ANTIDIARRHOEAL ACTIVITY OF AQUEOUS AND ALCOHOLIC EXTRACTS OF HEMIDESMUS INDICUS ROOT

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INTRODUCTION

Diarrhea is one of the most common infections in developing countries like India. Incidence of diarrheal 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 microorganism was 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 diarrheal is the root of Hemidesmus indicus. It is a typical climbing vine found throughout India and belongs to Asclepiadaceae family. It has a very important role in the ayurvedic and unani medicinal preparations against diarrheal, 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, diarrheal, 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, anti-inflammatory, immunomodulatory, anti-diabetic 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. The plant material was collected along with aerial parts. 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, diarrheal, 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].

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Ethanolic extract of 100 and 200 mg/kg bw dose effectively reduced the castor oil-induced intestinal fluid accumulation (VII), compared with control group. The results were expressed in percentage of inhibition.

**Castor oil induced entero pooling**

The Albino rats were divided into five groups and each group comprised 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 serves as disease control; Group II received standard drug of Loperamide 3 mg/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 served as disease control; Group II received standard drug of Loperamide 3 mg/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 200 mg/kg bw treated animals released diarrheal faecal droppings at 104.3 ± 1.9 mins only. Ethanol extracts at 200 mg/kg bw revealed effective control of diarrhea when compared to other plants extracts 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.

**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 disease control; Group II received standard drug of Loperamide 3 mg/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].

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

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment and Dose</th>
<th>Onset time</th>
<th>Total number of faecal matter</th>
<th>Number of wet faecal matter</th>
<th>% of wet stool</th>
<th>% Protection</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Normal control</td>
<td>-</td>
<td>3.6±0.8</td>
<td>0.2±0.4</td>
<td>5.5%</td>
<td>-</td>
</tr>
<tr>
<td>II</td>
<td>Disease control</td>
<td>42.8±1.7</td>
<td>21.2±2.6</td>
<td>15.3±2.2</td>
<td>72.2%</td>
<td>-</td>
</tr>
<tr>
<td>III</td>
<td>Loperamide 4mg/kg bw</td>
<td>100±1.23</td>
<td>7.8±0.7</td>
<td>2.3±0.8</td>
<td>29.5%</td>
<td>84.9%</td>
</tr>
<tr>
<td>IV</td>
<td>HIRAEE 100mg/kg bw</td>
<td>80±8.2</td>
<td>13.8±0.8</td>
<td>9.6±0.8**</td>
<td>69.6%</td>
<td>37.3%</td>
</tr>
<tr>
<td>V</td>
<td>HIRAEE 200mg/kg bw</td>
<td>89.2±5.3</td>
<td>0.8±0.7</td>
<td>5.3±0.5**</td>
<td>60.2%</td>
<td>65.4%</td>
</tr>
<tr>
<td>VI</td>
<td>HIRE 100mg/kg bw</td>
<td>85.0±4.3</td>
<td>10.3±1.2</td>
<td>6.3±0.8**</td>
<td>61.2%</td>
<td>58.8%</td>
</tr>
<tr>
<td>VII</td>
<td>HIRE 200mg/kg bw</td>
<td>104.3±1.9</td>
<td>8.3±1.2</td>
<td>3.3±0.5**</td>
<td>39.8%</td>
<td>78.5%</td>
</tr>
</tbody>
</table>

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

MIRAEE and MIREEE decreased the intestinal propulsion. This study evidenced the antidiarraheal action of extracts with reference to intestinal peristaltic movement. It was analysed by making use of marker meal charcoal. Aqueous extract at 200 mg/kg bw, ethanol extract 100 & 200 mg/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**

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

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

Ethanolic extract of 100 and 200 mg/kg bw dose effectively decreased the castor oil-induced intestinal fluid accumulation upto 45.2% and 56.6% respectively, which was better than (36.4%) the effect produced by the known antidiarraheal agent loperamide (Gp. III). HIRAEE 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. II). HIREE at 200 mg/kg bw showed significantly and comparatively better diarrheal protection in experimental animals. This extract showed more than 50% protection in all types of antidiarraheal study i. e., 75.5% protective effect in faecal score, 51.2% in intestinal dropping and 56.6% for intestinal fluid secretion.
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 prostaglandins. This prostaglandins may in the secretion of mucus in the small intestine area. Prostaglandins released by inhibiting the release of autocoids and prostaglandins, by stimulates calcium pumping system, which induces muscle relaxation [27].

Ricinolic acid of castor oil also induces the release of nitric acid in the inflamed intestinal cells. Nitric acid stimulates gastric secretions 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 autooids and prostaglandins, by inhibiting contractions caused by spasmsgenes, 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 GM1, 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.

Anti-diarrheal 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 extract 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 hydroelectrolyt 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 Ca2+ 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

**REFERENCES**