

**INTERNATIONAL JOURNAL OF ADVANCES IN PHARMACY,  
BIOLOGY AND CHEMISTRY****Research Article****Antidiarrhoeal Activity of Ethanolic Extract of  
*Onosma bracteatum wall*****GP.Choudhary**

School of Pharmacy, Ring road, Devi Ahilya University, Indore, Madhya Pradesh, India.

**ABSTRACT**

The present study was carried out to evaluate the antidiarrhoeal effect of ethanolic extract of *Onosma bracteatum* (Family: Combretaceae) using castor oil and magnesium sulphate induced diarrhoea models in mice. At the doses of 250 and 500 mg/kg b.w, the ethanolic extract showed significant antidiarrhoeal activity in both models. The extract, at the dose of 250 and 500 mg/kg, retarded the intestinal transit of charcoal meal in mice as compared to the control.

**Keywords:** *Onosma bracteatum*, Antidiarrhoeal activity, Loperamide, Atropine sulphate.

**I. INTRODUCTION**

In developing countries, a majority of people living in rural areas almost exclusively use traditional medicine for treating diseases of different types including diarrhoea. 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 due to 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. According to W.H.O. estimates for 1998, about 7.1 million deaths were caused by diarrhoea<sup>1</sup>. The incidence of diarrhoeal diseases still remains high despite the efforts of many governments and international organizations to curb it. It is therefore important to identify and evaluate available natural drugs as alternatives to currently used anti-diarrhoeal drugs.

Diarrhoea is characterized by increased frequency of bowel movement, wet stool and abdominal pain<sup>1</sup>. It is a leading cause of malnutrition and death among children in the developing countries of the world today. 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<sup>2</sup>. In recent years, there has been a great interest in herbal remedies for the treatment of number of ailments. A range of medicinal plants with anti-diarrhoeal properties is widely used by traditional healers. However, the effectiveness of many of these antidiarrhoeal traditional medicines has not been scientifically evaluated.

*Onosma bracteatum* (OB), Wall (Family Boraginaceae, commonly known as Gaozaban, Gojihva) which has been reported to be used in the treatment of asthma and bronchitis. The drug is used as tonic, alterative, demulcent, diuretic and is considered cooling. It is useful as a spasmolytic. A decoction is used in the treatment of rheumatism, syphilis and leprosy. The plant is considered to be useful in relieving excessive thirst and restlessness in febrile excitement, and also to be useful in relieving functional palpitation of the heart, irritation of the bladder and stomach, and strangury<sup>3</sup>.

Hence, the present study was under taken to evaluate the antidiarrhoeal activity of ethanolic extract of aerial parts of *Onosma bracteatum*.

**II. MATERIALS AND METHODS****Plant Material**

The dried aerial parts of *Onosma bracteatum* was purchased from Dravid Herbs World, Pondicherry, India, and identified by Dr.A.B.Sheerwani. (Retd. Prof. and Head), Deptt. of Botany, Holkar Science College, Indore. A voucher specimen has been deposited in our laboratory for further reference.

**Extraction**

*Onosma bracteatum* aerial parts extracted with 90% ethanol in a soxhlet extractor. The extract was concentrated under reduced pressure at a temperature below 60°C to yield a syrupy mass

(Yield -7.45%), which was used for the present investigation

#### **Preliminary phytochemical analysis**

The phytochemical tests were performed using various reagents<sup>4</sup>. The ethanolic extract was tested for the presence or absence of alkaloid, glycosides, tannins, steroids, proteins, amino acids, phenolic compounds and flavonoids. Preliminary phytochemical analysis shows the presence of glycosides, phenolic compounds and flavonoids.

