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EVALUATION OF ANTIDIARRHEAL ACTIVITY BY USING ETHANOLIC LEAF EXTRACT OF *ADENOSTEMMA LAVENIA*

Sachin Nath*, Dr. Govind Nayak, Dr. Abhishek Sharma, Dr. Mehta Parulben D.

Lakshmi Narain College of Pharmacy, Raisen Road, Bhopal, Madhya Pradesh, India-462022.

***Corresponding Author: Sachin Nath**

Lakshmi Narain College of Pharmacy, Raisen Road, Bhopal, Madhya Pradesh, India-462022.

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ABSTRACT

To evaluate the pharmacological activity against diarrhea of ethanolic extract of *Adenostemma Lavenia* (Family: Asteraceae) leaves. The present study was undertaken to evaluate the effects of EEAL on castor oil-induced diarrhea and magnesium sulphate-induced diarrhea method were examined. Each model consisted five groups, normal control, disease control,standard (loperamide), *Adenostemma Lavenia* leaf extract 250 mg/kg and 500 mg/kg were used. It was noticed that the fecal weight was reduced in the doses 250 and 500 mg/kg significantly in comparison to control. The extract dose (300 mg/kg) was significantly chosen for reducing the diarrheal condition in experimental rats. The extract was found to inhibit peristaltic movements of secretions in castor oil induced and magnesium sulphate induced model confirming its antidiarrheal activity, which might be due to its high flavonoid content. The results provide evidence that the ethanolic extract of *Adenostemma Lavenia* leaf possesses antidiarrheal activity and could be accounted for pharmacological effects and Herbal medicine.

KEYWORDS: *Adenostemma Lavenia*, Diarrhea, Castor oil, Pharmacological activity, Antidiarrheal.

INTRODUCTION

Among the death causing diseases, diarrhea is the leading one, especially in developing countries. Every year, millions of people die of diarrhea in third world's countries, so this is the most concerning issue for these countries. Children are more susceptible to this disease which is accounted as the second leading causes of death of children under five years old.[1] Diarrhea is gastrointestinal disorder, characterized by an increase in stool frequency and change consistency.[2] To combat this problem, the World Health Organization (WHO) has initiated a diarrhea disease control program to study traditional medicine practices and prevention approaches.[3,4] From a long time ago, plant kingdom played an important role for discovering new drug source. Number of therapeutics drug isolated from plant species. For the treatment of diarrhea, medicinal plants are a potential source of anti-diarrheal drugs. Drug from plant sources has negligible adverse effect and for their potential activities against diarrhea. Many international organizations have encouraged studies pertaining to the treatment and prevention of diarrheal diseases using traditional medical practices.[5,6] Plant kingdom provides an enormous reservoir of biologically active compounds with distinctive chemical properties which can prevent or cure diseases.

A. lavenia can be found in almost every province in Indonesia, but it is not widely cultivated. They described *A. lavenia* as a glandular-hairy or subglabrous stem; ovate, obtuse or acute apex, dentate or serrate leaves; grows in humid environments, areas with some shade, woodlands, brushwood, ditches, and along the roadside.[7] The phytochemical screening of the *Adenostemma Lavenia* leaves and other part revealed the presence of alkaloids, glycosides, carbohydrates, starches, phenolic compounds, flavonoids, proteins, pectin, mucilage, saponins, lipids, tannins, sterols and steroids.[8] A. lavenia is traditionally known to have several properties. The leaves effectively treat dysuria, aphthae, sore throat, sunburned skin, dysentery, and are used as an antispasmodic (as a reliever of muscle pain). Crushed leaves and stems are applied topically and believed to be effective for healing wounds, skin diseases, ulcers, headaches, toothaches, chest pain, diarrhea (rubbed on the stomach), and insect and caterpillar bites. A mixture of leaf paste and milk is used to treat dizziness.[9]

It showed Anti-Melanogenic, Antiaging, antimicrobial, anticancer, antioxidant, anti-inflammatory, antispasmodic, antitumor, lung injury, hypolipidemic and many other pharmacological effects.[10-13] No scientific work has so far evaluated the antidiarrheal activity of this plant. The main objective of this work is to evaluate the activities of *Adenostemma Lavenia* leaf extract on castor oil-induced and Magnesium sulphate induced diarrhea in rats.

MATERIAL AND METHODS

Collection and Authentication of plant

Adenostemma Lavenia leaves were collected from Sanjeevani Gardan, Bhopal the state of Madhya Pradesh during the month of March. The plant has been identified and authenticated by Dr. Saba Naaz Head of the Department Botany at the Safia college of science, Bhopal (M.P.).

Drying, size reduction and storage of plant material

The plants parts were dried under shade. It was pulverized to coarse powder with the help of mixer grinder. The coarse powder was passed through sieve No. 20 to maintain uniformity and packed into airtight container and stored in cool and dry place. This material was used for the further study.

Preparation of *Adenostemma Lavenia* leaves extract

Extraction of *Adenostemma Lavenia* was done by Soxhlet extraction method.[14]

Soxhlet Extraction: The coarse powder was packed tightly in the Soxhlet apparatus and extracted with 500ml 70% ethanol for 72 hours with occasional shaking maintained at 78°C throughout the extraction process. The extract was concentrated to ¼ of its original volume by evaporation. The resulting extract of *Adenostemma Lavenia* was subjected to phytochemical study.

Phytochemical Analysis of Crude Extracts

The crude extracts of plants obtained by solvent extraction were subjected to various qualitative tests to detect the presence of common chemical constituents as: alkaloid, glycoside, carbohydrate, phytosterols, saponins, tannin, flavonoids and protein etc.

Experimental Work

Animals: Adult Wistar rats of 150-200 g were used for the study. The animals were maintained under controlled conditions of temperature (23 ± 2°C), humidity (50 ±5%) and 12 h light-dark cycles. All the animals were acclimatized for seven days before the study. The animals were randomized into experimental and control groups and housed individually in sanitized polypropylene cages containing sterile husk as bedding. They had free assessed to standard pellets as basal diet and water *ad libitum*. Animals were habituated to laboratory conditions for 48 h prior to experimental protocol to minimize if any of non-specific stress.

Preparation of dose: The ethanolic extract of ***Adenostemma Lavenia* leaf part** was dissolved in suspending agent (1% CMC) before orally administered to the Rats. Standard drug was dissolved in suspending agent (1% CMC) before orally administered to the Rats.

Antidiarrheal Activity

Castor - oil induced diarrhea

The induction of diarrhea with castor oil results from the action of ricimoleic acid formed by hydrolysis of oil which produces changes in the transport of water and eletrolyte results in hypersecretory responses. Wistar rats weighing 200-350gms were fasted for 18hrs with free access to water and libitum. They were housed individually in cages and divided into five groups of six animals. Group I served as control and received normal saline 1ml/kg orally. Group II served as Disease control and received castor oil 1 ml/rat, orally. Group III served as standard drug loperamide 3 mg/kg p.o. Group IV and V received ethanolic extract 250 and 500 mg/kg p.o.

Group I: Normal control (Normal saline solution 1 ml/kg), orally.
Group II: Disease control (Castor oil 1 ml/rat), orally.
Group III: Standard (Loperamide 3mg/kg), orally.
Group IV: EEAL (250mg/kg), orally.
Group V: EEAL (500mg/kg), orally
After 1 hour later, castor oil 0.1 ml/ rat was administered orally. The animals were then caged singly in cages lined with white blotting paper. The total number of both dry and wet feces excreted were measured after 4 hours and compared with the control group.

Magnesium sulphate induced diarrhea

Wistar rats weighing 200-350gms were fasted for 18hrs with free access to water and libitum. They were housed individually in cages and divided into Five groups of six animals. Group I served as control and received normal saline 1ml/kg orally. Group II served as Disease control and received magnesium sulphate 2 mg/kg, orally. Group III served as standard drug loperamide 3 mg/kg p.o. Group IV and V received ethanolic extract 250 and 500 mg/kg p.o.

Group I: Normal control (Normal saline solution 1 ml/kg), orally.
Group II: Disease control (magnesium sulfate 2 mg/kg), orally.
Group III: Standard (Loperamide 3mg/kg), orally.
Group IV: EEAL (250mg/kg), orally.
Group V: EEAL (500mg/kg), orally

After 1 hour later, magnesium sulphate 1 ml/ rat was administered orally. The animals were then caged singly in cages lined with white blotting paper. The total number of both dry and wet feces excreted were measured after 4 hours and compared with the control group.

Evaluation: Every hour, total weight of faecal output, total weight of wet faeces, total number of faecal outputs, and number of wet faeces were recorded. A numerical score based on stool consistency was assigned as follows: normal stool= 1, semi-solid stool = 2 and watery stool = 3. And % inhibition of diarrhea was calculated as follows:

$$\% \text{ Inhibition of Diarrhea} = \frac{\text{Mean Number of wet defecation (control – test)}}{\text{Mean wet defecation of control}} \times 100$$

Statistical analysis: The data were expressed as mean±SEM. Results were analysed statistically by One-way ANOVA (analysis of variance) followed by Dunnett's t-test using standard statistical software. All the groups were compared with the control group in each model. The difference was considered significant if $p<0.05$.

RESULTS

Morphology and Phytochemical investigation

Morphological characteristics of *Adenostemma Lavenia* Leaves shown in Table 01. The percentage yields of the ethanolic extracts were found to be 13.25% w/w. The ethanolic extract showed the presence of alkaloids, carbohydrates, flavonoids, glycosides, proteins and saponins in *Adenostemma Lavenia* leaf part. Phytochemical screening of ethanolic extract of *Adenostemma Lavenia* shown in Table 02.

Table 01: Morphological characteristics of *Adenostemma Lavenia* leaves.

S. No.	Character	Observation
1	Color	Greenish
2	Odor	None
3	Taste	Characteristic
4	Size	4-7 cm. length

Table 02: Phytochemical screening of ethanolic extract of leaf part of *Adenostemma Lavenia.*

S. No.	Identification Test	Test name	Results
1	Alkaloids	Mayer's test	-
		Dragendroff's test	+
		Wagner's test	+
2	Glycosides	Killer-killani test	+
3	Carbohydrates	Molisch's test	+
		Fehling test	+
4	Tannins & Phenols	Gelatin test	+
		Ferric chloride test	+
5	Flavonoids	Shinoda test	+
		Alkaline reagent test	+
6	Steroids	Libermamm-Burchard test	+
		Salkowski test	+
7	Saponins	Foam test	+
8	Protein	Xanthoprotic	+
9	Gums & Mucilage	With 95% alcohol	-

(+) = Present, (–) = Absent

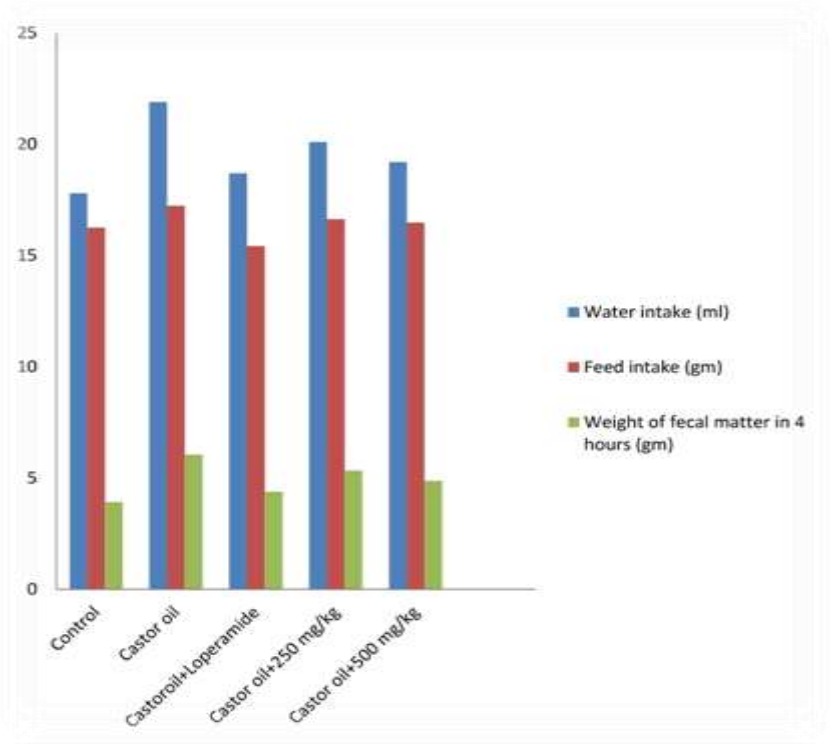
Effects of the ethanolic extract of *Adenostemma Lavenia* on castor oil-induced diarrhea

