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Original Article

ANTIDIARRHOEAL ACTIVITY OF AQUEOUS AND ALCOHOLIC EXTRACTS OF *HEMIDESMUS INDICUS* ROOT

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ABSTRACT

Objective: To analyse the antidiarrhoeal activity of *Hemidesmatus indicus* (L) R. Br root aqueous (HIRAE) and ethanolic (HIREE) extracts.

Methods: HIRAE and HIREE at the dose of 100 and 200mg/kg bw was used to assess antidiarrheal activity in albino rats. Antidiarrheal activity was studied with reference to castor oil diarrhea for assessing faecal score, intestinal transit and enteropooling and compared with loperamide.

Results: HIRAE and HIREE significantly reduced the diarrheal effect by decreasing faecal droppings, intestinal transit and intestinal fluid secretions. Ethanolic extract at 200mg/kg bw showed 75.5% protective effect in faecal score, 51.2% in intestinal dropping and 56.6% for intestinal fluid secretion.

Conclusion: Results of this study suggests that *Hemidesmus indicus* root could be considered as a better antidiarrheal agent

Keywords: *Hemidesmus indicus root,* Antidiarrhoeal activity, Enteropooling, faecal score, Castor oil, Intestinal transit.

INTRODUCTION

Diarrhea is one of the most common infections in developing countries like India. Incidence of diarrhea is high among the children under the age of 5-10 years every year [1]. It is a third leading killer of children in India, higher incidence is still continuing through WHO and GOI taken a vulnerable step to avoid it. Multiple etiological agents are responsible for causing diarrhea. Diarrhea is treated with multiple antimicrobial agents along with symptomatic therapy and ORT. These modern drugs against diarrhea have not been much applauded by the scientists. Drug resistance is one among the global problem because treatment may fail if infected strain is resistant to the prescribed antibiotics. Poongothai *et al*., [2] showed that outbreaks caused by antimicrobial resistant microorganisms were associated with an increase rate of hospitalization. To overcome these, scientists turned to search the drug from the nature [3].

The WHO also includes studies of traditional medicinal practices for treatment of diarrhea [4]. Traditionally many plants have been used for the treatment of diarrhea [5]. One among the common plant used for the treatment of diarrhea is the root of *Hemidesmus indicus*. It is a typical climbing vine found throughout India and belongs to Ascleipediaceae family. It has a very important role in the ayurvedic and unani medicinal preparations against diarrhea, appetite and fits [6]. It is commonly called as Indian sarsaparilla, anantamul, sariva, nannari. It has been used as folk medicine and as ingredient in Ayurvedic and Unani preparations against diseases of blood, inflammation, diarrhea, respiratory disorders, skin diseases, syphilis, fever, bronchitis, asthma, eye diseases, epileptic fits in children, kidney and urinary disorders, loss of appetite, burning sensation and rheumatism etc., [7].

The root is described as tonic, diuretic, and alterative. Root decoction helps in skin diseases, syphilis, elephantiasis, loss of appetite, blood purification and for kidney and urinary disorders [8]. Several biological activities like hepato protective, antithrombotic, anti-ulcerogenic, antiinflammatory, immunemodulatory, antidiabetic etc. Have been reported from various root extracts [9, 11, 12]. Lack of scientific support revealed that the antidiarrheal activity of aqueous extracts of *Hemidesmus indicus* (Linn.). In view of the above fact, in the present study, it is possible to evaluate the *in-vivo* antidiarrheal activity of root of this plant using standard methods.

MATERIALS AND METHODS

Plant material

The root of *Hemidesmus indicus* was collected along with aerial parts. The plant material was authenticated by Dr. John Britto, the Department of Botany, Rapinet Herbarium, St. Joseph's College, Trichy - 620002. The roots were shade dried and coarsely powdered in a mechanical grinder. The aqueous and ethanolic extract was prepared by soaked the plant material in water and ethanol for 3 days in the ratio of 1:10 (100 gm of root powder in 1000 ml ethanol/ water). The filtrate was taken and allowed to condense at 50° C and refrigerated for further use [12].

