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Evaluation of Acute and Sub-Chronic Oral Toxicity of Entoban: A Polyherbal Drug on Experimental Mice

Sadia Shakeel[1*], Zeeshan Ahmed Sheikh[2], Saira Bano[2] and Khan Usmanghani[2, 3]

[1]*Dow College of Pharmacy, Dow University of Health Sciences, Karachi, Pakistan*

[2]*Herbion Pakistan (Pvt.) Ltd., Korangi Industrial Area, Karachi, Pakistan*

[3]*Faculty of Pharmacy, Jinnah University for Women, Karachi, Pakistan*

***Corresponding author**: Sadia Shakeel, Dow College of Pharmacy, Dow University of Health Sciences, Karachi-75270, Pakistan, Tel: 6595229237; E-mail: sadia.shakeel@duhs.edu.pk

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Abstract

Objectives: The current preclinical study aimed at appraising the claim of anti diarrheal activity, acute and sub chronic oral toxicity of the polyherbal formulation Entoban to acknowledge its safety and efficacy.

Methods: In order to investigate the antidiarrheal activity albino mice were treated with Entoban at doses of 2.5, 5, 10 mg/kg. For evaluation of acute toxicity the animals were treated orally with doses (1 or 5 g/kg) of the Entoban capsule aqueous extract, maintained under standard laboratory conditions. Entoban was administered at doses of 50, 100 and 200 mg/kg body weight for 28 days for determining sub chronic oral toxicity. The data collected were summarized as mean ± SEM. Student's t- test was used to determine significant differences.

Results: Entoban showed significant inhibition of diarrhoea in dose dependent manner. Entoban did not cause any mortality in albino mice at the given doses of 1 or 5 g/kg. Other signs of toxicity like hair loss, weight reduction, mucus membrane (nasal), lacrimation, drowsiness, gait and tremors were also not observed.

Conclusion: Results of the present study gave evidence of good tolerance of Entoban and the absence of detrimental effects on the functional state of the vital organs of the experimental animals in acute and sub chronic oral toxicity test. Future prospects include the clinical trials of the finished product as the clinical efficacy is proven in animal studies.

Keywords: Entoban; Diarrhoea; Acute toxicity; Sub-chronic toxicity; Safety

Introduction

Acute gastroenteritis remains a common complaint in infants and children worldwide, epitomized by diarrhoea, coupled with nausea, vomiting, fever, and abdominal pain [1]. In 1992, CDC prepared the first national guidelines for the management of childhood diarrhoea [2]. Diarrhoea is a sign of increased water content, whether due to impaired absorption and / or secretion of active ions, organic substrates [3,4]. According to UNICEF (United Nations Children's Fund), diarrhoea kills 1.5 million children under five years every year [5]. Diarrhoea caused 16% of child deaths in Pakistan [6]. The main causes of diarrhoea are dependent on the socioeconomic status of the population. In developing countries, chronic diarrhoea is often due to infection with functional disorders, malabsorption and inflammatory bowel disease [7]. Although diarrhoea can be represented as a simple symptom at one end, it may be life threatening at the other. The proper approach to the diagnosis and treatment of acute infectious diarrhoea is determined by the frequency and intensity of disease [8].

Although there are splendid advancements in modern medicine, yet traditional medicine has always been accomplished for treating gastrointestinal infections. The traditional medicine sector has become an imperative resource in health care, particularly in rural and tribal areas of the country [9]. According to the World Health Organization (WHO), the utilization of plant based medicines has exceeds to that of conventional medicines all over the globe by two to three times [10]. Plant derived drug is still the mainstay of about 75 - 80% of the global populace for primary health care; owing to the general belief that herbal drugs are devoid of any side effects besides being economical and easily accessible [10, 11]. Herbal remedies are remarkably successful in curing chronic diarrhoea and acute diarrheal diseases. It is obligatory to set up logical evidences for rational utilization of such traditional medicinal products. Hence, the present preclinical study aimed at appraising the claim of its anti-diarrheal activity, acute and sub chronic oral toxicity to acknowledge the safety and efficacy of the formulation.

Materials and Methods

Plant Materials

Herbs used were stored in dark at 23˚C. Every herb was evaluated for their macro and microscopic descriptions.

Drugs

Entoban capsules – Herbion Pak. Pvt. Ltd, Magnesium sulphate – Merck, Castor oil (refined pure) and Loperamide hydrochloride were obtained from a local outlet in Karachi.

Extract preparation

The collected herbs were washed. Air dried and extracted by continuous extraction using Soxhlet apparatus and ethanol 96 %. After exhaustive extraction, the collected extract was dried under reduced pressure using rotavapor and dried at water bath.

Animal handling

In order to investigate the antidiarrheal activity, acute and sub chronic oral toxicity of Entoban, 3 to 4 weeks aged NMRI albino mice of both sex, of around 25 to 35 g weight, were collected from the animal house facility of Herbion Pak. Pvt. Ltd. They were kept under standard environmental conditions i.e. 25 ± 1°C and 12 h dark / light cycle dark for the entire study time. Food and water were available ad libitum.

