EVALUATION OF ANTI-DIARRHEAL PROPERTY OF CRUDE EXTRACTS AND DIFFERENT FRACTIONS OF STEM BARK AND FRUITS OF *OROXYLUM INDICUM*

Md. Mahfuzur Rahman¹, Majedul Islam², Syed Abu Hasnat², Md. Rasal Hossain Khan³, Nazmul Hasan Sumon² and Dr. Md. Abdul Muhit⁴

¹Department of Clinical Pharmacy and Pharmacology, Faculty of Pharmacy, University of Dhaka.
²Department of Pharmaceutical Technology, University of Dhaka, Dhaka, Bangladesh.
³Department of Clinical Pharmacy and Pharmacology, Faculty of Pharmacy, University of Dhaka.
⁴Department of Pharmaceutical Chemistry, University of Dhaka, Dhaka, Bangladesh.

ABSTRACT

Globally one in nine children deaths occur due to diarrheal diseases, making diarrhea the second leading cause of death among children under the age of five.[¹] Specially, for Third World countries, millions of people are dying of it every year.[²] From the ancient time plants have been using as source of new drugs.[³] In this experiment, crude Methanolic extract of *Oroxylum indicum* bark and fruits with different soluble parts were subjected to investigate for the evaluation of antidiarrheal activity on mice in vivo experiment. Twenty eight adult Swiss albino mice (mus domesticus) were divided into seven groups for the experiment. Each group received a particular treatment i.e. control, standard, single dose of Methanolic crude extract and its different fractions of bark and fruits of *Oroxylum indicum*. Castor oil was used for the induction of diarrhea in mice. The methanolic crude extract and its different fractions (Ethyl acetate fraction, Dichloromethane fraction, Hexane fraction, Carbon tetrachloride fraction) (400mg/kg) cause reduction of diarrheal feces by 50%, 31.4%, 52.3%, 34.9%, 53.55%. The methanolic crude extract of fruit and its different fractions (Ethyl acetate fraction, Dichloromethane fraction,
Hexane fraction, Carbon tetrachloride fraction) (400mg/kg) cause reduction of diarrheal feces by 46.5%, 32.6%, 45.3%, 33.7%, 51.2%. This experiment reveals that methanol extracts of bark and fruits of *Oroxylum indicum* showed significant anti-diarrheal property.

**KEYWORDS:** Diarrhea, *Oroxylum indicum*, Swiss albino mice, methanolic extract.

**INTRODUCTION**
Abnormal passage of liquid or unformed stool at an increased frequency is termed as diarrhea: Infectious agents, certain medications, plant and animal toxins, gastrointestinal disorders, and substances that enhance gastrointestinal tract secretions are, may be, the triggers of diarrhea. It can also be caused by the ingestion of poorly absorbable resources or inflammatory and dysmotility troubles of the gastrointestinal tract.[4]

1 in 9 child deaths worldwide occurs due to Diarrheal diseases, making diarrhea the second leading cause of death among children under the age of 5. Diarrhea kills more than two thousand children every day—more than AIDS, malaria, and measles combined.[1] Diarrhea is even more deadly for children with HIV; for these children, the death rate is 11 times greater than that for children without HIV.[5]

Diarrheal diseases are a major setback in Third World countries and millions of people are dying of it every year.[2] From the ancient time plants have been using as source of new drugs. Many plant species have been screened for substances with therapeutic activity and many of them are found as promising source of antidiarrheal drugs.[3] For this rationale, World Health Organization (WHO) and many other international organizations have encouraged studies pertaining to the treatment and prevention of diarrheal diseases using traditional medical practices.[6]

*Oroxylum indicum* (Linn.) Vent, the plant used in this study is one among the group of ten drugs named Dasamool, widely used in Ayurvedic system of medicine.[7] Locally in Bangladesh, it is known as Khona, Sona, Hona, Nasona. Patti, Dinga, Kanak, Kanaidinga, Bhinga, Thona etc. English names of the plant include, Indian trumpet flower, broken bones plant, Indian calosanthes, Indian Trumpet, Indian trumpet flower, midnight horror, etc.[8]

*Oroxylum indicum*, a deciduous tree, has small or medium size, up to 12 m in height with soft light brown or grayish brown bark with corky lenticels. The leaves are extremely large, 90-180 cm long 2-3 pinnate with 5 or additional pairs of primary pinnate, rachis especially fast,
cylindrical, swollen at the connection of branches, leaflets 2-4 pairs ovate or elliptic, acuminate, glabrous. The fresh root bark is malleable and juicy in nature; it’s sweet, but becomes bitter later. On drying, the bark shrinks, stick closely to the wood and becomes faintly fissured.\(^8\) The tree has long fruit pods (Fig. 1) that curved downward; hang down from the branches, looks like the wings of a large bird or dangling sickles or sword in the night.

\[\text{Fig. 1: Oroxylum indicum (Fruits)}\]

*Oroxylum indicum* grows in India, Bangladesh, Sri Lanka, Philippines, Indonesia, China, Bhutan, Malaysia and Mallaca. In Bangladesh, it is distributed in Chittagong hill tracts, Cox’s bazaar and Sylhet. It is mostly sighted along the river banks or slopes of the hills.\(^7,9,10\)

**MATERIAL AND METHODS**

**Principle**

The antidiarrheal activity of the crude extract and its different fractions of *Oroxylum indicum* were evaluated using the method of castor oil induced diarrhea in mice.\(^8\) According to this method, each mouse was fed with 10 mm of highly pure analytical grade castor oil which would induce diarrhea. The number of fecal stools was recorded for each individual mouse.\(^11\) The observations of the experiment groups were compared against that of the control to evaluate the antidiarrheal activity of the samples.

**Collection and preparation of plant material**

The plant samples (bark and fruits) of *Oroxylum indicum* were collected from Teknaf, Cox’s bazar in April 2012. The plant was identified by National Herbarium, Mirpur, Dhaka (Accession No- 37883) Dhaka, for further reference. The bark and fruit were dried under sun
and oven, then dried parts were ground in coarse powder using high capacity grinding machine in the Phytochemical Research Laboratory, Faculty of Pharmacy, University of Dhaka.

**Experimental animal**

Twenty eight adult Swiss albino mice (*mus domesticus*) of either sex weighing between 30-35g, aged 4-5 weeks obtained from the Jahangirnagar University, Bangladesh were used for the experiment. The animals were housed in polypropylene cages (30x20x7 cm) in standard conditions for (21± 1ºc with a 12 h light and dark cycle) for 3 days before experiment.

**Experimental design**

Mice were divided into seven groups denoted as group-I, group-II, group-III, group- IV, group –V, group-VI and group-VII consisting of 4 mice in each group. Each group received a particular treatment i.e. control, standard, single dose of Methanolic crude extract and its different fractions of bark and fruits of *Oroxylum indicum*.

**Preparation of test materials**

In order to administer the crude extract at doses of 400 mg/kg body weight of mice, 50 mg of the extract Tween-80 and normal saline were mixed to make final volume of the suspension 2.5 ml.

For the preparation of at the dose of 5 mg/kg-body weight, 50 mg of Loperamide was suspended in 2.5 ml normal saline.

**Procedure**

The method described by Shoba & Thomas,[2] was followed for this study with slight modification. The animals were divided into control, positive control and test groups containing four mice in each group. Control group received vehicle (1% Tween 80 in water) at dose of 10ml/kg orally. The positive control group received Loperamide at the dose of 50 mg/kg orally; test groups received methanolic extract, ethyl acetate fraction, dichloromethane fraction, hexane fraction carbon tetrachloride fraction at the dose of 400 mg/kg b.w. orally. Diarrhea was induced by oral administration of castor oil to each mouse, 30 min after above treatments. During an observation period of 4 hours, the numbers of diarrheic feces excreted by the animals were recorded.
RESULTS AND DISCUSSION

Statistical analysis
All values were expressed as mean± standard error of mean (SEM) and the results were analyzed statistically by one way analysis of variance (ANOVA) followed by Dunnett’s test by using SPSS Ver. No. 16.

RESULTS

For bark
The methanolic extract of *Oroxylum indicum* bark showed profound anti-diarrheal property in castor oil induced diarrheal mice. The methanolic crude extract and its different fractions (Ethyl acetate fraction, Dichloromethane fraction, Hexane fraction, Carbon tetrachloride fraction) (400mg/kg) cause reduction of diarrheal feces by 50%, 31.4%, 52.3%, 34.9%, 53.551.2%, respectively compared to standard Loperamide (82.6%).

Table: 1 effect of methanolic extract and different fractions on castor oil induced diarrhea in mice

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (b.w)</th>
<th>Number of diarrheal feces (Mean + SEM)</th>
<th>% Reduction of diarrhea</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (Saline)</td>
<td>10 ml/kg</td>
<td>21.5 ± 1.76</td>
<td>---</td>
</tr>
<tr>
<td>Standard</td>
<td>50 mg/kg</td>
<td>3.75 ± 0.853</td>
<td>82.6</td>
</tr>
<tr>
<td>Crude</td>
<td>400 mg/kg</td>
<td>10.75 ± 0.95</td>
<td>50</td>
</tr>
<tr>
<td>Ethyl acetate</td>
<td>400 mg/kg</td>
<td>14.75 ± 1.15</td>
<td>31.4</td>
</tr>
<tr>
<td>Dichloro methane</td>
<td>400 mg/kg</td>
<td>10.25 ± 1.03</td>
<td>52.3</td>
</tr>
<tr>
<td>Hexane</td>
<td>400 mg/kg</td>
<td>14 ± 1.13</td>
<td>34.9</td>
</tr>
<tr>
<td>Carbon tetrachloride</td>
<td>400 mg/kg</td>
<td>10 ± 0.885</td>
<td>53.5</td>
</tr>
</tbody>
</table>

Fig 2: % reduction of diarrheal feces by different fractions
Note: STD= Standard sample, Crude= Methanolic crude extract, EAOI= Ethyl acetate fraction of *Oroxylum indicum*, DCMF= Dichloromethane fraction of *Oroxylum indicum*, CTOI= Carbon tetrachloride fraction of *Oroxylum indicum*

**For fruits**

The methanolic extract of *Oroxylum indicum* fruits showed profound anti-diarrheal property in castor oil induced diarrheal mice (Table-2). The methanolic crude extract and its different fractions (Ethyl acetate fraction, Dichloromethane fraction, Hexane fraction, Carbon tetrachloride fraction) (400mg/kg) cause reduction of diarrheal feces by 46.5%, 32.6%, 45.3%, 33.7%, 51.2%, respectively compared to standard Loperamide (82.6%).

Table: 2: effect of methanolic extract and different fractions on castor oil induced diarrhea in mice

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (b.w)</th>
<th>Number of diarrheal feces (Mean + SEM)</th>
<th>% Reduction of diarrhea</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (Saline)</td>
<td>10 ml/kg</td>
<td>21.5 ± 1.76</td>
<td>---</td>
</tr>
<tr>
<td>Standard</td>
<td>50 mg/kg</td>
<td>3.75 ± 0.853</td>
<td>82.6</td>
</tr>
<tr>
<td>Crude</td>
<td>400 mg/kg</td>
<td>11.5 ± 1.08</td>
<td>46.5</td>
</tr>
<tr>
<td>Ethyl acetate</td>
<td>400 mg/kg</td>
<td>14.5 ± 0.408</td>
<td>32.6</td>
</tr>
<tr>
<td>Dichloromethane</td>
<td>400 mg/kg</td>
<td>11.75 ± 0.288</td>
<td>45.3</td>
</tr>
<tr>
<td>Hexane</td>
<td>400 mg/kg</td>
<td>14.25 ± 0.408</td>
<td>33.7</td>
</tr>
<tr>
<td>Carbon tetrachloride</td>
<td>400 mg/kg</td>
<td>10.5 ± 0.707</td>
<td>51.2</td>
</tr>
</tbody>
</table>

**Fig 3: % Reduction of Diarrheal Feces By Different Fractions**

Note: STD= Standard sample, Crude= Methanolic crude extract, EAOI= Ethyl acetate fraction of *Oroxylum indicum*, DCMF= Dichloromethane fraction of *Oroxylum indicum*, CTOI= Carbon tetrachloride fraction of *Oroxylum indicum*
DISCUSSION

Castor oil causes diarrhea due to its active metabolite, ricinolic acid,\textsuperscript{[13,14]} which stimulates peristaltic activity in the small intestine, leading to changes in electrolyte permeability of the intestinal mucosa. Its action also stimulates the release of endogenous prostaglandin.\textsuperscript{[14]} Several other mechanisms have been reported to cause diarrhea by castor oil including inhibition of intestinal Na\textsuperscript{+}/K\textsuperscript{+}-ATPase activity, activation of adenyl cyclase or mucosal cAMP-mediated active secretion and platelet activating factor.\textsuperscript{[15]}

The previous reports have demonstrated the antidiarrheal activity of tannin,\textsuperscript{[15]} flavonoids,\textsuperscript{[14]} alkaloids\textsuperscript{[4]} Saponins, reducing sugars and sterols and or terpenes containing plant extracts.

The present study showed that methanolic crude extract of both bark and fruits and their different fractions (Ethyl acetate fraction, Dichloromethane fraction, Hexane fraction, CCl\textsubscript{4} fraction) (400mg/kg) cause reduction of diarrheal feces compared to standard Loperamide.

CONCLUSION

The result of this study shows that the methanolic extract of both bark and fruits of \textit{Oroxylum indicum} possesses strong antidiarrheal activity. But this study is preliminary type and it would be interesting to carry out further study for isolating the possible phytococonstituent and characterization of the active constituents which may be responsible for the Anti-diarrheal activity.

REFERENCE


