EVALUATION OF THE ANTIDIARRHEAL ACTIVITY OF AQUEOUS EXTRACT OF *LYCOPUS EUROPÆUS* IN MICE

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ABSTRACT

The plant, *Lycopus europaeus* is a small, ever green plant which is reported to possess a number of medicinal properties. The purpose of the present study was to evaluate the anti-diarrhoeal activity of the aqueous extract of *Lycopus europaeus* which is used traditionally as folk medicine, by using a castor oil and MgSO₄ (Magnesium Sulphate) induced diarrhoea model. The aqueous extract of the plant at graded doses (100mg/kg, 200mg/kg body weight) was investigated for its anti-diarrhoeal activity in terms of the reduction in the rate of defaecation and the consistency of faeces in castor oil, MgSO₄ induced diarrhoea. To understand the mechanism of its anti-diarrhoeal activity, its effect was further evaluated on the gastro-intestinal transit time with charcoal meal. The extract showed significant (p<0.05) inhibitory activity against castor oil and MgSO₄ induced diarrhoea. There was a significant reduction in the gastro-intestinal motility which was observed by using the charcoal meal test in mice. The results which were obtained in this study substantiated the anti-diarrhoeal effects of the aqueous extract of *Lycopus europaeus* and its use by the traditional practitioners in the treatment of diarrhoea.

Keywords: Anti-diarrhoeal activity, *Lycopus europaeus*, Castor oil, MgSO₄, Charcoal meal.

INTRODUCTION

Diarrhoea is characterized by an increased frequency of bowel movements, wet stools and abdominal pain [1]. It is a leading cause of malnutrition and deaths among children in the developing countries of the world today [2]. According to the World Health Report, diarrhoea is the cause of 3.3% of all the deaths worldwide. The worldwide distribution of diarrhoea accounts for more than 5-8 million deaths each year in children who were aged less than 5 years. The use of traditional medicine to combat the consequences of diarrhoea has been emphasized by the WHO in its diarrhoea control programme [3]. Many synthetic chemicals are available for the treatment of diarrhoea, but they have some side effects. The natural drugs are used as anti-diarrhoeal drugs, which are not always free from adverse effects [4].

The approach towards evaluating medicinal plants has been based on the chemical
extraction of the plants which are then tested on various experimental models. In developing countries like Pakistan, a majority of people who live in the rural areas almost exclusively use traditional medicines in treating all sorts of diseases, including diarrhoea.

Scientists have played their important role for the evaluation of traditional uses of *Lycopus europaeus* on different animals. For example, extracts of *L. europaeus* administered to healthy rats reduced the weight of the thyroid, decreased thyroid hormone activity, and increased absorption and storage of iodine. The extract retarded goiter formation in propylthiouracil-treated rats. All animals treated with the extract demonstrated reduced metabolism[5]. Other studies in rats have shown inhibition of serum thyrotropic hormone and thyroxine after oral administration [6]. Cardiac signs of hyperthyroidism were reduced in an experiment in rats treated with *L. europaeus* extract [7]. The plant was also reported for its antitussive activity [8].

Hence, the present study was undertaken to evaluate the possible anti-diarrhoeal activity of the extract of *Lycopus europaeus* which is used commonly in Pakistan traditional medicine, by using various validated models and to find out if the folk medicinal use has a scientifically justified basis.

**MATERIALS AND METHODS**

**Collection of plant and Preparation of crude extract:**

The plant was collected from the tropical regions of Pakistan and was identified by senior taxonomists. The plant material was made free from soil and other adulterants and vegetative debris. The dried plant material was grinded to coarse powder with the help of a special herbal grinder. 10g of finely-powdered *Lycopus europaeus* was weighed and mixed with 100ml of water and this was kept on a water bath at 60°C for two hours and filtered. This extract was diluted with distilled water and was administered orally to mice.

**Animals:**

Albino mice which weighed between 25-30 gms was used in this study. The cages of the animals were placed at room temperature with controlled cycles of 12 hours of light and 12 hours of darkness. The relative humidity was maintained
at 44-45 %. All the animals were fed with a standard pellet diet and water ad libitum. The standard pellet diet comprised of 21% protein, 5% lipids, 4% crude fiber, 8% ash, 1 % calcium, 0.6% phosphorous, 3.4% glucose, 2 % vitamin, and 55% nitrogen-free extract (carbohydrate) and it provided a metabolizable energy of 3600 kcal /kg. The study protocol was approved by the institutional animal ethical committee. The animal beds in the cages were renewed thrice a week to ensure hygienic conditions and the maximum comfort of the animals.

**Phytochemical Screening:**

The phytochemical analysis of the crude extract was carried out to determine the active phytochemical constituents which were responsible for the anti-diarrhoeal activity.

**Acute Toxicity Study:**

Different doses (50–2000mg/kg, p. o) of the aqueous extract of the *Lycopus europaeus* were administered to groups of mice and they were observed continuously for 1 hour and then at half– hourly intervals for 4 hours, for any gross behavioural changes and further up to 72 hours, followed 14 days for any mortality. The extract of *Lycopus europaeus* was found to be non-toxic up to the maximum dose of 2000mg/kg body weight.

**Castor Oil Induced Diarrhoea:**

The method which was proposed by Galvez et al., was modified to suit the experimental needs [9]. The animals were kept in fasting for 24 hours before the test, with free access to water. The mice were divided in to 4 groups of 5 animals each. Diarrhoea was induced by administering 0.5ml of castor oil orally. Group I was taken as the control group (0.5ml of distilled water), Group II which received Loperamide (5mg/kg) served as the standard group, and Groups III and IV received the extract (100, 200 mg/kg, oral) 30 minutes before the castor oil administration. Each animal was placed in an individual cage, the floor of which was lined by blotting paper. The floor lining was changed every hour. The consistency of the faecal matter and the number of both the wet and the dry diarrhoeal droppings were counted every hour for a period of 4 hours. During an observation period of 4 hours, the total number of faeces which were excreted by
the animals was recorded. The numerical score which was based on the stool consistency was assigned as follows; normal stool=1, semi solid=2, and watery stool=3 [10].

**Magnesium Sulphate-Induced Diarrhoea:**
A similar protocol, as the one which was used for castor oil-induced diarrhoea, was followed. Diarrhoea was induced by the oral administration of Magnesium sulphate at a dose of 2g/kg to the animals, 30 minutes after the pre-treatment with distilled water to the control group, after the pre-treatment with Loperamide (5mg/kg) to the positive control group and after the pre-treatment with the aqueous extract at the doses of 100 and 200 mg/kg to the test groups. All the administrations were carried out through the oral route [11].

**Effect on Gastrointestinal Transit Time:**
The mice were kept in fasting for 24 hours and were divided into four groups of five mice each and each animal was given 0.1ml of 1% charcoal suspension orally, 60 min after an oral dose of the test drug, the standard and the vehicle. Group I was administered 0.5ml distilled water, Group II received Loperamide 5mg/kg and Groups III and IV received the extract at the dose of 100mg/kg and 200mg/kg body weight respectively. The faecal boluses which were expelled were collected. Each faecal bolus was pressed on a white sheet of paper to examine the presence of the charcoal meal. The time for the appearance of the 1st faecal bolus with the charcoal meal was recorded.

**Statistical Analysis:**
The data which was obtained in the studies were subjected to one way analysis of variance (ANOVA) for determining the significant difference. The inter group significance was analyzed by using Dunnet’s t-test. A p value of < 0.05 was considered to be significant. All the values were expressed as mean ± SEM

**RESULTS**
In the castor oil-induced diarrhoea experiment, the extract of *Lycopus europaeus* produced a marked anti-diarrhoeal effect in the mice, as shown in Table 1. At doses of 100 and 200 mg/kg, the extract significantly decreased (p<0.05) the total number of faeces which was produced upon the administration of castor oil.
(58.03% at 100mg/kg, 69.64% at 200mg/kg) as compared to that in the control group. Similarly, the extract, at 100 and 200 mg/kg dose levels, significantly (p<0.05) reduced the extent of the diarrhoea (68.96%, 79.88%) in the test animals in the magnesium sulphate-induced diarrhoea as shown in Table 2. However, both the doses were shown to reduce the total number of faeces in the test groups as compared to that in the control.

In the gastro-intestinal transit test, the extract, at the doses of 100 and 200 mg/kg, retarded the gastro-intestinal transit of the charcoal meal in mice, where a significant (p<0.05) retardation of the intestinal transit was observed at the doses of 100 and 200 mg/kg dose as compared to that in the control as shown in Table 3.

