ANTIDIARRHEAL ACTIVITY OF METHANOLIC EXTRACT OF Rumex Nervosus.

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ABSTRACT

Rumex nervosus vahl leaves were evaluated for antidiarrheal potential against experimental models of diarrhea in mice and rats. Methanolic extract of leaves of Rumex nervosus vahl (LRN) was evaluated against charcoal meal test in mice and castor oil induced diarrhea in rats. LRN was given at two different doses of 0.5 g/kg p.o and 1 gm/kg p.o. The effects obtained were compared with atropine (5 mg/kg s.c). LRN (1 gm/kg p.o) produced significant reduction in gastrointestinal motility following charcoal meal in mice. It also showed significant inhibitory activity against castor oil induced diarrhea in rats. The effect produced by LRN was less when compared to atropine (5 mg/kg s.c). LRN (0.5 gm/kg p.o) did not show any significant reduction in castor oil induced diarrhea. However, it significantly decreased gastrointestinal motility when tested by charcoal meal test. The results obtained established the efficacy of LRN as an antidiarrheal agent.

Key words: Rumex nervosus; Diarrhea; Charcoal meal; Castor oil.

INTRODUCTION

Diarrhea is among the major diseases for which traditional medicine is utilized at large-scale in developing countries. Of the estimated 15 million deaths per year among children under the five years of age, 29% to 39% is due to diarrhea1. Majority of Ethiopian population in the rural areas rely on traditional medicines. A large number of plants are used for the treatment of diarrhea. One of the plants used for the treatment of diarrhea in Ethiopia is Rumex nervosus vahl. The plant is commonly known as ‘Embwacho’ in most parts of Ethiopia. It is a shrub of 2 mm height or more. It is found in Tigray, Gondar, Gonjam and Wello regions of Ethiopia. The plant is also found in Eriteria in the western region2.

The leaves of the plant are usually boiled with water, filtered and the water extract is consumed to reduce non-specific diarrhea1. The plant is also used for the treatment of wounds, eczema, typhus and rabies. Apart from this, it is used for treatment of acne, as a hypoglycemic agent and as ophthalmic antiseptic in Yemen24.

Rumex nervosus vahl contains three flavonoids; kaempferol, quercetin and isohamnetin. Quercetin is present in greater amount than kaempferol and isohamnetin5. Quercetin is known to inhibit the release of acetylcholine in gastrointestinal tract6. In addition to flavonoids, it contains tannins, alkaloids, saponins, amino acid (12 free and 11 protein amino acids), glucuronic acid and glucose5.

Since, there is no study to substantiate the traditionally claimed antidiarrheal activity of the plant, the present study was undertaken to evaluate antidiarrheal activity of leaves of Rumex nervosus vahl.

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Experimental:

Collection of plant material: The whole plant was collected in April 2003 from the Addis Ababa city, Ethiopia. Natural Herbarium, Department of Biology, Faculty of Science, Addis Ababa University identified the plant. The leaves separated from the plant, were dried in open air and powdered.

Preparation of the extract: The dried powdered leaves of Rumex nervosus (116.5 gm) were macerated in 550 ml of methanol (80%). The maceration was carried out at room temperature in a closed vessel with infrequent shakings for 72 hours. The extract was then filtered and the marc obtained after filtration was macerated twice with 300 ml of 80% methanol each once for 48 hours and then for 24 hour. All the three extracts obtained were put together and the solvent was removed using rota-vapour at 55°C, a reddish brown semisolid mass was obtained. This was stored in a vacuum drier at 40°C for three days and finally a resin like reddish brown semisolid (yield 16.2% w/w with respect to dry starting material) was obtained.

Animals: Albino mice weighing between 28-34 gm and Wistar rats weighing between 113-218 gm of either sex were used. The animals were maintained at Animal House, School of Pharmacy, Addis Ababa University for 10 days prior to study. The animals were fed with standard diet (Kaliti Food Processing, Addis Ababa, Ethiopia) and water ad libitum during the maintenance period

Acute toxicity study: Acute toxicity study was performed in different groups of mice. Each group consisted of six animals. The animals were fasted for 12 hours prior to study. The methanolic extract of leaves of Rumex nervosus (LRN) was administered in the form of aqueous suspension made using tragacanth (1%) as suspending agent, the volume of the extract administered did not exceed 1 ml/100g p.o body weight. Doses of 250 mg/kg, 500 mg/kg, 1000 mg/kg and 2000 mg/kg p.o body weight were administered. The animals were observed for 48 hours after drug administration.

Gastrointestinal motility test: Each mouse was administered orally with 0.3 ml of charcoal meal, animals were either treated with solvent, which was a suspension of tragacanth (1%) in water (1 ml/100 gm p.o), LRN at a dose of 0.5 gm/kg p.o or 1 gm/kg p.o or atropine (5 mg/kg s.c). Fifteen minutes after the administration of charcoal meal, animals were sacrificed by cervical dislocation. The abdomen was opened and the whole small intestine was removed. The total length of the intestine and the length traversed by the charcoal were measured. The percentage of the movement of the charcoal was calculated by using the formula

\[
\text{Distance traversed by the charcoal} \times 100 = \frac{\text{Distance traversed by the charcoal}}{\text{Total length of the small intestine}}
\]

Castor oil induced diarrhea: Female Wistar rats were fasted for 18 hours with water ad libitum in cages having mesh bottom to prevent coprophagy. Solvent (1% aqueous tragacanth at the dose of 1 ml/300 gm p.o), LRN (0.5 gm/kg p.o or 1 gm/kg p.o) or atropine (5 mg/kg s.c) was administered to different groups of animals. Thirty minutes later, castor oil (1 ml/rat p.o) was administered. The animals were then caged individually and examined for diarrhea for a period of 4 hours after castor oil challenge. Diarrhea was defined as the presence of fluid material in the stools. The time of onset of diarrhea, total number of stool passed and the number of wet stool passed during 4-hour period were recorded for each animal.

