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Antidiarrhoeal activity of *Rotula aquatica* in ratsSunder singh<sup>1\*</sup>, Rai AK<sup>2</sup>, Praveen Sharma<sup>3</sup>, Yogesh Barshiliya<sup>4</sup>, Mohini Sihare<sup>5</sup>, Anju Negi<sup>6</sup><sup>1</sup> Jodhpur Pharmacy College, Jhanwar road, Boranada Jodhpur– 342006, India<sup>2</sup> Praveer Singh Institutes of Pharmaceutical Science, Kanpur, India<sup>3</sup> College of Pharmacy, IPS Academy, Indore, India<sup>4</sup> Vinayaka College of Pharmacy, Kullu, India<sup>5</sup> Malhotra College of Pharmacy, Bhopal MP, India<sup>6</sup> Doon Paramedical College, Dehradun UK, India

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## ABSTRACT

**Objective:** To evaluate the antidiarrhoeal effect of alcoholic extract of *Rotula aquatica* by using different experimental models. **Methods:** Antidiarrhoeal effect was evaluated by castor oil induced diarrhea, charcoal meal test and PGE<sub>2</sub> induced diarrhea. Loperamide (2 mg/kg) and atropine (0.1mg/kg) were used as standard drugs. Alcoholic extract was used in 100 and 200 mg/kg dose. **Results:** In case of castor oil induce diarrhea dose 200 mg/kg showed delay in defecation time (186.980±15.660) min and 100 mg/kg showed delay in defecation time (97.779±4.952) min, while loperamide (2 mg/kg) showed delay in defecation time (221.148±21.919) min. Finally the reading showed that doses 200 mg/kg showed significant antidiarrhoeal effect from 100 mg/kg of alcoholic extract of *Rotula aquatica* in different model used for antidiarrhoeal activity. **Conclusions:** This study revealed that the alcoholic extracts has potential pharmacological activity against diarrhea and this may possible explain the use of the plant in traditional medicine.

## 1. Introduction

Diarrhoea is a major health problem especially for children under the age of 5 and up to 17% of children admitted in the paediatrics ward die of diarrhoea. Worldwide distribution of diarrhoea accounts for more than 5–8 million deaths each year in infants and children below 5 years old especially in developing countries[1].

Diarrhea and the associated fecal urgency and incontinence result from an imbalance between the absorptive and secretory mechanisms in the intestinal tract accompanied by hypermotility. This results in excess loss of fluid and electrolytes in feces[2]. It is an important health problem especially in developing countries where it is hyper-endemic with parasitism. A variety of factors such as consumption of contaminated food/water and reactions or intolerance to certain foods are implicated as causes.

A range of medicinal plants with anti-diarrhoeal properties is widely used by traditional healers, but still there is an urgent need for research on medicinal plant in the management of diarrhoeal diseases.

*Rotula aquatica* belongs to the family Borogenaceae, and is reported to contain baunerol, steroid, alkaloid[3]. The root of *Rotula aquatica* is also used as a laxative, and used in treatment of piles and veneral disease[3]. A decoction of roots of *Rotula aquatica* showed antilithic activity in glycolic acid induced nephrolithiasis rats, and the results were summarized based on the ionic change in both urine and serum[4]. Antidiabetic activity of the aqueous extract of *Rotula aquatica* was shown in alloxan induced diabetic rat[5]. The effect of the alcoholic extract of *Rotula aquatica* against ethylene glycol induced urolithiasis in albino rats[6]. The root of *Rotula aquatica* is reported to contain antimutagenic activity[7].

However, literature review failed to offer any scientific validation on the antidiarrhoeal activity of *Rotula aquatica*. Hence, this leads us to study antidiarrheal activity of *Rotula aquatica* in different diarrhoeal models.

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## 2. Materials and methods

### 2.1. Plant material

Root of *Rotula aquatic* was collected and authenticated. The whole plant was then dried, powdered and stored in airtight containers for further use. The powdered material was subjected to soxhlet extraction with various solvents ranging from non-polar to polar. The solvents used were petroleum ether, benzene, chloroform, alcohol and water. Each time before extraction with next solvents the marc was air-dried. All the extracts were concentrated by distilling the solvent at low temperature. They were then weighed and percentages of different extractive values were calculated with respect to air-dried substance. Alcoholic extract was selected for antidiarrheal activity on the basis of phytochemical screening and TLC pattern.

### 2.2. Experimental animals

Albinos Wister rats of both sex weighing between 160–260 g were used. Institutional Animal Ethics Committee approved the experimental protocol; animals were housed under standard conditions of temperature [ $24 \pm 2$  °C] and relative humidity (30%–70%) with a 12:12 light: dark cycle. Animal handling was performed according to Good Laboratory Practice. The animals were given standard diet and water *ad libitum*.

### 2.3. Evaluation of antidiarrheal activity

#### 2.3.1. Castor oil induced diarrhea

Rats of either sex (140–200 g) were fasted for 18 h, and were divided into 4 groups (Group 1–4) of 6 animals each. Diarrhea was induced by administering 1 mL of castor oil orally to rats. Group 1 served as control (2 mg/kg, *p.o.* saline); Group 2 received loperamide (2 mg/kg, *p.o.*)<sup>[1]</sup>, and served as standard; Group 3, 4 received the alcoholic extract of *Rotula aquatica* (100 and 200 mg/kg, *p.o.*), 1 h before castor oil administration<sup>[8]</sup>.

After that the animals were placed in separate wired cages for observation. The consistency of faecal matter and frequency of defecation were observed for 4 hours.

#### 2.3.2. Charcoal meal test

Rats of either sex (170–250 g) were fasted for 18 h<sup>[9]</sup>. They were divided into four groups<sup>[9]</sup> ( $n=6$ ). Group 1 which served

as control was administered with aqueous 1% tragacanth suspension. Group 2 received standard drug atropine (0.1 mg/kg) subcutaneously. The alcoholic extract of *Rotula aquatica* was administered orally at 100 mg/kg to Group 3 and 200 mg/kg to Group 4 as suspension. The animals were given 1 mL of 10% activated charcoal suspended in 10% aqueous tragacanth powder *p.o.*, 30 min after treatment. Animals were euthanized 30 min after charcoal meal administration by ether anesthesia. The abdomen was cut off and the small intestine carefully removed. The distance travelled by charcoal plug from pylorus to caecum was measured, and expressed as percentage of the distance traveled by charcoal plug for each of animal.

#### 2.3.3. PGE<sub>2</sub> induced enteropooling

Rats of either sex (150–250 g) were fasted for 18 h<sup>[8]</sup>. They were then divided into four groups ( $n=6$ ). A solution of PGE<sub>2</sub> was made by mixing 5% v/v alcohol with the normal saline. Group 1, which served as control, was administered with PGE<sub>2</sub> (100  $\mu$ g/kg, *p.o.*) only. Group 2, which served as vehicle control was administered with aqueous 1% tragacanth suspension by oral route. The alcoholic extract of *Rotula aquatica* was administered orally at 100 mg/kg to Group 3 and 200 mg/kg to Group 4 as suspension. Immediately after extract administration PGE<sub>2</sub> was administered. After 30 min following administration of PGE<sub>2</sub> each rat was sacrificed and whole length of the intestine from pylorus to caecum was dissected out, its content collected in measuring cylinder and volume measured.

### 2.4. Statistical analysis

The data are represented as mean  $\pm$  SEM. and statistical significance between treatment and control groups was analyzed using one-way ANOVA, followed by Dunnett's test where  $P < 0.05$  was considered statistically significant.

## 3. Results

Result of antidiarrhoeal activity in castor oil induced diarrhea test was shown in Table 1.

