

## Research Article

**Evaluation of *In-Vivo* Anti-diarrheal and Cytotoxic Activity of Ethanolic Extract of *Alstonia scholaris* Leaves**

B.Nagaraju\*, A.Ramu, S.Vidyadhara, Ch.ArunaKumar, D.Sandeep, B.Venkateswara rao.

Department of Pharmacology, Chebrolu Hanumaiah Institute of Pharmaceutical Sciences, Chandramoulipuram, Chowdavaram, Guntur, AP, India.

\*Corresponding author's E-mail: [bnrajupharma@gmail.com](mailto:bnrajupharma@gmail.com)

Accepted on: 10-08-2016; Finalized on: 31-10-2016.

**ABSTRACT**

This study investigated the antidiarrheal and cytotoxic effects of Ethanolic extract of *Alstonia scholaris* leaves. Antidiarrheal effect was measured in castor oil induced- diarrhea, and enteropooling models in albino mice. Cytotoxicity was investigated by zebra fish lethality bioassay. In castor oil induced diarrhea we measure the parameters like total no fecus, % inhibition of defecation, total no diarrheal feces,% inhibition of diarrhea, volume of intestinal content(ml) and % inhibition of intestinal content. In cytotoxic activity we measure parameters like number of alive larvae,% mortality and LC50. The extract (500 mg/kg) showed a remarkable antidiarrheal activity by reducing the number of defecation and maintaining the consistency of feces. Ethanolic extract inhibited the diarrheal incidence at the dose of 500mg/kg and Castor oil induced enteropooling and fluid accumulation was significantly reduced. Loperamide was used as a reference drug in the above mentioned models. In cytotoxicity test, the lowest LC50 was found to be 50.25µg/ml by the Ethanolic extract. The results demonstrated that *Alstonia scholaris* leaves could be used as pharmaceutical preparation of antidiarrheal and cytotoxic agent.

**Keywords:** Antidiarrheal activity, castor oil, enteropooling, cytotoxicity, Alstonia, Zebra fish.

**INTRODUCTION**

Diarrheal diseases are a major problem in Third World countries and are responsible for the death of millions of people each year.<sup>1</sup> Diarrhoea is an alteration in normal bowel movement and is characterized by an increase in the water content, volume, or frequency of stools.<sup>2</sup> Plants have long been a very important source of new drugs. Many plant species have been screened for substances with therapeutic activity. Medicinal plants are a promising source of antidiarrheal drugs.<sup>3</sup> For this reason, international organizations including the World Health Organization (WHO) have encouraged studies pertaining to the treatment and prevention of diarrheal diseases using traditional medical practices. *Alstonia scholaris* belongs to the Apocyanaceae family and is indigenous to the South and South-east Asia. The tree is commonly used for centuries in Ayurvedic medicines for treatment of various disorders. The leaves yield a tonic and antiseptic medicine which is used to treat anemia, menstrual cycle, malarial fever, colic, diarrhea, dysentery and acute arthritis. Decoction of the bark is used to treat diarrhea and malaria, anti-periodic, astringent and anthelmintic. Bark is also used in snakebite and produces protective effect on hepatotoxin induced acute liver damage, Sap, gum and roots are used in tumor and cancer. Various extracts of leaves was found to have antibacterial, immunostimulant, antimalarial and anticancer activities. Although the plant has widespread applications in traditional medicine, very few scientific studies have been performed to ascertain its medicinal potential. Therefore, the objective of the present investigation was to evaluate the in vivo anti-diarrheal

and cytotoxic activity of ethanolic extract of *Alstonia scholaris* leaves.<sup>4,5</sup>

**MATERIALS AND METHODS****Collection and Identification**

*Alstonia scholaris* leaves were collected from the local medicinal garden of Chebrolu Hanumaiah Institute of Pharmaceutical Sciences, Guntur, Andhra Pradesh, India. Authenticated by the Department of Botany, ANU, Guntur, voucher specimen are for future reference.

**Drying and Grinding**

The collected plant part (leaves) was separated from undesirable material and the leaves were dried under shade at room temperature for two week. The dried leaves were ground into a coarse powder with a suitable grinder. The powder was stored in air tight container until the analysis was commenced.

**Extraction**

Coarsely powdered leaves (500 g) were successively extracted with petroleum ether (60-80°C) for 7 days to remove fatty matter. The defatted marc was then subjected to soxhlet extraction with 95% ethanol to obtain ethanolic extract. The ethanolic extract was evaporated under reduced pressure at low temperature (30°C) to dryness and brownish yellow colour extracts of was obtained.<sup>6</sup>

**Preliminary Phytochemical Screening**

Ethanolic extract of *Alstonia scholaris* was subjected to preliminary phytochemical for the detection of various constituents.<sup>7</sup>



## Experiment

Albino mice of weighing between 20-25 g were employed in the experiment taking five in a group. All mice were feed with pelleted diet (Pranav Agro Industries Ltd, Sangli, India) and water ad libitum. Institutional Animals Ethics Committee (IAEC) approved the experimental protocol and care of animals was taken as per guidelines of CPCSEA, IAEC NO: 439/PO/01/a/CPCSEA.

### Assessment of In -Vivo Anti-diarrheal activity

#### Castor oil induced diarrhea

The experiment was carried out according to the method described by Awouters et al.<sup>8,9</sup> Mice were fasted for 18 hrs before the test with free access to water and randomly divided into five groups' five animals in each group. Group I was treated as control (saline 2 ml/kg bw, i.p.), Group II received 3ml of castor oil Group III received standard drug (loperamide, 5 mg/kg bw, i.p.) Group IV received 250 mg/kg EEAS and Group V received 500mg/kg EEAS. One hr later, castor oil was administered orally to these animals to induce diarrhea. The mice were then housed individually in cages with white blotting paper. The papers were changed every hour. The total number of both dry and wet feces excreted was counted every hour for a period of 4 h and compared with the control group. The total number of diarrheal feces of the control group was considered 100%. A numerical score based on stool consistency was assigned as follows: normal stool =1, semisolid stool =2 and watery stool =3. Percent inhibition (PI) was calculated as follows: results were shown in table 2.

$$PI = \frac{\text{Mean defecation (control group-treated group)} \times 100}{\text{Mean defecation of control}}$$

#### Castor oil induced enter pooling

Intraluminal fluid accumulation was determined by the method of Robert et al.<sup>10,11,12</sup> 18 hrs fasted mice were divided into six groups five animals in each group. Group I was treated as control (saline 2 ml/kg bw, i.p.), Group II (toxic control) received 3ml of Castor oil Group III received standard drug (loperamide, 5 mg/kg bw, i.p.) Group IV received 250 mg/kg EEAS and Group V received 500mg/kg EEAS. After one hr, castor oil was administered orally to these animals to induce diarrhea. Two hrs later, the mice were sacrificed by overdose 100-120 mg/kg of chloroform anesthesia, and the small intestine was ligated both at the pyloric sphincter and at the ileocecal junctions and dissected out. The small intestine was weighed. The intestinal contents were collected by milking into a graduated tube and the volume was measured. The intestines were reweighed and the differences between full and empty intestines were calculated and results were shown in table 3.

#### Zebra fish lethality assay for cytotoxicity

Zebra fish lethality bioassay was used to determine the cytotoxic activity of the extract.<sup>13, 14,15</sup> Zebra fish (*Danio*

*rerio*) eggs were collected from local aquarium shop (Guntur, Andhra Pradesh) and hatched in artificial seawater (3.8% NaCl solution) for 48 hr to have the grown-up larvae called veligers at 37°C temperature with continuous oxygen supply. 500mg of dried ethanolic extract of *Alstonia scholaris* was dissolved in DMSO and made the volume 10 ml. The concentration of this solution was 50µg/ml. 38g Sea salt (pure NaCl 20g and table salt 18g) was weighed accurately, dissolved in distilled water to make one liter and then filtered off to get a clear solution. Sea water was taken in the small tank and zebra eggs were added. The eggs were allowed for 3 days to hatch and mature as larvae called veligers. 20 clean test tubes were taken, 10 of which were for the samples in five concentrations (two test tubes for each concentration) and 10 for control test. Then with the help of micropipette specific volumes (2,4,8,16 and 32µl) of extract solution were transferred from the stock solution to the test tubes to get final sample concentrations of 10,20,40,80,160µg/ml respectively. Sea water was added to adjust the volume of each test tube to 10 ml. For the control, same volumes of DMSO (as in the sample test tubes) were taken in the rest of 10 test tubes. Finally 10 living mature larvae (veligers of zebra fish) were transferred to each test tube. These were incubated at 37°C for 24 hr, after incubation each tube was examined and surviving of larva were counted and recorded. The percentage of mortality was calculated at each concentration. LC50 values were calculated by using Microsoft Excel and results were shown in table 4.