Statistical analysis: Statistical significance was determined using one-way ANOVA followed by Dunnett's test using Graphpad Instat software (version 3.0 for Windows 3.00 (Graphpad software, SanDiego, California, USA). P<0.05 was used to indicate statistical significance.

Results:

Acute toxicity study:

No sign of toxicity was observed till 48 hours after drug administration. Hence, the drug was considered as safe and a working dose of 1 gm/kg p.o body weight was selected for the present study.

Gastrointestinal motility test: LRN (1 gm/kg p.o) and atropine (5 mg/kg s.c) significantly decreased propulsion of charcoal meal through the gastrointestinal tract when compared with control (P<0.01). LRN at a dose of 0.5 gm/kg p.o was also effective in decreasing the gastrointestinal motility (P<0.05) when compared with control (Table 1).
Table 1: Inhibition of gastrointestinal motility in mice.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Movement of charcoal meal (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Solvent (1 ml/100 gm p.o)</td>
<td>44.25 ± 1.697</td>
</tr>
<tr>
<td>LRN (0.5 gm/kg p.o)</td>
<td>35.00 ± 2.408</td>
</tr>
<tr>
<td>LRN (1 gm/kg p.o)</td>
<td>31.50 ± 2.306</td>
</tr>
<tr>
<td>Atropine (5 mg/kg s.c)</td>
<td>28.60 ± 1.892</td>
</tr>
</tbody>
</table>

All values are mean ± SEM. n=6, *P<0.05, **P<0.01 when compared with solvent treated group.

Castor oil induced diarrhea: Castor oil (1 ml/rat p.o) produced severe diarrhea in control animals. The stools were wet and viscous. Treatment of rats with LRN (1gm/kg p.o) reduced significantly the total number of stools and number of wet stools (P<0.05 and P<0.01 respectively). The treatment also caused a significant increase (P<0.05) in the latent period when compared with solvent treated control (Table 2).

Table 2: Effect of castor oil induced diarrhea.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Latent period</th>
<th>Total Number of Defecation</th>
<th>Number of wet defecation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Solvent (1 ml/300 g.p.o)</td>
<td>59.345 ± 4.456</td>
<td>10.78 ± 1.548</td>
<td>10.78 ± 1.548</td>
</tr>
<tr>
<td>LRN (0.5 gm/Kg p.o)</td>
<td>63.341 ± 5.341</td>
<td>11.45 ± 3.135</td>
<td>11.45 ± 3.135</td>
</tr>
<tr>
<td>LRN (1 gm/Kg p.o)</td>
<td>76.25 ± 4.033</td>
<td>4.80 ± 0.969*</td>
<td>4.4 ± 0.881*</td>
</tr>
<tr>
<td>Atropine (5 mg/Kg s.c)</td>
<td>83.24 ± 3.609</td>
<td>4.98 ± 0.992</td>
<td>4.4 ± 0.341*</td>
</tr>
</tbody>
</table>

All values are mean ± SEM. n=6, *P<0.05, **P<0.01 when compared with solvent treated group.

Similar results were obtained in animals treated with atropine (5 mg/kg s.c). However, LRN at a dose of 0.5 gm/kg did not show any significant reduction in total number of stools and number of wet stools. LRN (0.5 gm/kg p.o) was also not effective in increasing the latent period.

Discussion: Charcoal meal test is one of the most widely used methods to evaluate the effect of drugs on gastrointestinal motility. LRN decreased the gastrointestinal motility in the above-mentioned test.

Castor oil is a purgative, which is used to induce experimental diarrhea. Recinoleic acid liberated from castor oil irritates and inflames the intestinal mucosa leading to prostaglandin release, which stimulates motility and secretion. The castor oil model therefore incorporates both motility and secretion. LRN (1gm/kg p.o) decreased castor oil induced diarrhea suggesting that it may be effective against both motility and secretory components of diarrhea.

The exact mechanism of action and constituent responsible for antidiarrheal activity is not known. However, as mentioned earlier, Rumex nervosus leaves contain quercetin, which has been shown to inhibit release of acetylcholine in gastrointestinal tract. Inhibition of gastrointestinal release of acetylcholine leads to inhibition of motility and secretion. Furthermore, the antidiarrheal activity may also be due to the presence of tannins in the leaves of Rumex nervosus.

Conclusion

LRN (1 gm/kg p.o) decreased gastrointestinal motility in charcoal meal test. The extract also reduced severity of diarrhea when animals were challenged with castor oil. Therefore, LRN possess antidiarrheal activity.

References: