Antidiarrhoeal activity of *Rhus javanica* ripen fruit extract in albino mice

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**Abstract**

The antidiarrhoeal effects of the methanolic extract of *Rhus javanica* ripen fruits (MERJ) were investigated by employing four experimental models of diarrhoea in Swiss albino mice. MERJ treated mice, showed significant reduction in the faecal output and protected them from castor oil-induced diarrhoea. The extract also reduced the intestinal fluid secretion induced by MgSO$_4$ and gastrointestinal motility after charcoal meal administration in the albino mice. No mortality and visible signs of general weakness was observed in the mice following the test extract administration up to 2000 mg/kg dose.

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**Keywords:** *Rhus javanica*; Antidiarrhoeal activity

1. **Introduction**

In order to combat the problems of diarrhoea globally, the World Health Organisation in its Diarrhoeal Disease Control programme has given a special emphasis on the use of traditional folklore medicines in the control and management of diarrhoea [1]. *Rhus javanica* is a small tree, which is abundant in the hilly areas of Manipur, north-east India. The ripen fruits of this plant have a long history of traditional medicine use among the traditional healers of Naga tribal community in Manipur, to treat dysentery and diarrhoea as well as the other gastrointestinal disorders. Barring a few studies related to testing of its efficacy against herpes
simplex and cytomegalus virus [2–5], there is apparently no reference available in the literature regarding the antidiarrhoeal effects of this plant either in humans or in any animal models. Employing experimentally induced diarrhoea in Swiss albino mice, in the present study we were interested to investigate whether the acclaimed antidiarrhoeal effect of *R. javanica* has any scientific justification.

2. Experimental

2.1. Plant material

The ripen fruits of *R. javanica* L. (Anacardiaceae) were collected from Paoyi village (Manipur, India) in the month of January, 2002. The plant was identified and authenticated by Dr P.B. Gurung, Department of Botany, North-Eastern Hill University (NEHU), Shillong. A voucher specimen of the plant was deposited at NEHU Herbarium.

2.2. Preparation of the extract

The shade dried ripen fruits of *R. javanica* pulverized were Soxhlet extracted with MeOH [6] to give an extract (yield: 13.78%), which was stored at –4 °C until use.

2.3. Animals

Swiss albino mice, weighing 25–30 g, were used for the study. They were kept at standard environmental conditions and fed with standard rodent diet (Pranav Agro Industries Ltd., Delhi) and water ad libitum.

2.4. Preliminary acute toxicity test

The test extract was administered orally in 2% gum acacia at a dose—62.5, 125, 250, 500, 1000 and 2000 mg/kg to a group of 5 animals each. Simultaneously, the controls were given 2% gum acacia. The general signs and symptoms of toxicity, intake of food and water and mortality were recorded for 48 h.

2.5. Antidiarrhoeal experiments

The antidiarrhoeal efficacy of *R. javanica* fruits methanolic extract (MERJ) was assessed using the following four experimental models.

2.5.1. Measurement of faecal output

Five groups of animals were housed in separate cages having paper placed below for collection of faecal matters. Group 1 (control) received 0.5 ml of 2% gum acacia; groups 2–4 were treated with MERJ at 100, 200 and 500 mg/kg, p.o.,
respectively. The 5th group received 0.5 ml of 5-mg/kg loperamide. The faecal material collected for 12 h post treatment was dried in an incubator and weighed. The percentage reduction in the faecal output was determined [7].

2.5.2. Castor oil-induced diarrhoea

In overnight fasted male mice, diarrhoea was induced by oral administration of castor oil (0.5 ml/mouse, p.o.). The animals were randomized into five groups of 5 mice each. Group 1 served as control and received 0.5 ml of 2% of gum acacia. Groups 2–4 were given orally the test extract (100, 200 and 500 mg/kg) 1 h prior to castor oil administration. The remaining group 5 received 5 mg/kg of loperamide as standard. The percentage protection from diarrhoeal droppings was calculated as described by Akah [8].

2.5.3. Enteropooling assay

Five groups of 5 animals each fasted overnight were used. Group 1 was used as control, while groups 2–4 received the test extract (100, 200 and 500 mg/kg p.o., respectively). The last group 5 was given the standard drug, loperamide (5 mg/kg). One hour later, all groups were given the diarrhoeal agent (0.5 ml/mouse of a 10% aq MgSO₄, orally). They were killed 30 min later and the small intestines were collected and weighed to find out the accumulation of intestinal fluid secretion evoked by MgSO₄ [9].

2.5.4. Gastrointestinal transit test

Five groups of 5 animals each fasted overnight were used. The test extract was given orally to group 2–4 (100, 200 and 500 mg/kg, respectively), while group 1 was used as control; the 5th group received the loperamide (5 mg/kg) as a standard. Five minutes later, 0.5 ml of a 3% charcoal suspension in 5% suspension of tragacanth powder was administered orally to each mouse. All the mice were killed by cervical translocation 30 min later and the distance travelled by the charcoal plug from pylorus to caecum was determined and expressed as a percentage of the total length of the small intestine [10].

2.6. Statistical analysis

The significance of difference between the means was determined by the Student’s ‘t’-test and the results were regarded as significant when \( P < 0.05 \).

3. Results

3.1. Preliminary acute toxicity test

It was observed that oral administration of methanolic extract of *R. javanica* fruits to the mice up to 2000 mg/kg dose neither showed any mortality or any visible clinical signs of general weakness in the animals.
Table 1
Effect of the methanolic extract of *R. javanica* ripen fruits (MERJ) on faecal output in male albino mice

<table>
<thead>
<tr>
<th>Experimental group</th>
<th>Dose (mg/kg, p.o.)</th>
<th>Dried faecal output per 100 g of mice*</th>
<th>% Reduction in faecal output</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>–</td>
<td>0.482±0.071</td>
<td>0.00</td>
</tr>
<tr>
<td>MERJ 100</td>
<td>100</td>
<td>0.335±0.009*</td>
<td>30.50</td>
</tr>
<tr>
<td>MERJ 200</td>
<td>200</td>
<td>0.253±0.017*</td>
<td>47.51</td>
</tr>
<tr>
<td>MERJ 500</td>
<td>500</td>
<td>0.246±0.006*</td>
<td>48.96</td>
</tr>
<tr>
<td>Loperamide</td>
<td>5</td>
<td>0.208±0.002*</td>
<td>56.85</td>
</tr>
</tbody>
</table>

* Values are mean±S.E.M. (n=5).
* P<0.05 vs. control, Student’s ‘t’-test.

3.2. Measurement of faecal output

MERJ tested at the concentration of 100, 200 and 500 mg/kg reduced the faecal output of the mice by 30.50%, 47.51% and 48.96%, respectively, while the reduction in the faecal output by loperamide (5 mg/kg) was noted to be 56.85% when compared to the control group (Table 1).

3.3. Castor oil-induced diarrhoea

The extract (100, 200 and 500 mg/kg) protected the mice against castor oil-induced diarrhoeal droppings by 60–80%. Whereas, the protection was noticed to be 100% in the case of treatment by loperamide (5 mg/kg) (Table 2).

3.4. Enteropooling assay

The extract reduced the intestinal fluid secretion induced by MgSO₄, in a dose-dependant fashion (Table 3). The reduction in the intestinal fluid secretion at 500 mg/kg of plant extract treatment was found to be almost comparable with that of treatment by 5 mg/kg dose of loperamide.

Table 2
Effect of the methanolic extract of *R. javanica* ripen fruits (MERJ) on castor oil-induced diarrhoea in mice

<table>
<thead>
<tr>
<th>Experimental group</th>
<th>Dose (mg/kg, p.o.)</th>
<th>No. of mice with diarrhoeal droppings within 4 h</th>
<th>Protection (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>–</td>
<td>5/5</td>
<td>0</td>
</tr>
<tr>
<td>MERJ 100</td>
<td>100</td>
<td>2/5</td>
<td>60</td>
</tr>
<tr>
<td>MERJ 200</td>
<td>200</td>
<td>1/5</td>
<td>80</td>
</tr>
<tr>
<td>MERJ 500</td>
<td>500</td>
<td>1/5</td>
<td>80</td>
</tr>
<tr>
<td>Loperamide</td>
<td>5</td>
<td>0/5</td>
<td>100</td>
</tr>
</tbody>
</table>
Table 3
Effect of the methanolic extract of \textit{R. javanica} ripen fruits (MERJ) on enteropooling assay in mice

<table>
<thead>
<tr>
<th>Experimental group</th>
<th>Dose (mg/kg, p.o.)</th>
<th>No. of mice in the group</th>
<th>Wt. of the small intestine per 100 g of mice$^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>–</td>
<td>5</td>
<td>8.996 ±0.477</td>
</tr>
<tr>
<td>MERJ</td>
<td>100</td>
<td>5</td>
<td>8.244 ±0.421$^*$</td>
</tr>
<tr>
<td>MERJ</td>
<td>200</td>
<td>5</td>
<td>7.710 ±0.625$^*$</td>
</tr>
<tr>
<td>MERJ</td>
<td>500</td>
<td>5</td>
<td>7.564 ±0.158$^*$</td>
</tr>
<tr>
<td>Loperamide</td>
<td>5</td>
<td>5</td>
<td>6.766 ±0.567$^*$</td>
</tr>
</tbody>
</table>

$^a$ Values are mean ± S.E.M. ($n=5$).
$^*$ $P<0.05$ vs. control, Student’s ‘$t$’-test.

3.5. 	extit{Gastrointestinal transit test}

The results revealed that the methanol extract (100, 200 and 500 mg/kg) inhibited the small intestinal motility of the charcoal marker in mice by 6.09–25.62% whereas the inhibition was noted be 58.15% in the case of treatment by loperamide (Table 4).

4. Discussion

In traditional medicine system, many plants or herbs are claimed to have antidiarrhoeal efficacy without any scientific basis. The aim of the present study was to evaluate the putative antidiarrhoeal effects of the ripen fruits of \textit{R. javanica}, which are consumed very commonly by the local people in Manipur, north-east India in their traditional medicine system to treat the diarrhoea. In establishing the pharmacological evaluation of a potential antidiarrhoeal agent, the inhibition of experimentally induced diarrhoea, reduction in the faecal output and gastrointestinal motility tests have remained the most common parameters in several studies [11–14]. The present study revealed that the methanolic extract of ripen fruits of \textit{R. javanica} inhibited significantly the frequency of defecation and reduced greatly the wetness of the faecal excretion like the standard antidiarrhoeal agent, loperamide. The therapeutic effect of loperamide is believed to be due to its antimotility and

Table 4
Effect of the methanolic extract of \textit{R. javanica} ripen fruits (MERJ) on gastrointestinal transit in mice

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Dose (mg/kg, p.o.)</th>
<th>Distance travelled by marker as % of total length of small intestine$^a$</th>
<th>% Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>–</td>
<td>65.92 ±2.01</td>
<td>0.00</td>
</tr>
<tr>
<td>MERJ</td>
<td>100</td>
<td>61.92 ±3.67$^*$</td>
<td>6.07</td>
</tr>
<tr>
<td>MERJ</td>
<td>200</td>
<td>54.16 ±3.71$^*$</td>
<td>17.84</td>
</tr>
<tr>
<td>MERJ</td>
<td>500</td>
<td>49.03 ±4.40$^*$</td>
<td>25.62</td>
</tr>
<tr>
<td>Loperamide</td>
<td>5</td>
<td>27.59 ±2.57$^*$</td>
<td>58.15</td>
</tr>
</tbody>
</table>

$^a$ Values of mean ± S.E.M. ($n=5$).
$^*$ $P<0.05$ vs. control, Student’s ‘$t$’-test.
antisecretory properties [15]. From this, it is likely that the extract may mediate its effect through similar mechanism. The extract also significantly protected the mice from diarrhoeal droppings evoked by castor oil administration. Drugs affecting motility, frequency and consistence of diarrhoea also affect secretion [16]. The intraluminal fluid accumulation induced by castor oil was blocked by the test extract in dose-related manner. Further, the experiments carried out on the gastrointestinal tract motility after charcoal meal administration also showed a reduction in the propulsive movement of small intestine after pre-treatment with the extract of R. javanica. Intestinal fluid secretion has been analyzed by enteropooling assay in mice, evoked by MgSO$_4$ (a standard laxative agent).

In conclusion, the results of this study seem to provide a support for the use of R. javanica ripens fruits as anti diarrhoeal agent in the local medicine system of Naga tribes in Manipur north-east India. Further study, however, is necessary to isolate and identify the active ingredients of fruits and their precise mechanism of action.

Acknowledgments

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References