There was a significant anti-diarrheal effect according to the parameters observed and the results analysed. The castor oil treated rats showed a significant ($p<0.01$) increase in total number of faces and weight of fecal matter when compared to normal animals. The ethanolic leaf extract of *Adenostemma Lavenia*, showed a significant ($p<0.01$) decrease in total number of faces and weight of fecal matter when compared to castor oil treated rats. The maximum anti-diarrheal effect of the extract 500 mg/kg (0.10 ± 0.01), 71.50 % and that of 250 mg/kg extract (0.20±0.02), 64.92 % but it was lower than standard drug Loperamide treated animals. (Table no. 03)

Table 03: Results of Castor oil Induced Diarrhea of EEAL.

Group	Dose (mg/kg)	Time of onset of diarrhea (min)	Total no. of faeces in 4hrs	Weight of stool (gm)	% Inhibition of defecation
Normal Control	Normal saline solution (1 ml/kg)	60.0±0.6	1.8±0.2***	0.05±0.02***	-
Disease Control	Castor oil (1 ml)	143.8±2.0	6.9±1.0	0.72±0.01	-
Standard Control	Loparamide (3 mg/kg) + castor oil (1 ml)	198.2±0.2***	1.6±0.2***	0.01±0.02***	76.81 %
Test Control	Ethanolic extract (250 mg/kg) + castor oil (1ml)	165.0±0.1***	3.8±0.4**	0.20±0.02***	64.92 %
	Ethanolic extract (500mg/kg) + castor oil (1 ml)	185.0±0.3***	2.1±0.5**	0.10±0.01***	71.50 %

Results are Mean ± SEM and significantly different when compared with that of the control at * $P<0.05$, ** $P<0.01$, *** $P<0.001$.



Graph 01: Effect of EEAL on Anti-diarrhoeal effect on castor oil induced diarrhea in wistar rats.

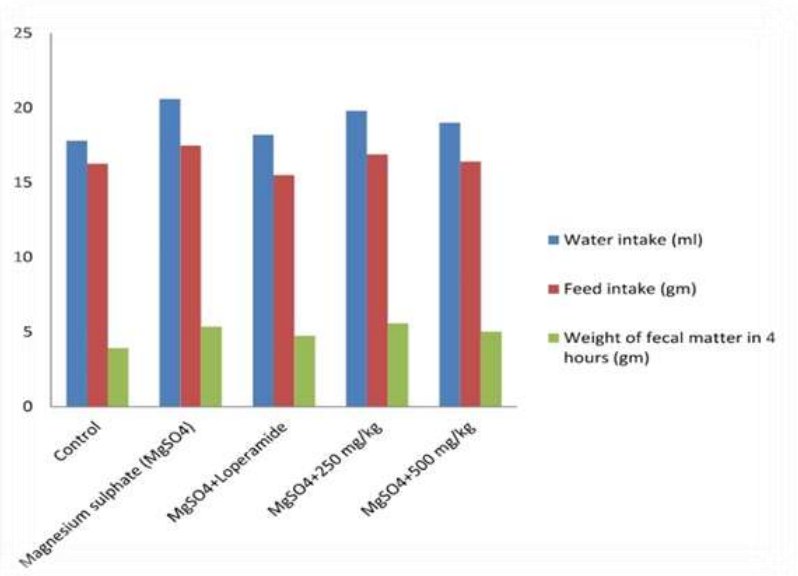
Effects of the ethanolic extract of *Adenostemma Lavenia* on Magnesium sulfate -induced diarrhea

The anti-diarrheal activity of the ethanolic leaf extract of *Adenostemma Laveniae*valuated by magnesium sulphate induced diarrhea in wistar rats. The anti-diarrheal activity was analysed by evaluating the parameters such as total number of faces in 4 hrs and weight of fecal matter. The magnesium sulphate induced diarrhea was confirmed by significant (P<0.01) elevation in total number of faces and weight of fecal matter when compared to normal rats. The EEAL at 250 and 500 mg/kg treatment showed significant decrease in total number of faces in 4 hrs and weight of fecal matter when compared to magnesium sulphate treated rats. The weight of the fecal matter in 4 hours reduced significantly (0.20±0.03) at 500 mg/kg as compared to (0.39±0.01) at 250 mg/kg EEAL treatment. The maximum anti-diarrheal effect of the extract 500 mg/kg, 73.54% and that of 250 mg/kg extract, 62.88 % but it was lower than standard drug Loperamide treated animals. (Table no. 04)

Table 04: Results of Magnesium sulfate induced diarrhea.

Group	Dose (mg/kg)	Time of onset of diarrhea (min)	Total no. of faeces in 4hrs	Weight of stool (gm)	% Inhibition of defecation
Normal control	Normal saline solution (1 ml/kg)	60.0±0.6	1.8±0.2***	0.12±0.14***	-
Disease control	Magnesium sulfate (2 mg/kg)	136.8±2.0	6.80 ± 0.34	0.68±0.02	-
Standard control	Loparamide (3mg/kg) + castor oil (1 ml)	202±0.1***	1.43 ± 0.77	0.10±0.98	79.97 %
Test control	Ethanolic extract (250mg/kg) + castor oil (1 ml)	176.0±0.3***	3.00 ± 0.98	0.39±0.01*	62.88 %
	Ethanolic extract (500mg/kg) + castor oil (1 ml)	197±0.1***	2.12± 0.45	0.20±0.03*	73.54%

Results are Mean ± SEM and significantly different when compared with that of the control at *P<0.05, **P<0.01, ***P<0.001



Graph 02: Effect of EEAL on Anti-diarrhoeal effect on magnesium sulphate induced diarrhea in wistar rats.

DISCUSSION

Diarrheas have long been recognised as one of the most common health problems in the developing countries. Imbalance in the absorption and secretion mechanisms in the intestinal tract result in frequent loss of watery stool (diarrhea). There are several mechanisms proposed to explain the diarrheal effect of castor oil including inhibition of intestinal Na+ K+ ATPase activity, consequently reducing normal fluid absorption, activation of adenylatecyclase or mucosal cAMP-mediated active secretion, and stimulation of prostaglandin formation and platelet activating factor.

The present study was conducted to assess the anti-diahorreal properties of ethanolic extract of leaf part of *Adenostemma Lavenia*. Castor oil induced and magnesium sulphate induced diarrhea model for anti-diahorreal activity.

The ethanolic extract of *Adenostemma Lavenia* leaves contains phenols, tannins, flavonoids, saponins, terpenoids, glycosides, and anthraquinones as asserted by preliminary phytochemical screening tests, and most of these secondary metabolites were reported to have an antidiarrheal activity. In the preliminary phytochemical screening, the extract was positive for flavonoids, saponins, tannins, phenols, terpenoids, and alkaloids. These secondary metabolites are effective as antioxidant, antineoplastic, anti-ulcer, anti-inflammatory, and immune stimulating agents. Flavonoids are thought to increase mucosal prostaglandin content, decrease histamine secretion from mast cells by inhibition of histidine decarboxylase, inhibit Helicobacter pylori growth, act as free radical scavengers, and inhibit H+/K+- ATPase.

As per literature review in acute oral toxicity study there were no behavioral changes seen up to 4 hrs and no mortality was observed up to the end of 24 hrs even at the maximum tested dose level of 2000 mg/kg per oral. The extract dose (500 mg/kg) was significantly choose for reducing the diarrheal condition in experimental rats. For the treatment of rats standard drug for diarrhea loperamide was used. However, it is well documented that castor oil produces diarrhoea due to its most active component ricinoleic acid through a hypersecretory response. Therefore, it can be assumed that the antidiarrheal activity of flavonoids has been ascribed to their ability to inhibit intestinal motility and hydro-electrolytic secretions which are altered in this intestinal condition. Antidiarrheal action of the extract was mediated by an antisecretory mechanism. The present study sought to assess the antidiarrheal activity of leaf extracts of *Adenostemma Lavenia*.

CONCLUSION

The present study "Evaluation of anti-diarrheal activity by using ethanolic leaf extract of ***Adenostemma Lavenia***." in experimental animal models was found effective in the anti-diarrhea activity due to the presence of phytoconstituents.

In conclusion we can say that extract dose (500 mg/kg) of ***Adenostemma Lavenia leaf*** show significantly reducing the condition of diarrhea with the help of flavanoids, saponin, alkaloid, tannins, phenols and some other phyto-constituents. The plant extract was also found to have optimal safety margin based on the limit test at 2000 mg/kg dose level acute toxicity test. Therefore, the plant is potentially useful to develop plant based products after further studies to identify the active principle and the mechanism of action.

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