Evaluation of antidiarrhoeal activity of aqueous bulb extract of *Allium cepa* against castor oil-induced diarrhoea.

K. Rajesh Kumar, Afsar Shaik, J. Venu Gopal, P. Raveesha

ABSTRACT

The objective of this study was to evaluate antidiarrhoeal activity of aqueous bulb extract of *Allium cepa*. The Antidiarrhoeal effect was evaluated by castor oil-induced diarrhoeal model in rats. Loperamide (3 mg/kg, p.o.) was taken as standard, aqueous bulb extract of *Allium cepa* 150 & 300 mg/kg was used as a test doses. The results showed significant (P<0.05) antidiarrhoeal activity on gastrointestinal motility with castor oil-induced diarrhoeal model in rats. The extract tested at 150 and 300 mg/kg shown similar effect as that of standard drug (loperamide) by significantly inhibiting the frequency of defecation droppings compared to untreated control rats. This result is in support of previous claims in respect of antidiarrhoeal herbs. The study revealed that the aqueous bulb extract possess Pharmacological activity against diarrhoea and may possibly explain the use of the plant in traditional medicine.

Keywords: Antidiarrhoeal activity, *Allium cepa*, Castor oil, Loperamide.

1. Introduction

Diarrhoea is characterized by increased frequency of bowel movement, wet stool and abdominal pain [1]. Diarrhoeal diseases caused several million of deaths in the world annually [2]. In developing countries they are the most common causes of morbidity and mortality [3]. At the beginning of the 1980s, deaths caused by diarrhea were estimated at 4.6 millions every year for children under the age of 5 years [4]. Infants younger than 1 year account for more than half of these deaths, and the risk can be 2 - 3 times higher among infants who are not exclusively breast-fed [5].

According to W.H.O. estimates for 1998, about 7.1 million deaths were caused by diarrhoea [6] and the cause of 3.3% of all deaths [7]. Around 88% of diarrheal related deaths are caused due to inadequate sanitation and poor hygiene [8].

*Allium cepa* is the common onion. It is a member of the Liliaceae, which consists of over 250 genera and 3700 species. Because of their bulbs, tubers and rhizomes, these plants are able to survive under harsh conditions, e.g. winter or dryness.

In Indian folk medicine, the bulb of *Allium cepa* is used to treat dysentery, fever, chronic bronchitis, insect bites, stings, skin diseases [9]. Tannins have been found to form irreversible complexes with proline rich protein resulting in the inhibition of cell protein synthesis. Tannins are known to react with proteins to provide the typical tanning effect which is important for the treatment of inflamed or ulcerated tissues. Herbs that have tannis as their main components are astringent in nature and are used for treating intestinal disorders such as diarrhea and dysentery [10]. These observations therefore support the use of *Allium cepa* in herbal cure remedies.

The present study was therefore conducted to evaluate the Antidiarrhoeal activity of aqueous bulb extract of *Allium cepa*. 
2. Materials and methods
2.1 Collection of Plant material:
The Bulbs of *Allium cepa* were collected from the local market of Tirupati, A.P, India. And identified by assistant professor in the Department of Botany, Sri Venkateswara University, Tirupati.

2.2 Preparation of plant extract:
The onions were washed with clean sterile distilled water and allowed to air dry for one hour. The outer coverings were manually peeled off and the aqueous extract was obtained from the bulbs by the method used by earlier workers [11]. Exactly 200gms of fresh onion bulbs were blended and soaked in 100ml of distilled water for 24hrs. The pulp obtained was left in a clean sterile glass container and shaken vigorously to allow for proper extraction and was filtered using muslin cloth. The filtrate was concentrated using distillation to give the aqueous extract.

2.3 Phytochemical investigation:
Phytochemical tests were carried out to find the presence of phytoconstituents Viz., Carbohydrates, Flavanoids, Proteins, Glycosides, Saponins, Fats & oils, Alkaloids, Steroids and Tannins.

2.4 Experimental animals:
Wistar rats weighing between 150-175 gm were obtained from M/s. Venkateshwara Enterprises, Bangalore, Karnataka, India. The animals were housed in stainless steel cages at a controlled room temperature of 24°C, under a 12 h light and 12 h dark cycle. After one week of acclimatization, the animals were used for experimentation. The experimental protocol was approved by the Institutional Animal Ethical Committee.

### Table 1: Treatment schedule for assessing the Antidiarrhoeal activity of Aqueous Bulb Extract of *Allium cepa* (ABEAC).

<table>
<thead>
<tr>
<th>S.NO</th>
<th>Groups</th>
<th>Treatment</th>
<th>Purpose</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Control</td>
<td>Castor oil 1 ml +Vehicle (0.5% v/v aqueous Tween 80)</td>
<td>To serve as control</td>
</tr>
<tr>
<td></td>
<td>N=6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>Standard</td>
<td>loperamide (3 mg/kg, p.o.)</td>
<td>To serve as standard</td>
</tr>
<tr>
<td></td>
<td>N=6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>Treatment-1</td>
<td>ABEAC (150 mg/kg)</td>
<td>To assess the antidiarrhoeal activity of ABEAC at a dose of 150mg/kg</td>
</tr>
<tr>
<td></td>
<td>N=6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IV</td>
<td>Treatment-2</td>
<td>ABEAC (300 mg/kg)</td>
<td>To assess the antidiarrhoeal activity of ABEAC at a dose of 300mg/kg</td>
</tr>
<tr>
<td></td>
<td>N=6</td>
<td></td>
<td></td>
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</table>

2.5 Acute toxicity study
Swiss albino mice of either sex weighing (18-22g) and of 90 days age were used for acute oral toxicity study. The study was carried out as per the guidelines set by OECD. The animals were starved overnight were divided into six groups (n=3) and were fed with increasing doses (10, 30, 100, 300, 1000, 2000, 3000 mg/kg B.W.) of the aqueous extract. The animals were continuously observed for mortality and behavioural responses for 48 h and thereafter one daily for 14 days after administration. The 1/10th of the lethal dose was taken as effective dose ED50 (therapeutic dose).

2.6 Evaluation of Antidiarrhoeal Activity
2.6.1 Castor oil-induced diarrhoea:
The treatment schedule is as shown in the table 1. 24 rats were allowed to fast for 18 h and divided into 4 groups of 6 animals each. One group received 10 ml/kg 0.5% v/v aqueous Tween 80 orally and served as a negative control. Another group received the standard drug loperamide (3 mg/kg, p.o.) as positive control, third and fourth groups received aqueous bulb extracts of *Allium cepa* at a dose of 150 and 300 mg/kg body weight, respectively After 1 h of treatment, all the animals were challenged with 1 ml of castor oil orally, by oral gavage and observed for consistency of fecal material. After this administration, the animals were placed separately in metabolic cages with filter paper, which was changed every hour. The severity of diarrhea was assessed each hour for 6 hours. The total number of diarrhoeal droppings excreted and the total weight of feces were recorded within a period of 24 h and compared with the control group. The total number of diarrheal droppings of the control group was considered 100%. The results were expressed as a percentage of inhibition of diarrhea.

2.6.2 Statistical analysis:
All the data was expressed as Mean ± S.E.M. Statistical significance between more than two groups was tested using one way ANOVA followed by the Tukey test using computer based fitting program (Prism graph pad 5.0). Statistical significance was set accordingly.

3. Results
3.1 Acute toxicity:
Acute toxicity studies show that drug is safe up to the dose of 3000 mg/kg body weight. No mortality was observed at 14th day of the acute toxicity study.

3.2 Phytochemical Screening:
*Allium cepa* was examined for the presence of various phytoconstituents by performing qualitative phytochemical tests and the results are recorded in Table 2.
Table 2: Phytochemical screening of aqueous bulb extract of *Allium cepa*.

<table>
<thead>
<tr>
<th>S.no</th>
<th>Phyto Chemical</th>
<th>Aqueous bulb extract of <em>Allium cepa</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Alkaloids</td>
<td>- ve</td>
</tr>
<tr>
<td>2</td>
<td>Carbohydrates</td>
<td>+ve</td>
</tr>
<tr>
<td>3</td>
<td>Flavanoids</td>
<td>- ve</td>
</tr>
<tr>
<td>4</td>
<td>Fats &amp; oils</td>
<td>-ve</td>
</tr>
<tr>
<td>5</td>
<td>Saponins</td>
<td>- ve</td>
</tr>
<tr>
<td>6</td>
<td>Steroids</td>
<td>-ve</td>
</tr>
<tr>
<td>7</td>
<td>Tannins</td>
<td>+ve</td>
</tr>
<tr>
<td>8</td>
<td>Proteins</td>
<td>- ve</td>
</tr>
<tr>
<td>9</td>
<td>Glycosides</td>
<td>-ve</td>
</tr>
</tbody>
</table>

3.3 Anti-diarrhoeal activity:
In the castor oil-induced diarrhoea experiment, aqueous bulb extract of *Allium cepa* significantly prolonged the time of diarrhoeal induction in a dose dependent manner. The frequency of stooling (number of wet faeces and total number of faeces) as well as fresh weight and water content of the faeces decreased significantly as shown in Table 3. There was more reduction in these parameters at 300 mg/kg body weight when compared with loperamide. There was also increase in the percentage inhibition of defecation. However, the highest dose (300 mg/kg body weight) produced inhibition of defecation that compared favourably with the loperamide.

