A study on the evaluation of anti-diarrhoeal efficacy of a polyherbal formulation versus loperamide in rat models

Santanu Munshi¹, Ranjita Santra (Dhali)*², Manab Nandy³, Swati Bhattacharya³, Tapas Sur⁴

¹Professor, Department of Pharmacology, Calcutta National Medical College, Kolkata, India
²Assistant Professor, Department of Clinical & Experimental Pharmacology, Calcutta School of Tropical Medicine, Kolkata, India
³Associate Professor, Department of Pharmacology, Calcutta Medical College, Kolkata, India
⁴Research Associate, Department of Pharmacology, Institute of Post Graduate Education & Research, Kolkata, India

Corresponding author*
Dr. Ranjita Santra (Dhali) MD,
Assistant Professor,
Department of Clinical & Experimental Pharmacology,
Calcutta School of Tropical Medicine, Kolkata, India
E-mail: dsdranjita@gmail.com

Abstract
Objective: To assess the efficacy of a new polyherbal formulation LQ14 for acute diarrhea in rats and to explore its mechanism of action as an anti-diarrhoeal agent.
Materials and methods: Wistar albino rats (average body weight 150g) were divided into five groups of six animals each. Group 1 control (2 ml/kg distilled water), group 2 received loperamide (3 mg/kg) served as standard and group 3, 4, and 5 received test drug LQ14 (1 ml/kg, 2 ml/kg and 4 ml/kg respectively) 1 hour before castor oil administration. All drugs were given by oral route. Diarrhea was induced by administering 1 ml of castor oil. Castor Oil-Induced Diarrhea, Castor Oil-Induced Enteropooling and Small Intestinal Transit were the models used in this study.
Results: LQ14 possess significant anti-diarrhoeal activity due to its effect on reduction of frequency of diarrhoea episodes, delay in gastrointestinal propulsion and inhibition of fluid accumulation in the intestinal tract of rats.
Conclusion: The new polyherbal formulation was found to possess anti-diarrhoeal activity as it showed significant reduction of number of diarrhoea stools, delayed gastrointestinal propulsion and inhibition of fluid accumulation in the intestinal tract of rat.
Keywords: polyherbal formulation, anti-diarrhoeals, rat models, percentage inhibition, peristaltic index, efficacy

1. Introduction
In the developing countries, diarrhoea is one of the main causes of morbidity and mortality especially in children below 5 years of age. Infants account for more than half of these deaths, and the risk amounts to 2-3 times higher among infants who are not exclusively breast-fed. One out of every five children diarrhea worldwide is an Indian.[1] Administration of oral fluids in the form of oral rehydration solution (ORS) reverses the dehydration, but does not seem to have any effect on the frequency and duration of diarrhoeal episodes. In view of this problem, the World Health Organization (WHO) has a Diarrhoea Disease Control Program, which includes studies of traditional medical practices together with the evaluation of health education and prevention approaches. Most of the studies on anti-diarrhoeal medicinal plants have focused on intestinal motility and/or antimicrobial activity. The formulation, LQ14 was claimed for anti-diarrhoeal properties, but its efficacy has not been studied earlier. This piece of work was the first attempt to find out its anti-diarrhoeal efficacy.

2. Materials & Methods
The proprietary test formulation LQ14 was prepared and supplied by the sponsor of the present research project. The drug LQ14 was formulated, prepared, and supplied by the sponsor in the liquid form in amber coloured bottle. The anticipated therapeutic action is anti-diarrhoeal. The formulation contains five herbal parts and other constituents, like thickener, preservative, stabilizer, solvent etc. The shelf-life is 36 months if it is preserved at 25±2°C in dark cool bottle, density is 1300 Kg/m³ and specific gravity is 1.3 when packed. The formulation was analyzed for heavy metals contamination and atomic absorption spectroscopic (AAS) reports informed that LQ14 contains 2.34 ppm lead, less than 0.05 ppm arsenic, less than 0.002 ppm cadmium and 0.248 ppm mercury.

2.1 Reagents and Chemicals
The test drug was supplied by the sponsor (M/s Parker Robinson Pvt. Ltd., Kolkata). Castor oil (M/s Dabur India
Limited, India), Charcoal (M/s Merck, India), Loperamide (Lopamide, M/s Torrent, India), Cell Diluting Fluids (M/s StanBio, India), Biological Commercial Kits (M/s Span Diagnostics Ltd, India) and other general reagents were used.

2.2 Ethics Clearance

The study was conducted after obtaining ethics clearance from the Institutional Animal Ethics Committee (IAEC) no: PR-HC/06 ETHICs/ 1019.

Table 1: List of ingredients used in test formulation LQ 14 [2-8]

<table>
<thead>
<tr>
<th>Sl No.</th>
<th>Name of Ingredients</th>
<th>Parts used</th>
<th>Activities</th>
<th>Amount (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Kurchi (Holarrhena antidysenterica)</td>
<td>Stem bark</td>
<td>Antimicrobial</td>
<td>100 mg</td>
</tr>
<tr>
<td>2.</td>
<td>Mango (Mangifera indica)</td>
<td>Leaves</td>
<td>Antimicrobial</td>
<td>100 mg</td>
</tr>
<tr>
<td>3.</td>
<td>Musta (Cyperus rotundus)</td>
<td>Rhizome</td>
<td>Antimicrobial</td>
<td>20 mg</td>
</tr>
<tr>
<td>4.</td>
<td>Mandukaparni (Centella asiatica)</td>
<td>Whole plant</td>
<td>Antimicrobial</td>
<td>20 mg</td>
</tr>
<tr>
<td>5.</td>
<td>Haridra (Curcuma longa)</td>
<td>Rhizome</td>
<td>Antimicrobial</td>
<td>10 mg</td>
</tr>
<tr>
<td>6.</td>
<td>Pectin</td>
<td>-</td>
<td>Thickener</td>
<td>4 mg</td>
</tr>
<tr>
<td>7.</td>
<td>Sodium benzoate</td>
<td>-</td>
<td>Preservative</td>
<td>6 mg</td>
</tr>
<tr>
<td>8.</td>
<td>Citric acid</td>
<td>-</td>
<td>Stabilizer</td>
<td>3 mg</td>
</tr>
<tr>
<td>9.</td>
<td>Purified water</td>
<td>-</td>
<td>Solvent</td>
<td>q.s.</td>
</tr>
</tbody>
</table>

2.3 Test Drug Preparation

The supplied test drug as a liquid formulation (shown in Table 1) was ready to use. No other solvent was mixed throughout the study.

2.4 Animals and animal care

Animals were allowed to be acclimatized for a period of 2 weeks in our laboratory environment prior to the study. The animals were housed in polypropylene cages (4 animals per cage), maintained under standard laboratory conditions (i.e. 12:12 hour light and dark sequence; at an ambient temperature of 25±2ºC; 35-60% humidity). They were fed with nutritionally balanced pellet diet for rodent and water ad libitum.

Basically, 3 models of acute diarrhea were studied for efficacy assessment namely Castor oil-induced diarrhea, Castor oil-induced enteropooling and Small intestinal transit.

2.5 Castor Oil-Induced Diarrhoea

Wistar/NIN(1B) albino rats (150 g) were randomly divided into three groups of six animals each. The animals were fed on rats pellets and water ad libitum. Diarrhoea was induced by administering 1 ml of castor oil orally to rats. Group 1 served as control (2 ml/kg distilled water), group 2 received Loperamide (3 mg/kg) served as standard and group 3, 4, and 5 received test drug LQ14 (1, 2 and 4 ml/kg respectively) 1 h before castor oil administration. All drugs were given by oral route.[9] The volume of the extract administered to each animal in the test group was calculated based on the body weight. The individual animal was placed under an absorbent sheet in a clean polypropylene cage. The number of characteristics diarrhoeal droppings were counted for a period of 4 h and wet weight of the droppings was measured.

