

EVALUATION OF ANTIDIARRHOEAL ACTIVITY OF *SIDA RHOMBIFOLIA* LINN. ROOT

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ABSTRACT

The roots of *Sida rhombifolia* are used in traditional medicine for the treatment of diarrhoea. Thus the methanolic extract of *Sida rhombifolia* (Malvaceae) root was investigated for its antidiarrhoeal property to substantiate folkloric claim. The methanolic extract of *Sida rhombifolia*, at graded dose (200 and 400 mg/kg body weight) was investigated for antidiarrhoeal activity in castor oil induced diarrhoea. Results were comparable to that of standard drug Diphenoxylate (50mg/kg body weight). A single oral dose of *Sida rhombifolia* extract of 400 mg/kg body weight produced a significant decrease in the severity of diarrhoea. To understand the mechanism of its antidiarrhoeal activity, its effect was further evaluated on intestinal transit and castor oil induced intestinal fluid accumulation (enteropooling). Extract produced profound decrease in intestinal transit (57.73-61.84%) and significantly inhibited castor oil induced enteropooling comparable to that of intraperitoneal injection of standard drug atropine sulphate. The results showed that the methanolic extract of *Sida rhombifolia* have a significant antidiarrhoeal activity and supports its traditional uses in herbal medicine.

KEYWORDS: *Sida rhombifolia*, antidiarrhoeal activity, castor oil, diphenoxylate and atropine sulphate.

INTRODUCTION

Diarrhoeal diseases constitute a major cause of morbidity and mortality worldwide; specially in developing countries. More than 5 million children under the age of 5 years die every year because of diarrhoea.¹

The factors which tend to cause diarrhoea may be listed as under: (a) Deficient or reduced functioning of the colonic mucosa (b) Massive increase in peristalsis and propulsive activity (c) Impairment in the colonic reabsorption of water and electrolytes, and (d) Increased outpouring of fluids from the small intestine into the colon. The causative factors may be bacterial, amoebic, viral, fungal, dietary, hormonal or neuronal in nature.

Diarrhoea is a symptom marked by a rapid passage of faecal material through the gastrointestinal tract and a frequent passage of semisolid or liquid faeces. Prolong diarrhoea, whatever the cause, lead to electrolyte loss, the correction of which is of utmost importance.² Therefore, the search for safe and more effective agents has continued to be important areas of active research. Since ancient times, diarrhoea has been treated orally with several medicinal plants or their extracts based on folklore medicines.

The induction of diarrhoea with castor oil results from the action of ricinoleic acid formed by hydrolysis of the oil. Ricinoleic acid produces changes in the transport of water and electrolytes resulting in a hypersecretory response. In addition to hyper secretion, ricinoleic acid sensitizes the intramural neurons of the gut.³

Sida rhombifolia commonly known as Arrow-leaf sida in English, Pitabala and Swetbarela in Hindi, Atibala or Bala in Sanskrit and Dholabadianla or Nalobadianla in Oriya belonging to the family Malvaceae has been widely used in indigenous systems of Indian medicine due to its various medicinal properties. It has considerable reputation for its medicinal value in traditional medicine. It is a perennial or sometimes annual plant, a persistent, semi woody weed, 0.3 to 1.0 m tall. It is native to the tropics and subtropics. Common in pastures, roadsides, sunny waste areas. It grows from sea level to 2000 m above sea level in many soil types and from fertile to degraded conditions. Plants grow best in non-disturbed sites but are also found in cultivated land.⁴

The root of *S. rhombifolia* was used orally as an abortifacient.⁵ The dried root of *S. rhombifolia* was used orally for the abdominal upset and for the treatment of diarrhoea.⁶ The root of *S. rhombifolia* was used as an antivenin in human adult.⁷ The root infusion of *S. rhombifolia* was used orally in human adult for dysentery and diarrhoea.⁸ The root of *S. rhombifolia* has been used orally for the treatment of boils.⁹

It thus becomes important to identify and evaluate commonly available natural drugs as an alternative to currently used anti-diarrhoeal drug, which are not completely free from adverse effect.¹⁰

The acceptance of traditional medicine as an alternative form of health care and the development of microbial resistance to the available antibiotics has led researchers to investigate the antimicrobial herbal extracts.¹¹

MATERIALS AND METHODS

The plant was identified by the Department of Pharmacognosy, Birla Institute of Technology, Mesra, Jharkhand, India (Authenticated identification No.BIT/OS/1102/2008-09). After authentication, fresh roots were collected in bulk from young matured plants at the rural belt of Ganjam district of Orissa in the month of August. The roots were washed, shade dried and milled in to coarse powder by a mechanical grinder. The powder materials were passed through sieve number 40 and used for further studies.