Alcoholic extract of *Rotula aquatica* (100 and 200 mg/kg) and the anti-muscarinic drug, atropine (0.1 mg/kg) decreased the propulsive movement in the charcoal meal study, both dose 100 mg/kg and 200 mg/kg showed moderate effect to prevent diarrhea (Table 2).

**Table 1.**

Evaluation of antidiarrheal activity of alcoholic extract of *Rotula aquatica* in castor oil induced diarrhea rats.

| Treatment                     | Mean wet defecation in 4 h | Mean increase in weight of paper (g) | Delay in defecation time (min) |
|-------------------------------|----------------------------|--------------------------------------|--------------------------------|
| Control                       | 8.242 $\pm$ 0.866          | 3.149 $\pm$ 1.018                    | 40.746 $\pm$ 3.774             |
| Loperamide(2 mg/kg)           | 0.886 $\pm$ 0.885*         | 0.436 $\pm$ 0.076                    | 221.148 $\pm$ 21.919*          |
| Alcoholic extract (100 mg/kg) | 3.482 $\pm$ 0.503*         | 2.282 $\pm$ 0.631                    | 97.779 $\pm$ 4.952*            |
| Alcoholic extract (200 mg/kg) | 1.337 $\pm$ 0.363*         | 0.955 $\pm$ 0.153*                   | 186.980 $\pm$ 15.660*          |

\*= $P < 0.001$ , significantly different compare to control group.

Both 100 mg/kg and 200 mg/kg of alcoholic extract showed protection against PGE<sub>2</sub> induced enteropooling (Table 3), which might be due to the inhibition of synthesis of prostaglandins. Anti-enteropooling effect of the extract was more relevant because the prevention of enteropooling helps in the inhibition of diarrhea, especially by PGE<sub>2</sub> induced diarrhea as it is involved in the onset of diarrhea in intestinal mucosal cells.

The underlying mechanism appears to be spasmolytic and an anti-enteropooling property by which the extract produced relief in diarrhea.

Extract also inhibited the onset time and severity of diarrhea induced by castor oil. Castor oil is reported to cause diarrhea by increasing the volume of intestinal content and by reduction of reabsorption of water.

**Table 2**

Evaluation of antidiarrhoeal activity of alcoholic extract of *Rotula aquatica* in charcoal meal test.

| Treatment                     | Movement of charcoal |
|-------------------------------|----------------------|
| Control                       | 70.430±2.981         |
| Atropine sulphate (2 mg/kg)   | 29.380±1.348*        |
| Alcoholic extract (100 mg/kg) | 63.105±1.722*        |
| Alcoholic extract (200 mg/kg) | 34.338±2.925*        |

\*= $P < 0.001$ , significantly different compare to control group.

**Table 3**

Evaluation of antidiarrhoeal activity of alcoholic extract of *Rotula aquatica* in PGE<sub>2</sub> induced enteropooling.

| Treatment                     | Movement of charcoal |
|-------------------------------|----------------------|
| PGE <sub>2</sub> control      | 2.427±0.625          |
| Vehicle control               | 1.316±0.197*         |
| Alcoholic extract (100 mg/kg) | 1.102±0.113*         |
| Alcoholic extract (200 mg/kg) | 0.957±0.068*         |

\*= $P < 0.001$ , significantly different compare with PGE<sub>2</sub> control group.

#### 4. Discussion

Castor oil brings about changes in electrolyte and water transport and increases peristaltic activity. These changes are associated with prostaglandins that contribute to the patho-physiological functions in the gastro intestinal tract. Release of prostaglandins is also a major cause of arachidonic acid-induced diarrhea. This is characterized by an increase in the secretion of water and electrolytes, an increase in intestinal transit time and an increase in wet faeces.

The antidiarrhoeal effect of alcoholic extract is due to reduction of gastrointestinal motility, inhibition of the

synthesis of prostaglandin. The extract has potential effect on the reduction of gastrointestinal motility than the other effects. The above effects may also be due to the presence of alkaloids and flavanoids in the extract.

#### Conflict of interest statement

We declare that we have no conflict of interest.

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