2.6 Castor Oil-Induced Enteropooling

Intraluminal fluid accumulation was determined by the method of Robert et al., (1976). Overnight fasted rats were divided into five groups of six animals each. Group 1 served as control (2 ml/kg saline), group 2 received Loperamide (3 mg/kg) served as standard and group 3, 4, and 5 received the test drug extract (1ml/kg, 2ml/kg and 4 ml/kg respectively) 1 h before castor oil administration. All drugs were given by oral route. Two hours later the rats were sacrificed, the small intestine was removed after tying the ends with thread and weighed. [10-12] The intestinal contents were collected by milking into a graduated tube and their volume was measured (Fig. 1 & 2). The intestine was re-weighed and the difference between full and empty intestines was calculated.
2.7 Small Intestinal Transit

Rats were fasted for 18 h divided into six groups of six animals each. Group 1 served as control (2 ml/kg saline), group 2 received Loperamide (3 mg/kg) served as standard and group 3, 4, and 5 received the test drug extract (1, 2 and 4 ml/kg respectively) 1 h before castor oil administration. All drugs were given by oral route. One ml of marker (10% charcoal suspension in 5% gum acacia) was administered orally 1 h after castor oil treatment. The rats were sacrificed after 1 h and the distance travelled by charcoal meal from the pylorus was measured and expressed as percentage of the total length of the intestine from the pylorus to caecum (Fig 3 & 4).

Fig.3: Charcoal Transit

Fig.4: Intestinal length

The peristaltic index (PI) for each mouse was calculated, expressed as percentage of the distance traveled by the charcoal meal relative to the total length of the small intestine.

$$PI = \frac{LM}{LSI} \times 100\%$$

[Where: PI = Peristaltic Index; LM = Length traveled by Charcoal Meal; LSI = Length of Small Intestine]

The percentage inhibition [12,13] relative to the control was also calculated as:

$$\% \text{ Inhibition} = \frac{(\text{Control} - \text{Test})}{\text{Control}} \times 100$$

2.8 Statistical analysis

Results were represented as Mean ± SEM; N=6 in each group; One way ANOVA followed by Dunnett test (SPSS, IBM, USA, V17); Computed F values =3.501, 1.829, 4.962; * means p<0.05 and ** means p<0.01; a=compared with control, b=compared with Loperamide, c=LQ14 1ml/kg; d= LQ14 2ml/kg; e= LQ14 4ml/kg.

3. Results

The percentage inhibition of the total weight and number of wet faeces indicates the presence of anti-diarrhoeal activity in extract as compared with that of control group. Experimental result reflects that the activity is more pronounced at the dose of 4 ml/kg body weight (Table 2-4). The percentage of inhibition of number of wet faeces were 27.4% (p<0.05) at the dose of 1 ml/kg, 36.9% (p<0.01) at the dose of 2 ml/kg, 63.7% (p<0.01) at the dose of 4 ml/kg body weight while that of standard drug Loperamide (3 mg/kg) was 83.3% than control of castor oil-induced diarrhoea (Table 2). LQ14 not only diminished the number of faecal dropping but also reduced the weight of diarrhoeal (28.2-69.5%) wet faeces, similar to standard anti-diarrhoeal agent, Loperamide (84.5%), which are now exhibited in Table 2. The severity score also confirmed that the test drug has pronounced anti-diarrhoeal action (Table 2). LQ14 also reduced the incident of diarrhoeal findings (35.1-54.9%). In this study, it exhibited a significant dose-dependent anti-diarrhoeal activity. The results were comparable to that of the standard drug Loperamide (3 mg/kg) with regard to the severity of diarrhoea. The test drug also led to a marked reduction in the volume of the intestinal contents on castor oil-induced enteropooling. It significantly reduced both the weight (20.9%, 33.9% and 44.3%) and volume (25.1%, 35.8% and 47.8%) of intestinal content (Table 3). LQ14 produced profound decrease in intestinal transit of 18.2%, 29.7 and 36.1% at the doses of 1, 2 & 4 ml/kg body weight respectively (Table 4) and while that of Loperamide produced 55.3% inhibition of intestinal transit at dose of 3 mg/kg body weight. Similar results also revealed when the data are represented as peristaltic index (Table 4). This may be due to the fact that the LQ14 test formulation may increase the re-absorption of water by decreasing intestinal motility as observed in by the decrease in intestinal transit of charcoal meal.
Table 2: Effect of LQ14 on castor oil induced diarrhoea in rats in 4 hours

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment/dose</th>
<th>Number of wet faeces</th>
<th>Total weight of wet faeces (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control (2ml/kg)</td>
<td>14.6±1.11</td>
<td>5.35±0.16</td>
</tr>
<tr>
<td>2</td>
<td>Loperamide (3mg/kg)</td>
<td>2.0±0.93** (a,c,d)</td>
<td>0.83±0.36* (a,c,d)</td>
</tr>
<tr>
<td>3</td>
<td>LQ14 (1ml/kg)</td>
<td>9.2±0.75** (a,b,c)</td>
<td>2.71±0.39* (a,b,c)</td>
</tr>
<tr>
<td>4</td>
<td>LQ14 (2ml/kg)</td>
<td>5.3±0.88** (a,b,c,d)</td>
<td>1.63±0.26* (a,b,c,d)</td>
</tr>
</tbody>
</table>

Results were represented as Mean ± SEM; N=6 in each group; One way ANOVA followed by Dunnett test (SPSS, IBM, USA, V17); Computed F values = 4.962; * means p<0.05 and ** means p<0.01; a=compared with control, b=compared with Loperamide, c=LQ14 1ml/kg; d=LQ14 2ml/kg; e= LQ14 4ml/kg; Percent inhibition in comparison with control in parenthesis.

Table 3: Effect of LQ14 on castor oil induced intestinal fluid accumulation in rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment/dose</th>
<th>Wt. of intestinal content (g)</th>
<th>Vol. of intestinal content (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control (2ml/kg)</td>
<td>2.86±0.10</td>
<td>2.51±0.12</td>
</tr>
<tr>
<td>2</td>
<td>Loperamide (3mg/kg)</td>
<td>1.27±0.06* (a,c,d)</td>
<td>0.92±0.05* (a,c,d)</td>
</tr>
<tr>
<td>3</td>
<td>LQ14 (1ml/kg)</td>
<td>2.26±0.08* (a,c,d)</td>
<td>1.88±0.07* (a,b,c,d)</td>
</tr>
<tr>
<td>4</td>
<td>LQ14 (2ml/kg)</td>
<td>1.89±0.07* (a,b,c,d)</td>
<td>1.61±0.08* (a,b,c,d)</td>
</tr>
<tr>
<td>5</td>
<td>LQ14 (4ml/kg)</td>
<td>1.62±0.07* (a,b,c,d)</td>
<td>1.31±0.08</td>
</tr>
</tbody>
</table>

Results were represented as Mean ± SEM; N=6 in each group; One way ANOVA followed by Dunnett test (SPSS, IBM, USA, V17); Computed F values =1.829; * means p<0.05 and ** means p<0.01; a=compared with control, b=compared with Loperamide, c=LQ14 1ml/kg; d=LQ14 2ml/kg; e= LQ14 4ml/kg; Percent inhibition in comparison with control in parenthesis.

Table 4: Effect of LQ14 on castor oil induced gastrointestinal transit in rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment/dose</th>
<th>Intestinal length (cm)</th>
<th>Distance travel (cm)</th>
<th>Peristaltic Index</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control (2ml/kg)</td>
<td>96.3±1.02</td>
<td>76.3±1.42</td>
<td>79.05±0.78</td>
</tr>
<tr>
<td>2</td>
<td>Loperamide (3mg/kg)</td>
<td>95.8±0.94</td>
<td>34.1±0.94** (a,c,d)</td>
<td>35.93±1.08** (a,c,d)</td>
</tr>
<tr>
<td>3</td>
<td>LQ14 (1ml/kg)</td>
<td>96.1±0.85</td>
<td>62.4±1.12** (a,b,c,d,e)</td>
<td>64.5±0.94** (a,b,c,d)</td>
</tr>
<tr>
<td>4</td>
<td>LQ14 (2ml/kg)</td>
<td>97.2±0.74</td>
<td>53.6±1.40** (a,b,c,d)</td>
<td>55.25±1.14** (a,b,c,d)</td>
</tr>
<tr>
<td>5</td>
<td>LQ14 (4ml/kg)</td>
<td>96.6±1.09</td>
<td>48.7±1.48** (a,b,c,d)</td>
<td>49.13±1.70** (a,b,c,d)</td>
</tr>
</tbody>
</table>

Results were represented as Mean ± SEM; N=6 in each group; One way ANOVA followed by Dunnett test (SPSS, IBM, USA, V17); Computed F values =4.962; * means p<0.05 and ** means p<0.01; a=compared with control, b=compared with Loperamide, c=LQ14 1ml/kg; d=LQ14 2ml/kg; e= LQ14 4ml/kg; Percent inhibition in comparison with control in parenthesis.

4. Discussion

A large variety of plants have been explored in the search of anti-diarrhoeal activity and some of them were found to possess the anti-diarrhoeal effect. [14, 15] In the present study, a single oral administration at various doses of LQ14 produced a significant decrease in the severity of diarrhoea in terms of reduction in the rate of defecation in Wister albino rats. The test drug formulation possesses significant anti-diarrhoeal activity due to its inhibitory effect on castor oil induced diarrhoeal secretion and that may be mediated through gastrointestinal propulsion. It may promote re-absorption of materials in the intestine due to decrease propulsion of material in the intestinal tract, and the extract might have exerted its anti-diarrhoeal action by anti-secretory mechanism. The delay in the gastrointestinal transit prompted by the extract might have contributed, at least to some extent, to their anti-diarrhoeal activity by allowing a greater time for absorption. The percentage inhibition for the number and weight of wet faeces indicates the presence of anti-diarrhoeal activity in extract as compared with that of control group. It is well evident that castor oil produces diarrhoea due to its most active component ricinoleic acid which causes irritation and inflammation of the intestinal mucosa, leading to release of prostaglandins, which results in stimulation of secretion. [16-18] LQ14 not only diminished the number of faecal dropping.
but also reduced the weight of diarrhoeal (28.2-69.5%) wet faeces, similar to standard anti-diarrhoeal agent, Loperamide (84.5%). The test drug formulation contains anti-diarrhoeal plants, like Holarrhena antidysenterica, Mangifera indica, Cyperus rotundus, Centella asiatica and Curcuma longa etc. Previous studies claimed that these plants have anti-diarrhoeal activities in castor oil induced diarrhoea in animals. [19,20]

Moreover, the test formulation was demonstrated to be safe in oral use in acute and 28-days repeated doses studies in rodents. [21] Since LQ14 inhibited the castor oil-induced diarrhoea, the formulation might have exerted its anti-diarrhoeal action via anti-secretory mechanism which was also evident from the reduction of total number and the weight of wet faeces in the test groups of the experiment. Pharmacological active constituents of plant origin, flavonoids, tannin, alkaloids present in the test formulation may inhibit release of autacoids and prostaglandins and thereby inhibit motility and secretion induced by castor oil. The formulation also significantly reduced intestinal transit as observed by the decrease in transit motility of charcoal meal.

5. Conclusion

The new test formulation LQ14 possesses significant anti-diarrhoeal activity due to its effect on reduction of number of diarrhoea stools, delayed gastrointestinal propulsion and inhibition of fluid accumulation in the intestinal tract of rats. It has been shown to be efficacious when compared to the standard anti-diarrhoeal drug loperamide.

Moreover, the test formulation was demonstrated to be safe in oral use in acute and 28-days repeated doses studies in rodents. Thus this study shows that, the polyherbal formulation LQ14 possesses significant anti-diarrhoeal activity in terms of efficacy in rat models.

Acknowledgments

This work was supported by the French-Ivory Coast cooperation project AMRUGE-CI (Campus France N° 854039K) and by a scholarship from the Ministry of Superior Education and Scientific Research of Ivory Coast (Decision N° 459/MERS/DB/SD-BHCI du 12/10/2015).

Declaration of conflict of interest: None as declared by the authors.

References