Preparation of Plant Extract

The dried root powder were successively extracted in soxhlet apparatus by using different solvents (Petroleum Ether, Chloroform and Methanol) with increasing order of polarity in the ratio of drug to solvent (1: 8) for 72 hours. Each extracts were concentrated at reduced pressure using rotary evaporator and stored in desiccator. The type and extractive yield of different extracts of *S. rhombifolia* root were observed. In the present study the methanolic extract of *S. rhombifolia* was selected for the antidiarrhoeal activity.

Preliminary Phytochemical Screening

Preliminary phytochemical screening for the presence of alkaloids, carbohydrates, proteins, amino acids, flavonoids, tannins, glycosides, resins, phenols, volatile oils, steroids and saponins were carried out using standard test procedures.¹²

Acute Toxicity Study

The experimental protocols were approved by the Institutional Animal Ethical Committee (IAEC) of Royal College of Pharmacy and Health Sciences (12/09/IAEC). The acute toxicities of the extracts of the roots of *Sida rhombifolia* was determined as per the CPCSEA guidelines using the oral route on both Wistar rats and Swiss albino mice. The animals were divided into different groups of ten animals each. The control group received 10 ml/kg body weight of 0.5 % v/v Tween 80 in distilled water orally. The other groups received the extracts of *Sida rhombifolia* at dose levels of 100, 250, 500, 1000, 1500 and 2000 mg/kg body weight through oral route. After administration of dose the animals were observed continuously for the first 4 hr for toxic symptoms like motor activity, tremors, convulsions, tonic extension, muscle spasm, loss

of righting reflex, ataxia, sedation, hypnosis, lacrimation, diarrhea, salivation, writhing, skin colour etc and for mortality, if any, at the end of 24, 48 and 72 hr.¹³

Antidiarrhoeal Activity

Castor oil-induced Diarrhoea

Rats were fasted for 18 h and divided into four groups of six animals each. All groups of animals were taken in separate cages having paper placed below for collection of faecal matters. Diarrhoea was induced by oral administration of castor oil (1ml/rat) following the method of Mondal *et al.* (2007).¹⁴ Thirty minutes after castor oil administration, the group I (control group) received vehicle (0.5% v/v Tween 80 in distilled water). The group II received Diphenoxylate (50mg/kg) as standard. Groups III and IV were given the test extract (200 and 400 mg/kg). The number of both total number of faeces and total number of diarrhoeal faeces were counted for 6 hr for each group and were recorded. The mean \pm SEM of the total number of faeces and diarrhoeal faeces for 6 hr of treated groups were compared with that of control group and standard group.

Castor oil-induced Enteropooling

Intraluminal fluid accumulation was determined by the method of Mangesh *et al.*, (2009).¹⁵ Animals were divided into four groups of six animals each fasted overnight. Group I which received the vehicle (0.5% Tween 80 in normal saline) (2 ml/kg i.p.) served as the control group. Group II received atropine sulphate (3 mg/kg i.p.) and groups III and IV received extract of 200 and 400mg/kg i.p., respectively, one hour before the oral administration of castor oil (1 ml/rat). After 2 h the rats were sacrificed. The two ends of intestine were tied with thread. The intestine was removed and weighed. The intestinal content was collected by milking into a graduated cylinder and their volume was measured. The intestine was reweighed and the difference between full and empty intestine was calculated.

Gastrointestinal Motility Test

This experiment was done by using charcoal meal as a diet marker¹⁶. The rats were divided into four groups of six animals each and fasted for eighteen hours before the experiment. The first group (the control group) was orally administered with the vehicle (0.5% Tween 80 in distilled water). The second and third groups orally received methanolic extract at doses of 200 and 400 mg/kg body weight respectively. The fourth group received the standard drug, atropine sulphate (0.1 mg/kg i.p.). Thirty minutes later each animal was given 1ml of charcoal meal (10% activated charcoal in 5% gum acacia) orally. Each animal was sacrificed thirty minutes after administration of charcoal meal. The distance covered by the charcoal meal in the intestine was expressed as a percentage of the total distance traveled from the pylorus to the caecum.

Statistical analysis

The statistical analysis was performed for all animal model experiments by using one-way ANOVA (and nonparametric) followed by post test Bonferroni: compare selected pairs of columns (Graph pad prism 5.0.3.0 version). The mean value \pm SEM was calculated for each parameter. All the test samples and standard drug's parameter were compared with control group at respective time. Finally, the test drugs were compared with standard drug to know the significant difference between two groups at respective time.

RESULTS

The colour of the methanolic extract of *S. rhombifolia* root was reddish brown and the extractive yield was found to be 8.10% (W/W). The extracts did not show any signs of toxicity or mortality even at 2000 mg/kg of body weight dose. Therefore, 1/10th (200 mg/kg of body weight) and 1/5th (400 mg/kg of body weight) of this dose were used for pharmacological studies.

The methanol extract of *S. rhombifolia* root was positive for reducing sugars, alkaloids, tannins, saponins, flavonoids, sterols and glycosides.

In the castor oil-induced diarrhoeal experiment in rat, the methanolic extract of root of *S. rhombifolia*, at the doses of 200 and 400 mg/kg, reduced the total number of faeces as well as the total number of diarrhoeal faeces in a dose dependent manner (Table 1). The frequency of defecation and the wetness of fecal droppings when compared with untreated group, indicates an anti-diarrhoeal activity. The methanolic extract of root of *S. rhombifolia*, at the doses of 200 and 400 mg/kg, retarded the intestinal transit of charcoal meal in rat when compared to the control (Table 3).

The extract also led to a marked reduction in the weight and the volume of the intestinal contents on castor oil-induced enteropooling (Table 2).

DISCUSSION

Majority of people living in rural areas and those that are homeless or working in automobile garages, refineries and lather industries in India often suffer from diarrhoea due to eating of contaminated food and/or drinking of contaminated water. In developing countries, a quarter of infant and childhood mortality is related to the diarrhoea.¹⁷ Diarrhoea is a symptom of infection caused by a host of bacterial, viral and parasitic organisms most of which can be spread by contaminated water. It is more common when there is a shortage of clean water for drinking, cooking and cleaning and basic hygiene is important in prevention. Water contaminated with human faeces for example from municipal sewage, septic tanks and latrines is of special concern. Animal faeces also contain microorganisms that can cause diarrhoea. Diarrhoea can also spread from person to person, aggravated by poor personal hygiene. Food is another major cause of diarrhoea when it is prepared or stored in unhygienic conditions. Water can contaminate food during irrigation, and fish and seafood from polluted water may also contribute to the disease. The highest mortality rates have been reported to be in children less than five years of age. There are several reports on the epidemiological and experimental issues pertaining to world-wide acute-diarrheal disease.¹⁸

Plants containing flavonoides, terpenoids, steroids, phenolic compounds and alkaloids have been reported to have antimicrobial activity.^{19, 20}

The methanolic extract of *S. rhombifolia* at doses of 200 and 400mg/kg, reduced significantly in a dose dependent way. The dose of 400 mg/kg of *S. rhombifolia* treated seems to show an equivalent effect of that of 50 mg/kg of diphenoxylate.