Castor oil induced diarrhoea

All procedures for testing were complying the Guide for the Care and Use of Laboratory Animals [12] and OECD guideline for acute toxicity [13, 14]. The animals used were divided in to control, test and positive groups. Each group contains six animals. Castor oil (0.2 ml) was used orally to induce diarrhoea to mice [15, 16]. The animals in control group received only distilled water (10 ml/kg) per oral (p.o); loperamide (2 mg/kg, p.o.) was given to the animals in positive control group; test group received Entoban at doses of 2.5, 5, 10 mg/kg, p.o., body weight thirty minutes earlier to the administration of castor oil. The parameters observed throughout an examination phase of 4 h, were: onset of diarrhoea, total weight of stool output, total weight of wet stools, total number of stool output, and number of wet stools (15). Percent inhibition of diarrhoea was calculated as follows:

$$\% \text{ inhibition of diarrhoea} = \frac{\text{Mean Number of wet defecation (control} - \text{test)}}{\text{Mean wet defecation of control}} \times 100$$

Magnesium sulphate induced diarrhoea

Analogous procedure as intended for castor oil induced diarrhoea was followed [15].Magnesium sulphate was administered 2 g/kg, p.o., to the animals 30 min following pre-treatment with distilled water (10 ml/kg,p.o.,) to the control group, loperamide (2 mg/kg, p.o.) to the positive control group, Entoban at doses of 2.5, 5, 10 mg/kg, p.o., to test group.

Acute toxicity studies

Healthy NMRI albino Mice of either sex (n = 15/sex) weighing between 25 – 35 g were treated orally with doses (1 or 5 g/kg) of the Entoban capsule aqueous extract, in standard laboratory setting. Animals were evaluated singly as a minimum for once for the duration of first 30 min after dosing, sporadically during the first 24 h and daily, thereafter, for a period of 7 days. Mortality and behavioural changes were observed for 1 week. Daily observations on the changes in skin and fur, eyes and mucus membrane (nasal), respiratory rate, heart rate, blood pressure, autonomic effects (salivation, perspiration, pilo erection, lacrimation, urinary incontinence and defecation) and central nervous system (ptosis, lethargy, gait, tremors and seizure) were noted.

Sub chronic toxicity

Healthy NMRI albino mice of either sex (n=40) were divided into 4 groups. Each group contains 10 animals of either sex. Group I was given water and standard diet. Group II was given standard diet, water and 50 mg/kg body weight of Entoban, group III was given standard feed, water and 100 mg/kg body weight of Entoban while group IV was given standard feed, water and 200 mg/kg body weight of Entoban. The drugs were administered for duration of 28 days. The treated animals were weighed up at 0, 7, 14 and 28 day of the doses.

Statistical analysis

The data collected were summarized as mean ± SEM. Student's t-test was used to determine significant differences.

Results

Effect of Entoban on castor oil induced diarrhoea

During the observation for 4 h. every mouse produced copious diarrhea in control group. Different doses of test product (Entoban) caused a significant dose dependent decrease in the rate of purging and weight of wet stools. Entoban showed 59 %, 79.51 %, 91.3 % inhibition of diarrhea at doses of 2.5 mg/kg, 5 mg/kg and 10 mg/kg while loperamide at dose of 2 mg/kg showed 93 % inhibition of diarrhea as shown in Table 1.

Group	Dose(/Kg)	Onset of diarrhoea (min)	Total weight of stools (g)	Weight of wet stools (g)	Total number of stools	Number of wet stools	% Inhibition
Control		52 ± 2.21	0.362 ± 0.010	0.325 ± 0.020	13.43 ± 0.23	12.00 ± 0.56	

Entoban	2.5 mg	85 ± 2.06	0.157 ± 0.005	0.14 ± 0.006	6.15 ± 0.31	4.92 ± 0.210	59*
Entoban	5 mg	112 ± 4.57	0.121 ± 0.048	0.081 ± 0.036	3.46 ± 0.24	2.46 ± 0.011	79.51**
Entoban	10 mg	172 ± 4.28	0.050 ± 0.012	0.035 ± 0.002	1.14 ± 0.26	1.04 ± 0.15	91.3***
Loperamide	2 mg	213 ± 5.12	0.036 ± 0.003	0.030 ± 0.003	1.04 ±0.23	0.84 ± 0.14	93 ***

Table 1: Effects of Entoban on Castor oil induced diarrhea in mice. Results were analyzed by Student't-test. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ vs control.

