg/kg)	1.533±1.15	0.433±0.26	188.00±22.500
Alcoholic extract (400 mg/kg)	4.557±0.95	2.340±0.27	71.833±2.855
Alcoholic extract (800 mg/kg)	3.227±0.77	1.107±061	133.33±39.261

Number of animals (N) = 6

Values are expressed as mean ± S.E.M.

Alcoholic extract of *Aerva lanata* (400 and 800 mg/kg) and the anti-muscarinic drug, atropine (0.1 mg/kg) decreased the propulsive movement in the charcoal meal study, both dose 800mg/kg and 400 mg/kg showed moderate effect to prevent diarrhea (**Table 02**).

Table No. 02

Evaluation of antidiarrhoeal activity of alcoholic extract of *Aerva lanata* by charcoal meal test

Treatment	MOVEMENT OF CHARCOAL
Control	64.25 ± 1.560
Atropine sulphate (2 mg/kg)	32.42 ± 3.10**
Alcoholic extract (400 mg/kg)	61.01 ± 5.55**
Alcoholic extract (800 mg/kg)	41.11 ± 3.14**

**=P<0.01= very significant

Number of animals (N) = 6, Values are expressed as mean ± S.E.M.

Both doses of alcoholic extract showed protection against PGE₂ induced enteropooling (**Table 03**), which might be due to the inhibition of synthesis of prostaglandins. Anti-enteropooling effect of the extract is more relevant because the prevention of enteropooling helps in the inhibition of diarrhea, especially by PGE₂ induced diarrhea as it is involved in the onset of diarrhea in intestinal mucosal cells. Although intraluminally administered PGE₂ is known to induce duodenal and jejunal secretion of water and of electrolytes such as Cl⁻ and Na⁽¹²⁾, fluid content is the principal determinant of stool volume and consistency. Net stool fluid content reflects a balance between luminal input (ingestion and secretion of water and electrolytes) and output (absorption) along gastrointestinal tract. Neurohumoral mechanisms, pathogens and drugs can alter these processes, resulting in changes in either secretion or absorption of fluid by the intestinal epithelium. Altered motility also contributes in a general way to this process, as the extent of absorption parallels the transit time.

The underlying mechanism appears to be spasmolytic and an anti-enteropooling property by which the extract produced relief in diarrhea.

Extract also inhibited the onset time and severity of diarrhea induced by castor oil. Castor oil is reported to cause diarrhea by increasing the volume of intestinal content by prevention of reabsorption of water.

Table No. 03

Evaluation of antidiarrhoeal activity of alcoholic extract of *Aerva lanata* by PGE₂ induced enteropooling.

Treatment	Volume of intestinal fluid (ml)
PGE ₂ control	2.84 ± 0.77
Vehicle control	1.66 ± 0.30
Alcoholic extract (400 mg/kg)	1.15 ± 0.15**
Alcoholic extract (800 mg/kg)	1.14 ± 0.15**

**= P<0.01 = very significant

Number of animals (N) = 6, Values are expressed as mean ± S.E.M.

Conclusion

The antidiarrhoeal effect of alcoholic extract is due to reduction of gastrointestinal motility, inhibition of the synthesis of prostaglandin. The extract has potential effect on the reduction of gastrointestinal motility than the other effects. The above effects of it may also be due to the presence of alkaloids and flavanoids in the extract.

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