### Statistical analysis

The results were expressed as mean ± SEM (n=5). Statistical analysis was performed using one way ANNOVA followed by Dennett's comparison test.

## RESULTS

### Phytochemical analysis

Ethanolic extract of *Alstonia scholaris* was subjected to preliminary phytochemical Screening results were shown in table 1.

**Table 1:** Phytochemical analysis of Ethanolic extract of *Alstonia scholaris*

Name of the Test	Results
Carbohydrates	++
Steroids	—
Alkaloids	++
Glycosides	++
Tannins	++
Flavonoids	—
Saponins	++
Gums	—

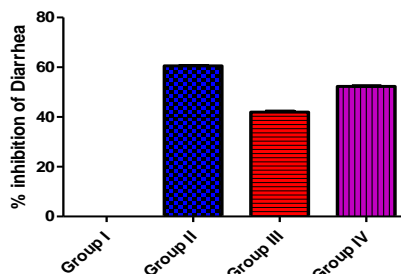
+ = Presence      — = Absence.



**Table 2:** Effect of Ethanolic extract of *Alstonia scholaris* on castor oil induced diarrhea in mice

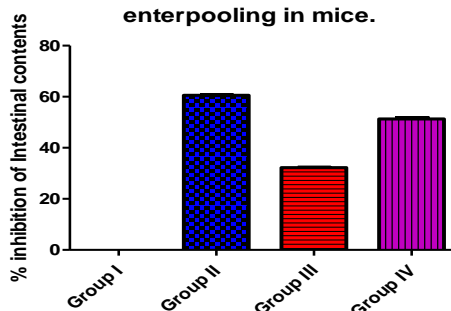
Group	Treatment	Dose	Total no.of feces	% inhibition of defecation	Total no,of diarrheal feces	% inhibition of diarrhea.
I	Saline + Castor oil	3ml/kg	17.56±0.88	—	14.76±1.20	—
II	LPM + Castor oil	5mg/kg	8.76±1.15	52.44	5.00±0.58	60.53±0.14
III	EEAS+ Castor oil	250mg/kg	12.67±0.29	30.25	8.00±.35	41.53±0.4
IV	EEAS+ Castor oil	500mg/kg	9.67±0.29	38.25	6.00±0.5	52.64±0.15

LPM= Loperamide, EEAS= Ethanolic extract of *Alstonia scholaris*. Data are shown as Mean ± SEM of five animals in each group. Values are significantly ( $p < 0.05$ ) different from each other. Data were analyzed by one-way ANOVA followed by Dunnett's test.

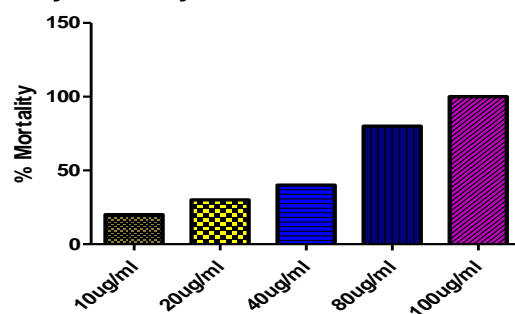
**Effect of Ethanolic extract of *Alstonia scholaris* on castor oil induced diarrhea in mice****Graph 1:****Table 3:** Effect of Ethanolic extract of *Alstonia scholaris* on castor oil induced enterpooling in mice.

Group	Treatment	Dose	Volume of intestinal content(ml)	Weight of intestinal content(g)	% inhibition of intestinal content
I	Saline + Castor oil	3ml/kg	0.67±0.04	1.76±0.03	--
II	LPM + Castor oil	5mg/kg	0.30±0.05	0.68±0.08	60.36 ±0.14
III	EEAS+ Castor oil	250mg/kg	0.48±0.04	1.18±0.08	32.95±0.14
IV	EEAS+ Castor oil	500mg/kg	0.4±0.05	0.85±0.09	51.70 ±0.52

LPM= Loperamide, EEAS= Ethanolic extract of *alstonia scholaris*. Data are shown as Mean ± SEM of five animals in each group. Values are significantly ( $p < 0.05$ ) different from each other. Data were analyzed by one-way ANOVA followed by Dunnett's test

**Effect of Ethanolic extract of *Alstonia scholaris* on castor oil induced enterpooling in mice.****Graph 2:****Table 4:** Results of zebra fish lethality bioassay of Ethanolic extract of *Alstonia scholaris*

Test sample	Conc.(µg/ml)	Log conc.	No.of alive larvae	%mortality	LC%50
EEAS	10	1.0	8	20	50.25
	20	1.3	7	30	
	40	1.6	6	40	
	80	1.9	2	80	
	160	2.2	0	100	

**Zebra fish lethality bioassay of Ethanolic extract of *alstonia scholaris*****Graph 3:****DISCUSSION**

Ethanolic extract of *Alstonia scholaris* leaves was evaluated for antidiarrheal effect in castor oil induced intestinal motility, intraluminal fluid accumulation as well as frequency of defecation and fluid accumulation in intestinal tract. Diarrhea can be induced by castor oil through the production of active metabolite ricinolic acid. Ricinolic acid increases peristaltic activity and produces permeability changes in the intestinal mucosal membrane to electrolytes and water. More precisely, castor oil elevates the biosynthesis of prostaglandin which results in irritation and inflammation of the intestinal mucosa to stimulate the motility and secretion. Castor oil model, therefore, incorporates both secretory and motility diarrhea.

The use of plant-derived medicines for the treatment of diarrhea is a common practice in many folk medications. Many people in the developing countries still rely on the treatment system employing medicinal plants. Relevantly, Ethanolic extract of *A.Scholaris* leaf used in our study were found to be significantly antidiarrheal in castor oil induced changes of gastrointestinal tract. However, 500mg/kg extract displayed the highest inhibitory action in the antidiarrheal models. The above mentioned function needs to be clarified with the possible mechanism how the extract works for exerted effects so that the plant species can be used for antidiarrheal formulation. As we discussed the central role of prostaglandin to cause diarrhea, several other mechanisms had been previously proposed to explain the diarrheal effect of castor oil which include inhibition of intestinal Na<sup>+</sup> K<sup>+</sup> ATPase activity, thus reducing normal fluid absorption.<sup>16</sup> It is possible that the EEAS were able to inhibit electrolyte permeability due to castor oil and prostaglandins release. Suppression of the intestinal fluid accumulation by the extract might also suggest the inhibition of gastrointestinal function. However, it is well proved that castor oil produces diarrhea due to its most active component ricinoleic acid through a hypersecretory response.<sup>17,18</sup> Therefore, it can be assumed that the antidiarrheal action of the extract was mediated by an antisecretory mechanism and also by reducing gastrointestinal motility contributed by the phytochemical agents possessed by the plant extract.

Phytochemical screening of the ethanolic extract of *A.scholaris* ensured the presence of carbohydrate, glycoside, alkaloids, tannins, flavonoids and steroids. The inhibitory activity of flavonoids on intestinal motility in a dose related manner was earlier reported. Apart from this, previous studies have shown that antidiarrheal and antidiarrheal properties of plants are due to tannins, alkaloids, saponins, flavonoids, sterol, triterpenes and reducing sugars.

The zebra fish lethality bioassay can be recommended as a guide for the detection of anti-tumor and pesticidal compounds because of its simplicity and low cost. The test is based on whether the zebra larvae are dead or alive at the end of the test. From the results of cytotoxicity test Ethanolic extract of *A.scholaris* an approximate linear correlation was found between logarithms of concentration VS % mortality. The results tend to suggest that its possible cytotoxic activity.

**CONCLUSION**

I concluded that the crude Ethanolic extract of *A.scholaris* possesses anti-diarrheal and cytotoxic properties, which correlates well with the traditional use of plants. Therefore further research is essential to find out the active principles responsible for those activities.

**Acknowledgment:** The authors are thankful to the Principal, Chairman and management of Chebrolu Hanumaiah Institute of Pharmaceutical Sciences, Chowadavaram, Guntur, for providing the facilities for conducting the study.

**REFERENCES**

1. Shoba FG, Thomas M. Study of antidiarrheal activity of four medicinal plants in castor oil-induced diarrhea. *J Ethnopharmacol*, 76, 2001, 73-76.
2. Guerrant RL, Van Gilder T, Steiner TS, Theilman MN, Slutsker L, Tauxe RV. Practice guidelines for the management of infectious diarrhea. *Clin Infect Dis*, 32, 2001, 331-35.
3. Maikere-Faniyo R, Van Puyvelde L, Mutwewingabo A, Habiaremye FX. Study of Rwandese medicinal plants used in the treatment of diarrhea. *JEthnopharmacol*, 26, 1989, 101-109.



4. Pharmacological activities of *Alstonia scholaris* linn. Apocynaceae - Pharmacognosy Reviews Vol 1, Issue 1, Jan-May, 2007.
5. Nadkarni A.K. Nadkarni's K.M. Indian Materia Medica, Vol. I, Popular Prakashan, Bombay, 80- 83, 1976. 3. The Wealth.
6. Kirtikar, K.R.; Basu, B.D.; Indian Medicinal Plants, Vol. II, P.565, 1975.
7. Sazada. S.Verma A, Rather A, A.Jabeen F, and Meghvansi M.K. Preliminary phytochemicals analysis of some important medicinal and aromatic plants. Adv. in Biol. Res., 3, 188-195 2009.
8. Shoba FG, Thomas M. Study of antidiarrheal activity of four medicinal plants in castor oil-induced diarrhea. J Ethnopharmacol, 76, 2001, 73-76.
9. Guerrant RL, Van Gilder T, Steiner TS, Theilman MN, Slutsker L, Tauxe RV. Practice guidelines for the management of infectious diarrhea. Clin Infect Dis, 32, 2001, 331-35
10. Maikere-Faniyo R, Van Puyvelde L, Mutwewingabo A, Habiyaremye FX. Study of Rwandese medicinal plants used in the treatment of diarrhea. J Ethonopharmacol, 26, 1989, 101-109.
11. Syder JD, Merson MH. The magnitude of the global problems of acute diarrheal disease: A review of active surveillance data. Bull WHO, 60, 1982, 605-613.
12. Lutterodt GD. Inhibition of gastrointestinal release of acetylcholine by quercetin as a possible mode of action of *Psidium guajava* leaf extracts in the treatment of acute diarrhea disease. J Ethnopharmacol, 25, 1989, 235-247.
13. Meyer, BN., Ferrign, RN., Putnam, JE., Jacobson, LB., Nicholas, DE., McLaughlin, JL., Brine shrimp: a convenient general bioassay for active plant constituents. Planta Medica. 45, 1982, 31-34.
14. McLaughlin, JL., Rogers, LL, Anderson, JE., The Use of Biological Assays to Evaluate Botanicals. Drug Information Journal, 32, 1998, 513-524
15. Goodman, LS., Gilman AG., Gilman A., Antiproliferative agents and immunosuppressive drugs. The Pharmacological basis of therapeutics. Macmillan Publishing Co., Inc., USA, 6th edition. 1980, 1299-1313.
16. Gaginella TS, Bass P. Laxatives: an update on mechanism of action. Life Sci, 23, 1978, 1001-1010.
17. Ammon HV, Thomas PJ, Phillips SF. Effect of the oleic acid and ricinoleic acid net jejunal water and electrolyte movement. J Clin Invest, 53, 1974, 374-379.
18. Gaginella TS, Stewart JJ, Olsen WA, Bass P. Action of ricinoleic acid and structurally related fatty acid on the gastrointestinal tract. II. Effect on water and electrolyte absorption in vitro. J Pharmacol Exp Ther, 195, 1975, 355-356.

Source of Support: Nil, Conflict of Interest: None.