Ricinoleic acid the active principle in castor oil caused changes in mucosal cell layer permeability, electrolyte transport and intestinal peristalsis, leading to prostaglandin secretion, which results in an increase in the secretion of water and electrolytes into the small intestine.²¹ The gut wall contains prostaglandins E and F with prostaglandin synthetase activity mainly in the mucosa that cause intestinal cramps and diarrhoea which might be due to effect on intestinal smooth muscle and secretions.²² Moreover prostaglandin contributes to the pathophysiological functions in gastrointestinal tract.²³ Further, the studies of Ferreria *et al.* (1972) also revealed an evidence for our study, that the prostaglandin biosynthesis inhibitors delayed castor oil induced diarrhoea.²⁴ The anti-diarrhoeal activity may thus be attributed to the presence of Tannins, the phytochemical which is known to reduce the effect through denaturing the proteins by the formation of protein tannate, thereby causing the intestinal mucosa more resistant and reduces secretion.²⁵

Methanolic extract also significantly reduced intestinal transit as observed by the decrease in transit motility of charcoal meal. This may be due to the fact that the extract may increase the reabsorption of water by decreasing intestinal motility as observed in the decrease

of intestinal transit by charcoal meal. The extract also led to a marked reduction in the weight and the volume of the intestinal contents on castor oil-induced enteropooling.

CONCLUSION

In conclusion, the results of this investigation revealed that methanolic extract contains pharmacologically active substance(s) with antidiarrhoeal properties. This provides the rationale for the use of the root extract of *Sida rhombifolia* as an anti-diarrhoeal drug by traditional healers. Further research is to be carried out to fractionate and purify the extract, in order to find out the molecule responsible for the anti-diarrhoeal activity observed.

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Table 1: Effect of methanolic extract of *S. rhombifolia* roots at different dose levels on castor oil- induced diarrhoea.

Groups	Total no. of faeces	Total no. of diarrhoeal faeces	Inhibition (%)	Total weight of faeces (g)	Inhibition (%)
Vehicle + Castor oil (1ml)	24.5±0.43	17.8±0.48	0	9.6±0.23	0
Diphenoxylate (50 mg/kg) + castor oil (1ml)	7.5±0.44 ^{***}	5.2±0.31 ^{***}	70.78	1.8±0.13 ^{***}	81.25
MeR (200mg) + castor oil (1ml)	13.5±0.42 ^{***}	[#] 8.7±0.88 ^{***}	51.12	4.2±0.13 ^{***}	56.25
MeR (400mg) + castor oil (1ml)	10.7±0.49 ^{***}	^{###} 5.8±0.40 ^{***}	67.41	^{###} 2.1±0.11 ^{***}	72.91

All values are expressed in Mean ± SEM, n=6; there is significant difference between control and test at ^{***}P <0.001, ^{**}P <0.01 and ^{*}P <0.05 significant level. There is no significant difference between standard and test drug at ^{###}P <0.001, ^{##}P <0.01 and [#]P <0.05 significant level

Table 2: Effect of methanolic extract of *S. rhombifolia* roots at different dose levels on castor oil enteropooling.

Group	Volume of intestinal content(ml)	Weight of intestinal content(gm)
Vehicle + castor oil (1ml)	4.2±0.10	4.5±0.07
Atropine sulphate + castor oil (1ml)	1.4±0.04 ^{***}	2.6±0.12 ^{***}
MeR (200mg) + castor oil (1ml)	[#] 2.0±0.09 ^{***}	[#] 3.3±0.11 ^{***}
MeR (400mg) + castor oil (1ml)	^{###} 1.6±0.12 ^{***}	^{###} 3.1±0.12 ^{***}

All values are expressed in Mean ± SEM, n=6; there is significant difference between control and test at ^{***}P <0.001, ^{**}P <0.01 and ^{*}P <0.05 significant level. There is no significant difference between standard and test drug at ^{###}P <0.001, ^{##}P <0.01 and [#]P <0.05 significant level.

Table 3: Effect of methanolic extract of *S. rhombifolia* roots at different dose levels on charcoal-induced gut transit changes.

Group	Distance traveled by charcoal meal (%)	Inhibition (%)
Vehicle(0.5% Tween 80 in distilled water)	71.03±1.23	0
Atropine sulphate (0.1 mg/kg)	26.22±0.47 ^{***}	63.08
MeR (200mg)	[#] 30.02±0.56 ^{***}	57.73
MeR (400mg)	^{###} 27.1±0.47 ^{***}	61.84

All values are expressed in Mean ± SEM, n=6; there is significant difference between control and test at ^{***}P <0.001, ^{**}P <0.01 and ^{*}P <0.05 significant level. There is no significant difference between standard and test drug at ^{###}P <0.001, ^{##}P <0.01 and [#]P <0.05 significant level.

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