Group	Dose (/Kg)	Onset of diarrhoea (min)	Total weight of stools (g)	Weight of wet stools (g)	Total number of stools	Number of wet stools	% Inhibition
Control		43 ± 1.20	0.392 ± 0.060	0.285 ± 0.060	11.14 ± 0.53	9.47 ± 0.32	
Entoban	2.5 mg	87 ± 3.01	0.251 ± 0.010	0.14 ± 0.003	6.15 ± 0.43	5.12 ± 0.120	45.9*
Entoban	5 mg	116 ± 1.45	0.136 ± 0.043	0.085 ± 0.054	3.46 ± 0.36	2.55 ± 0.310	73.07**
Entoban	10 mg	192 ± 2.12	0.045 ± 0.014	0.035 ± 0.024	2.23 ± 0.17	1.38 ± 0.09	85.42***
Loperamide	2 mg	232 ± 3.01	0.036 ± 0.023	0.020 ± 0.013	1.85 ±0.23	0.95 ± 0.34	89.96***

Table 2: Effects of Entoban on Magnesium sulphate induced diarrhoea in mice. Results were analyzed by Student't-test. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ vs control.

Effect of Entoban on magnesium sulphate induced diarrhoea

Every mice in control group produced diarrhea after magnesium sulphate administration during the entire examination. Entoban showed 45.9%, 73.07%, 85.42% inhibition of diarrhea at doses of 2.5 mg/kg, 5 mg/kg and 10 mg/kg while loperamide at dose of 2 mg/kg showed 89.96 % inhibition of diarrhea (Table 2).

Acute toxicity studies

The test product (Entoban) did not cause any mortality in NMRI albino mice at the given doses of 1 or 5 g/kg. Other signs of toxicity like hair loss, weight reduction, mucus membrane (nasal), lacrimation, drowsiness, gait and tremors were also not observed. The test product (Entoban) appeared to be safe on doses 1g/kg or 5g/kg.

Sub chronic toxicity

The tested product (Entoban) was administered for a period of 28 days. The body weights of the treated animals were evaluated at 0, 7, 14 and 28 day of the doses. The weight of animals in control group slightly increases. There was not any noticeable change in the weights of tested animals.

Discussion

World Health Organization has given specifics value to the use of traditional medicines in the management and treatment of diarrhoea, by virtue of its economic viability, availability and experience of our ancestors [17]. Diarrhoea occurs when the intestine secrete more electrolytes and water. Castor oil induced diarrhoea demonstrates secretory diarrhoea, since ricinoleic acid induces diarrhoea by a hyper secretory response. An active constituent of castor oil is the ricinoleic acid, produces inflammatory and irritating actions on the intestinal mucosa causing the release of prostaglandins. It enhances the permeability of the mucosal cells and changes electrolyte transport, which ultimately consequences a hypersecretory response and causes diarrhoea [18]. As Entoban effectively inhibited the castor oil produced diarrhoea, it can be concluded that the antidiarrheal action was put forthed by antisecretory mechanism. Magnesium sulphate induces diarrhoea by osmotic properties, preventing reabsorption of water ions, leading to increase in the volume of the intestinal content [19].

The present study was directed to a polyherbal formulation Entoban which incorporates an outstanding combination of herbs that have been used for decades to eliminate microbes and worms from gastrointestinal tract. It is the combination of Holarrhena antidysenterica, *Berberis aristata,Symplocos racemosa, Querecus infectoria and Helicteres isora. Holarrhena antidysenterica* wall is an important medicinal plant belonging to the class Apocynaceae. Research has shown that different parts of *H. antidysenterica* executed various medicinal properties [20, 21]. D Kavitha reported that alkaloids of *H. antidysenterica* reduced diarrhoea in castor oil induced rats[20]. Shamkuwar revealed that aqueous extract of *Berberis aristata* (BA) treated mice, considerably reduced the stimulation time of diarrhoea, number of wet stools and total number of stools in the diarrhoea produced by magnesium sulphate. It has also causes antisecretory and antimotility activity in castor oil induced intestinal transport and intraluminal fluid accretion in animals. These consequences point toward that BA produces its antidiarrhoeal effect all the way through decreasing intestinal secretions and antispasmodic effect by restraining the intestinal motility [22].

Entoban showed 59%, 79.51%, 91.3% inhibition of diarrhoea in castor oil induced diarrhoea and 45.9%, 73.07%, 85.42% inhibition of diarrhoea in magnesium sulphate induced diarrhoea at doses of 2.5 mg/kg, 5 mg/kg and 10 mg/kg. Entoban did not cause any mortality in NMRI albino mice at the given doses of 1 or 5 g/kg. Other signs of toxicity like hair loss, weight reduction, mucus membrane (nasal), lacrimation, drowsiness, gait and tremors were also not observed.

Entoban possesses antimotility and antisecretory activity due to the presence of different phytochemicals. Results of the present study gave evidence of good tolerance of Entoban and the absence of detrimental effects on the functional state of the vital organs of the experimental animals in acute and sub chronic oral toxicity test.

Conclusion

In the present study, the developed polyherbal Entoban capsules incorporating the herbs in standardized form provide an opportunity to validate its traditional claim regarding its therapeutic efficacy. Future prospects include the clinical trials of the finished product as the clinical efficacy is proven in animal studies.

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