#### **Animals**

Swiss mice of either sex, weighing 25-30g were obtained from the experimental animal house, School of life science, Devi Ahilya University, Indore. They were maintained under standard housing condition (Room temperature  $25\pm 2^{\circ}\text{C}$  and 45-55% RH with 10:14h, L:D cycles). The animals were given standard laboratory feed and water ad libitum. The study was cleared by Animal ethics committee (School of life science, Devi Ahilya University, Indore). All the animals received humane care according to criteria outlined in the guide for the care and use of laboratory animals prepared by the national academy of the sciences and published by national institute of health.

#### **Antidiarrhoeal activity study by castor oil induced diarrhea**

The method, described by Shoba and Thomas (2001)<sup>5</sup>. The animals were divided into control, positive control and test groups containing five mice in each group. Control group received vehicle (1% Tween 80 in water) at a dose of 10 ml/kg orally. The positive control group received loperamide at the dose of 3 mg/kg orally, test groups received the ethanolic extract at the doses of 250 and 500 mg/kg mg/kg b.w orally. Each animal was placed in an individual cage, the floor of which was lined with blotting paper. The floor lining was changed every hour. Diarrhoea was induced by oral administration of 1 ml castor oil to each mouse, 30 min after the above treatments.

During an observation period of 4 h, the total number of faecal output and the number of diarrhoeic faeces excreted by the animals were recorded.

#### **Antidiarrhoeal activity study by magnesium sulphate induced diarrhoea<sup>6</sup>**

A similar protocol as for castor oil induced diarrhoea was followed. Diarrhoea was induced by oral administration of magnesium sulphate at the dose of 2 g/kg to the animals 30 min after pretreatment with vehicle (1% Tween 80 in water, 10 ml/kg, p.o.) to the control group, loperamide (3 mg/kg) to the positive control group, the ethanolic extract at the doses of 250 and 500 mg/kg to the

test groups. All the administrations were carried out through oral route.

#### **Effect on Gastrointestinal Motility<sup>7</sup>**

Mice were fasted for 18h and divided into four groups of five mice each and each animal was given 1 ml of charcoal meal orally (5% activated charcoal suspended in 1% tween 80) 60 min after an oral dose of the test drugs and vehicle. Group I was administered 1% tween 80 (10 ml/kg) and groups III and IV received extract at the dose of 250 mg/kg and 500 mg/kg body weight respectively. Group II received atropine sulfate (0.1 mg/kg,) as the standard drug. Mice were sacrificed after 30 min and the intestine was removed without stretching and placed lengthwise on moist filter paper. The intestinal transit was calculated as a percentage of the distance travelled by the charcoal meal compared to the length of the small intestine .

#### **Statistical analysis**

Results are expressed as mean  $\pm$  S.E. Mean values were evaluated by One Way ANOVA followed by Dunnett test. Statistical significance was accepted at  $P < 0.05$ .

### **III. RESULTS AND DISCUSSION**

The ethanolic extract at the doses of 250 and 500 mg/kg, produced a dose dependent decrease in the number of faecal matters passed by the animals in castor oil-induced diarrhoeal model (Table I). At higher dose (500 mg/kg) of the extract, a significant ( $p < 0.05$ ) inhibition of characteristic diarrhoeal faeces was observed. Similarly, the extract at 500 mg/kg dose level significantly ( $p < 0.05$ ) reduced the extent of diarrhoea in test animals in magnesium sulphate-induced diarrhoea (Table II). However, both the doses were shown to reduce the total number of faeces when compared to control. In the gastrointestinal motility test, the extract at the doses of 250 and 500 mg/kg retarded the intestinal transit of charcoal meal in mice where a significant ( $p < 0.05$ ) retardation of intestinal transit was observed at 500 mg/kg dose when compared to the control (Table III).

Diarrhoea results from an imbalance between the absorptive and secretory mechanisms in the intestinal tract accompanied by hurry resulting in an excess loss of fluid in the faeces. In some diarrhoea the secretory component predominates while other diarrhoea is characterized by hypermotility. Castor oil causes diarrhoea due to its active metabolite, ricinolic acid<sup>8-9</sup>, which stimulates peristaltic activity in the small intestine, leading to changes in the electrolyte permeability of the intestinal ucosa. Its action also stimulates the release of endogenous prostaglandin<sup>10</sup>. In this study, the ethanolic extract of *Onosma bracteatum* exhibited a significant antidiarrhoeal activity. The results were similar to that of the standard drug

loperamide (3mg/kg) with regard to the severity of diarrhoea. Phytochemical screening revealed the presence of glycosides, phenolic compounds and flavonoids. Earlier studies showed that anti-dysenteric and anti-diarrhoeal properties of medicinal plants were due to glycosides, flavonoids, sterol and/or triterpenes<sup>11-13</sup>. Hence, glycosides and/or flavonoids may be responsible for the mechanism of action of anti-diarrhoeal activity. This can be due to the fact that the extract increased the reabsorption of water by decreasing intestinal motility.

As the previous phytochemical screening of extract showed the presence of alkaloids, flavonoids, tannins and gums<sup>14</sup>, these constituents may mediate the anti-diarrhoeal property of the extract. Flavonoids, present in the plant extract, are reported to inhibit release of autacoids and prostaglandins, thereby may inhibit motility and secretion induced by castor oil<sup>15</sup>. The anti-diarrhoeal activity of the extract may also be due to denature proteins forming protein tannates which make intestinal mucosa more resistant and reduce secretion. On the other hand, magnesium sulphate has been reported to induce diarrhoea by increasing

the volume of intestinal content through prevention of reabsorption of water. It has also been reported that it promotes the liberation of cholecystokinin from the duodenal mucosa, which increases the secretion and motility of small intestine and thereby prevents the reabsorption of sodium chloride and water<sup>16</sup>. The ethanolic extract was found to improve the diarrhoeic condition in this model. The extract may have increased the absorption of water and electrolyte from the gastrointestinal tract, since it delayed the gastrointestinal transit in mice as compared to the control. 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.

#### IV. CONCLUSION

The results of this investigation revealed that ethanolic extract contains pharmacologically active substance(s) with anti-diarrhoeal properties. Further research is to be carried out to fractionate and purify the extract, in order to find out the molecule responsible for the anti-diarrhoeal activity observed.

**Table 1: Effect of *Onosma bracteatum* on castor oil-induced diarrhoea in mice**

Treatment	Dose (mg/kg, p.o.)	Total number of faeces in 4 h	Total number of diarrhoeal faeces in 4 h
Control	-	16.24±1.0	12.1±1.44
Loperamide	3	2.88±0.8**	1.9±0.88**
Ethanolic extract	250	8.11±2.18**	4.8±0.8**
Ethanolic extract	500	5.1±1.20**	3.48±0.98**

Values are mean±S.E. (n =5). \*\*P < 0.01 vs. control, One way ANOVA followed by Dunnett test.

**Table 2: Effect of *Onosma bracteatum* on magnesium sulphate-induced diarrhea in mice**

Treatment	Dose (mg/kg, p.o.)	Total number of faeces in 4 h	Total number of diarrhoeal faeces in 4 h
Control	-	12.94±1.80	10.1±1.5
Loperamide	3	14.4±2.4**	7.6±0.44**
Ethanolic extract	250	25.3±3.24**	18.8±2.18**
Ethanolic extract	500	18.2±2.18**	9.9±1.38**

Values are mean±S.E. (n =5). \*\*P < 0.01 vs. control, One way ANOVA followed by Dunnett test.

**Table 3: Effect of *Onosma bracteatum* on the intestinal transit of charcoal meal in mice**

Treatment	Dose (mg/kg, p.o.)	% travelled by charcoal meal
Control	-	76.80 ± 3.88
Atropine sulphate	0.1 mg/kg	34.29 ± 2.62**
Ethanolic extract	250	56.64 ± 3.12**
Ethanolic extract	500	46.42 ± 2.78**

Values are mean ±S.E. (n =5). \*\*P < 0.01 vs. control, One way ANOVA followed by Dunnett test.

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