Table: 1. Effect of Le.cr on castor oil induced diarrhea in mice.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment</th>
<th>No. of faecal droppings in 4 h</th>
<th>% inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Castor oil (0.5ml,p.o) + Distilled water(0.5ml, p. o)</td>
<td>22.4±0.17</td>
<td>-</td>
</tr>
<tr>
<td>Loperamide</td>
<td>Castor oil (0.5ml, p .o) + Loperamide (5mg/kg, p. o)</td>
<td>3.5±1.41**</td>
<td>84.37**</td>
</tr>
<tr>
<td>Le.cr</td>
<td>Castor oil (0.5ml,p.o) + Le.cr (100mg/kg, p .o)</td>
<td>9.4±0.91**</td>
<td>58.03**</td>
</tr>
<tr>
<td>Le.cr</td>
<td>Castor oil (0.5ml, p .o) + Le.cr (200mg/kg, p .o)</td>
<td>6.8±0.97**</td>
<td>69.64**</td>
</tr>
</tbody>
</table>

Values are presented as mean ± SEM, (n=5); ** p<0.05, Dunnet’s t-test as compared to Control.

Table: 2. Effect of Le.cr on MgSO₄ in induced diarrhea in mice

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment</th>
<th>No. of faecal droppings in 4 h</th>
<th>% inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>MgSO₄ 2g / kg p.o) + Distilled water(0.5ml, p. o)</td>
<td>17.4±0.65</td>
<td>-</td>
</tr>
<tr>
<td>Loperamide</td>
<td>MgSO₄ 2 g/ kg , p .o) + Loperamide (5mg / kg, p. o)</td>
<td>2.1±0.2**</td>
<td>87.93**</td>
</tr>
<tr>
<td>Le.cr</td>
<td>MgSO₄ 2g/ kg ,p .o) + Le.cr (100mg / kg, p.o)</td>
<td>5.4±0.71**</td>
<td>68.96**</td>
</tr>
<tr>
<td>Le.cr</td>
<td>MgSO₄ 2g/ kg , p.o) + Le.cr (200mg / kg, p.o)</td>
<td>3.5±0.3**</td>
<td>79.88**</td>
</tr>
</tbody>
</table>

Values are presented as mean ± SEM, (n=5); ** p<0.05, Dunnet’s t-test as compared to Control.
Table 3: Effect of Aqueous extract of Le.cr on Charcoal meal stimulated gastro-intestinal transit.

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Dose (P.O)</th>
<th>Time (minutes) for the appearance of 1st faecal bolus with Charcoal meal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Distilled water</td>
<td>0.5ml</td>
<td>57±1.23</td>
</tr>
<tr>
<td>Standard</td>
<td>Loperamide</td>
<td>5mg/kg</td>
<td>225±2.54**</td>
</tr>
<tr>
<td>Test drug</td>
<td>Le.cr</td>
<td>100mg/kg</td>
<td>124±3.7**</td>
</tr>
<tr>
<td>Test drug</td>
<td>Le.cr</td>
<td>200mg/kg</td>
<td>172±1.6**</td>
</tr>
</tbody>
</table>

Values are presented as mean ± SEM, (n=5); ** p<0.05, Dunnet’s t-test as compared to Control.

DISCUSSION

Diarrhoea results from an imbalance between the absorptive and secretory mechanisms in the intestinal tract, which is accompanied by an excess loss of fluid in the faeces. In some types of diarrhoea, the secretory component predominates, while other types of diarrhoea are characterized by hyper motility. Castor oil causes diarrhoea due to its active metabolite, ricilonic acid [12], which stimulates the peristaltic activity in the small intestine, leading to changes in the electrolyte permeability of the intestinal mucosa. Its action stimulates the release of endogenous prostaglandins [13]. In this study, the aqueous extract of *Lycopus europaeus* exhibited a significant anti-diarrhoeal activity. The aqueous extract of *Lycopus europaeus* significantly reduced the intestinal transit, as was observed by a decrease in the intestinal motility of the charcoal meal. Phytochemical screening revealed the presence of glycosides, sugars, terpenes and flavonoids. Earlier studies have shown that the anti-dysenteric and anti-diarrhoeal properties of medicinal plants were due to the presence of tannins, saponins, flavonoids, sterols and/or tri terpenes and reducing sugars [14]. Hence, tannins, reducing sugars, sterols and/or tri terpenes may be responsible for the mechanism of action of the anti-diarrhoeal activity of *Lycopus europaeus*. This could be due to the fact that the extract increased the re-absorption of water by decreasing the intestinal motility, as was observed by the decrease in the intestinal transit in the charcoal meal test.
CONCLUSION

The results of this investigation revealed that the aqueous extract of *Lycopus europaeus* contained pharmacologically active substances with anti-diarrhoeal properties.

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