Animals

Albino rats of both sexes weighing 100-200g were utilized for the study. They were purchased from the TANUVAS, Chennai. They were stored in well equipped polypropylene cages at room temperature of 24°C and exposed to both light and also dark cycle. All the animals were allowed to acclimatise for two weeks in animal house of Srimad Andavan Arts and Science College, Tiruchirappalli. The animals were fed with a standard pelleted diet and water ad libitum. The container for the food and water was washed daily, as the food and water are renewed every day, to ensure hygiene and maximum comfort for the animal. The study protocol was approved by CPCSEA NO 790/03/ac/CPCSEA.

Castor oil-induced diarrhea

Albino rats of either sex (200-250g) were subdivided into seven groups of six animals each. They were fasted for 24h prior to the test, but allowed free access to water. Group I received normal saline, which serves as control; Group II received 2 ml of castor oil, which serves as disease control; Group II received standard drug of Loperamide 3mg/kg. Test groups IV and V received aqueous extract and test groups VI and VII received methanol extracts oral doses of 100 and 200 mg/kg b. w. All doses were administered orally. The animals were then housed singly in cages lined with transparent paper. One hour after pre-treatment with the extract, the animals were challenged with 1 ml of castor oil orally. Thereafter, they were observed for 4h for the presence of diarrhea defined as watery (wet), unformed stool [13]. The frequency of defecation and number of diarrheal faeces excreted in the recorded time was scored and

compared with control group. The results were expressed in percentage of inhibition.

Castor oil induced entero pooling

The Albino rats were divided in to five groups and each groups comprise of six rats only. They were fasted overnight and allowed the only access of water. Group I received normal saline, which served as control; Group II received 2 ml of castor oil, which serve as disease control; Group II received standard drug of Loperamide 3mg/kg. Test groups IV and V received aqueous extract and test groups VI and VII received methanol extracts oral doses of 100 and 200 mg/kg b. w. Castor oil was administered orally after 30 min of drug administration. Two hours later, rats were sacrificed, and the small intestine was removed after tying the ends with thread and weighed. The duodenal contents were collected by milking into a graduated tube and their volume was measured. The intestine was re weighed and the difference between full and empty intestine was calculated [14, 15]

Study of gastrointestinal tract mobility

Using charcoal meal as a diet marker. Albino rats of either sex (100-250g) were randomly subdivided into six groups of six rats each. They were fasted for 24 hours prior to the test, but were allowed free access to water. Group I received normal saline, which serves as control; Group II received 2 ml of castor oil, which serves as disease control; Group II received standard drug of Loperamide 3mg/kg. Test groups IV and V received aqueous extract and test groups VI and VII received methanol extracts oral doses of 100 and 200 mg/kg b. w. Thirty minutes after drug administration, 1 ml of charcoal meal (10% activated charcoal in 5% aqueous gum arabica was administered to all the animals in the study and thirty minutes later, all the rats were sacrificed and the small intestine was dissected out and the distance covered by the charcoal meal in the small intestine from the pylorus to the caecum was measured and expressed as a percentage of the distance traveled [16].

Statistical analysis

The experimental calculation was given as mean ± SD. The student t-test was used for the evaluation of significance.

RESULTS

Castor oil is used to induce diarrhea in experimental animals. Onset time of diarrhea was varied with the treatment group (table 1). It ranges from 42.8±1.7 mins to 104.3±1.9. Ethanol extracts at 200mg/kg bw treated animals released diarrheal faecal droppings at 104.3±1.9 mins only. Ethanol extracts at 200mg/kg bw revealed effective control of diarrhea when compared to other plants extract treated animal group with reference to total faecal output, release of wet faecal matter and percentage protection. By comparison, loperamide treated animal groups showed good percentage protection (84.9%). Similar effect was exhibited by ethanol extract 200 mg/kg bw treated animal group (Gp. VII). The extracts significantly reduced faecal droppings ($p<0.01$) after the administration of castor oil.

Table 1: Effect of *Hemidesmus indicus* root extracts on castor oil induced diarrhea in mice

Group	Treatment and Dose	Onset time	Total number of faecal metter	Number of wet faecal matter	% of wet stool	% Protection
I	Normal control	-	3.6± 0.8	0.2±0.4	5.5%	-
II	Disease control	42.8±1.7	21.2 ± 2.6	15.3±2.2	72.2%	-
III	Loperamide 4mg/kg bw	100.1±2.3	7.8 ± 0.7	2.3±0.8	29.5%	84.9%
IV	HIRAE 100mg/kg bw	80.5±2.4	13.8±0.8	9.6±0.8**	69.6%	37.3%
V	HIRAE 200mg/ kg bw	89.2±5.3	8.8±0.7	5.3±0.5**	60.2%	65.4%
VI	HIREE 100mg/kg bw	85.0±4.3	10.3 ± 1.2	6.3±0.8**	61.2%	58.8 %
VII	HIREE 200mg/kg bw	104.3±1.9	8.3 ± 1.2	3.3±0.5**	39.8%	78.5 %

Values are presented as Mean ± SD, (n=6); ** $p<0.01$

MIRAE and MIREE decreased the intestinal propulsion. This study evidenced the antidiarrheal action of extracts with reference to intestinal peristaltic movement. It was analysed by making use of marker meal charcoal. Aqueous extract at 200mg/kg bw, ethanolic extract 100 & 200mg/kg bw significantly reduced intestinal propulsion 58.7%, 54.8% and 41.7% respectively. Ethanolic extract reduced intestinal transit upto 41.7% with 51.2% protection (table 2).

Table 2: Effect of *Hemidesmus indicus* fruit pulp extracts on Charcoal induced gastrointestinal transit

Groups	Treatment and dose	Gastro intestinal transit			
		Mean intestinal length	Mean distance traveled	% GI transit	% Protection
I	Normal control	87.2 ± 1.56	56.46 ± 2.46	35.2	
II	Disease control Castor Oil 0.4 ml	86.5 ± 1.85	74.00 ± 3.60	85.5	
III	Loperamide 4mg/kg bw	84.7 ± 1.62	54.76 ±2.67	64.6	26
IV	HIRAE 100mg/kg bw	85.3±3.9	57.5±1.76	67.8*	22.3
V	HIRAE 200mg/ kg bw	88.0±3.3	49.7±3.32	58.7*	33.2
VI	HIREE 100mg/kg bw	86.0 ± 1.25	46.41 ± 2.95	54.8*	33.5
VII	HIREE 200mg/kg bw	85.1 ± 1.43	35.50 ± 3.10	41.7*	51.2

Values are expressed as mean ± SD. *($p< 0.05$) significant different when compared with the control.

Ethanolic extract of 100 and 200mg/kg bw dose effectively decreased the castor oil induced intestinal fluid accumulation upto 45.2% and 56.64% respectively, which was better than (36.47%) the effect produced by the known antidiarrheal agent loperamide (Gp. III). HIRAE also reduced intestinal fluid accumulation (table 3). Ethanolic extract shows higher reduction of the intestinal fluid (0.87±0.13 ml for 10mg/ml bw – Gp. VI and 0.69±0.21 ml – Gp. VII), accumulation when compared to loperamide (1.01±0.09 ml – Gp. III). HIREE at 200mg/kg bw showed significantly and comparatively better diarrheal protection in experimental animals. This extract showed more than 50% protection in all types of antidiarrheal study i. e., 75.5% protective effect in faecal score, 51.2% in intestinal dropping and 56.6% for intestinal fluid secretion.

Table 3: Effect of *Hemidesmus indicus* root extracts on castor oil induced Enteropooling rats

Groups	Treatment and Dose	Gastro intestinal transit		
		Mean weight of intestine (g)	Volume of intestinal content (ml)	% Protection
I	Normal control	5.13±2.16	1.31 ± 0.16	-
II	Disease control	6.32 ± 2.84	1.59 + 0.07	-
III	Loperamide 4mg/kg bw	7.29 ±1.69	1.01 ± 0.09	36.47
IV	HIRAE 200mg/kg bw	7.64 ±1.58	1.23 ±0.03*	22.67
V	HIRAE 400mg/ kg bw	7.31 ±1.76	1.12 ±0.12*	29.56
VI	HIREE 200mg/kg bw	8.15 ±2.32	0.87± 0.13*	45.28
VII	HIREE 400mg/kg bw	8.43 ±1.90	0.69 ± 0.21*	56.64

Values are expressed as mean ± SD. *($p< 0.05$) significant different when compared with the control.

DISCUSSION

In India, *Hemidesmus indicus* root infusion, decoction and sherbet were uses for the treatment of dysentery and diarrhea. The peoples of rural India experience the role of nannari root [7] Flavor of this plant root induce the people of India use this as a health drink. HIRAE and HIREE significantly reduced the number of faecal droppings, intestinal transit and secretions of the gastric system (table 1, 2 and 3). Ethanolic extract of this plant showed comparatively better antidiarrheal effect in experimentally induced diarrhoe in Albino rats.

Diarrhea is due to decrease in the absorption of water and lower level transport of electrolytes [17]. Excess of fluid loss is shown in the event of diarrhea. This is due to loss of absorptive mechanisms in the intestinal epithelium. Hyperactivity of colonic epithelium is evident during the course of diarrhea. It is evident that castor oil has the ability to produce diarrhea due to the presence of ricinolic acid in it. The ricinolic acid of castor oil releases body fluid and caused accumulation of electrolytes in the intestinal lumen by creating irritations and inflammation in the intestinal mucosa and involved in the release of inflammatory mediators like histamines and prostagladins. This prostagladins may in the secretion of mucus in the small intestine area. Prostagladins have been considered to be a best diarrhoegenic agent in animals and human. The prostagladin biosynthesis inhibition considered to cause delay castor oil induced diarrhea [18]. Several types of mechanical factors have been explained for the plants to have the antidiarrheal power [19]. These include the inhibition capacity of intestinal Na+, K+-ATpase activity to reduce normal fluid absorption.

Ricinolic acid of castor oil also induces the release of nitric acid in the inflamed intestinal cells. Nitric acid stimulates gastric secretions and increases epithelial permeability. It also causes oedema in the intestinal mucosa thereby preventing reabsorption of intestinal fluid [20]. One of our previous studies revealed that the phytochemical present in the plant reduces the release of prostaglandins and therefore considered to delay castor oil induced diarrhea. Plant extracts not only reduce castor oil induced diarrhea but also decrease microbial burden of the intestine and reduces the toxigenic effect created by the microorganisms [21].

Rajan *et al.,* [5], described the availability of phytochemical like tannins, flavonoids, coumarins, phenolic compounds in *Hemidesmus indicus* root extracts. These compounds act on the castor oil induced diarrhea in different mechanisms. Flavonoids exerts antidiarrheal activity by inhibiting the release of autocoids and prostaglandins, by inhibiting contractions caused by spasmogens, by stimulates normalization of the deranged water transport across the mucosal cells and also by inhibiting GI release of acetylcholine. Phenolic compounds makes intestinal mucosa more resistant and reduces secretion, stimulates normalization of deranged water transport across the mucosal cells and reduction of the intestinal transit, blocks the binding of B subunit of heat-labile enterotoxin to GM_1, resulting in the suppression of heat-labile enterotoxin-induced diarrhea, astringent action, increases supply of digestible proteins by animals by forming protein complexes in rumen, interferes with energy generation by uncoupling oxidative phosphorylation, causes a decrease in G. I. metabolism [22]. Steroids enhance intestinal absorption of Na+ and water.

Antidiarrheal activity of this extract may also be due to the presence of denatured proteins, which form protein tannates. Protein tannates make the intestinal mucosa more resistant and hence, reduce secretion [23]. This can be due to the fact that the extracts increased the reabsorption of water by reducing intestinal motility as observed in the decrease of intestinal transit by charcoal meal. Loperamide, apart from regulating the gastrointestinal tract, is also reported slowing down transit in the small intestine; reduce colon flow rate and consequently an effect on colonic motility [24]. Flavonoids present in the extract may be able to inhibit the bacterial motility and inhibit the prostaglandin secretion [25]. Anti-diarrhea activities of flavonoids have been ascribed to their ability to inhibit intestinal motility and hydroelectrolytic secretions which are deem altered in diarrheic conditions [26]. Tannins present in anti-diarrhea plants denature proteins in the intestinal mucosa by forming protein tannates which may reduce secretion. Studies on the functional role of tannins also reveal that they could also bring similar functions by reducing the intracellular Ca^{2+} inward current or by activation of the calcium pumping system, which induces muscle relaxation [27].

To conclude the study of *Hemidesmus indicus* root, it has been suggested to be used as medicine in folk as Siddha and Ayurvedha for the treatment of diarrhea. The compound to be separated for knowing the activity of diarrhea remains to be identified. Further more studies were adopted to know the mechanical action of antidiarrheal activity of this plant.

CONFLICT OF INTERESTS

Declared None

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