Table 3: Effect of ABEAC on castor oil induced diarrhea

<table>
<thead>
<tr>
<th>S.no</th>
<th>Groups</th>
<th>Treatment</th>
<th>Mean defecation in 4 hours. (no of stools)</th>
<th>Mean weight of faeces in 4 hours.(grams)</th>
<th>Percentage inhibition of defecation</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Control</td>
<td>Vehicle</td>
<td>19.5±3.594</td>
<td>1.5±0.178</td>
<td>_</td>
</tr>
<tr>
<td>II</td>
<td>Standard</td>
<td>Loperamide (3 mg/kg)</td>
<td>5.25±1.109***</td>
<td>0.525±0.9465**</td>
<td>73.077</td>
</tr>
<tr>
<td>III</td>
<td>Test I</td>
<td>ABEAC (150 mg/kg)</td>
<td>9.25±0.9646**</td>
<td>0.9375±0.1248*</td>
<td>52.564</td>
</tr>
<tr>
<td></td>
<td>Test II</td>
<td>ABEAC (300 mg/kg)</td>
<td>4.0±0.7071***</td>
<td>0.6375±0.1599**</td>
<td>79.492</td>
</tr>
</tbody>
</table>

All Values are shown in Mean ± S.E.M, N=6. *P<0.05, ***indicates significant anti-diarrhoeal activity at ** P< 0.01 Vs Control group.

4. Discussion
Diarrhoea results from an imbalance between the absorptive and secretory mechanisms in the intestinal tract accompanied by hury resulting in an excess loss of fluid in the feces. In some diarrhoea the secretory component predominates while other diarrhoea is characterized by hypermotility [12]. Castor oil causes diarrhoea due to its active metabolite, ricinoleic acid [13, 14], which stimulates peristaltic activity in the small intestine, leading to changes in the electrolyte permeability of the intestinal mucosa. Its action also stimulates the release of endogenous prostaglandin [15]. In this study, the aqueous bulb extract of *Allium cepa* significantly prolonged the time of diarrhoeal induction in a dose dependent manner. The results were comparable with that of standard loperamide. Hence, this plant material from this preliminary study may be claimed as a potent anti-diarrhoeal agent. The underlying mechanism appears to be spasmylytic and anti-enteropooling properties by which the plant extract produced relief in diarrhoea. Tannic acid and tannins are present in many plants and they denature proteins forming contents by preventing the re absorption of water. The liberation of ricinoleic acid results in irritation and inflammation of intestinal mucosa leading to release of prostaglandin [16]. In this study, the aqueous bulb extract of *Allium cepa* significantly prolonged the time of diarrhoeal induction in a dose dependent manner. The results were comparable with that of standard loperamide. Hence, this plant material from this preliminary study may be claimed as a potent anti-diarrhoeal agent. The underlying mechanism appears to be spasmylytic and anti-enteropooling properties by which the plant extract produced relief in diarrhoea. Tannic acid and tannins are present in many plants and they denature proteins forming
protein tannate, which makes the intestinal mucosa more resistant and reduces secretion by virtue of which so many different plant species has been reported to possess antidiarrheal potential [17,18]. The tannins present in the plant extract may be responsible for the anti-diarrheal activity. However isolation of the active constituent from the extract may further confirm this statement.

5. Conclusion
The results of this investigation revealed that aqueous bulb extract contains Pharmacologically active substance(s) with antidiarrheal properties. This provides the rationale for the use of the plant extract of *Allium cepa* as an anti-diarrheal drug by traditional healers. Further research is to be carried out to fractionate and purify the extract, in order to find out the molecule responsible for the anti-diarrheal activity.

6. Conflict of interest statement
We declare that we have no conflict of interest.

7. Acknowledgement
Authors wish to thank Principal, S.V University, Tirupati, for sparing the animals to carry out the research work.

8. Reference: