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Research Article

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Antidiarrhoeal activity of an ayurvedic formulation: Enterocin

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ABSTRACT

An ayurvedic formulation, Enterocin was tested for its antidiarrhoeal, antimotility and antisecretory activities. Antidiarrhoeal effect of Enterocin was evaluated in castor oil and magnesium sulphate induced diarrhoea, intestinal transit and intestinal secretion in mice at a dose of 2.5, 5, 10 ml/kg. Enterocin treated mice, significantly reduced the induction time of diarrhoea, weight of stools and number of stools in the diarrhoea induced by castor oil and magnesium sulphate. It has also produced antimotility and antisecretory activity in castor oil induced intestinal transit and intraluminal fluid accumulation in mice. Phytochemical analysis showed the presence of carbohydrates, steroids, triterpenoids, alkaloids, flavonoids and tannins as major constituents. These results suggest that Enterocin possesses antidiarrhoeal effect may be due to its antimotility and antisecretory effect. Antimotility and antisecretory effect of Enterocin may be due to the presence of different phytochemicals.

Key words: Enterocin, diarrhoea, intestinal transit, intestinal secretion.

INTRODUCTION

Diarrhoea includes increase in volume or fluidity of stools, change in consistency and increase frequency of defecation [1]. Diarrhoea involves both an increase in the motility of the gastrointestinal tract, along with increased secretion, and a decrease in the absorption of fluid and thus a loss of electrolytes and water [2]. The rapid movement of faeces through the intestine results in abnormally frequent and watery stools [3, 4].

Diarrhoea which could be infectious or non-infectious, is one of the principal cause of death, particularly in the malnourished infants [5]. Medicinal herbs constitute an indispensable component of the traditional medicine practiced world wide due to the economical viability, accessibility and ancestral experience. Despite the availability of a vast spectrum of approaches for diarrhoeal management, a vast majority of the people of the developing countries rely on herbal drugs for the management of diarrhoea [6]. World Health Organization has encouraged studies for the treatment and prevention of diarrhoeal diseases depending on traditional medical practices [7, 8]

As Enterocin is widely used Ayurvedic antidiarrhoeal pediatric syrup, present study was conducted to investigate its antidiarrhoeal, antimotility and antisecretory effect as well as Phytochemical constituents.

EXPERIMENTAL SECTION

Drugs

i) Enterocin – Ayurlab Herbals (P) Ltd., ii) Castor oil (refined pure) – Paras Chemical Industries, iii) Loperamide hydrochloride – Cipla Pharmaceuticals Ltd., iv) Chlorpromazine hydrochloride – Rhone Poulene (India) Ltd., v) Activated Charcoal – E. Merck, vi) Magnesium sulphate – Merck, vii) Atropine sulphate – Sigma chemicals Ltd.

Composition of Enterocin

Each 4 ml of Enterocin contains i) Vidangphal (1250 mg), ii) Daruhaladi chaal (1000 mg), iii) Dhaiphool (500 mg), iv) Kuda chaal (500 mg), v) Shodhit geirik pashan (500 mg), vi) Mustamool (500 mg), vii) Lodhara chaal (500 mg), viii) Ativishmool (250 mg), ix) Soonthimool (250 mg), x) Saindhav (10 mg), xi) Sanchal (10 mg), xii) Syrup base (q.s.).

Animals

"Swiss albino mice" of either sex, weighing; 20 – 25 gm obtained from VIPER, Pune, were used for the experiments. They were kept in standard environmental condition, fed standard food and water ad libitum. All experiments were performed after an overnight fast. The study was approved by Institutional Animal Ethical Committee of Government College of Pharmacy, Aurangabad, Maharashtra, India (GCPA/IAEC/2011/235, 11/03/2011).

Experimental procedure for antidiarrhoeal activity

Acute toxicity

Initially the Enterocin was studied for acute oral toxicity as per revised OECD guidelines number 423. Enterocin was devoid of any toxicity up to 20 ml/kg in albino mice by oral route. Hence for further studies 2.5 to 10 ml/kg doses of these formulations were used.

Castor oil induced diarrhea

The animals were divided in to control, positive and test groups containing six in each group. Each mouse was kept for observation under a glass funnel, the floor of which was lined with blotting paper and observed for 4 h. Diarrhea was induced by administering 0.2 ml. of castor oil orally to mice [9]. The control group received only distilled water (10 ml/kg, po); the positive control group received loperamide (2 mg/kg, po); test group received Enterocin at doses of 2.5, 5, 10 ml/kg, po, body weight 30 min before the administration of castor oil. During an observation period of 4 h, the parameters observed were: onset of diarrhoea, total weight of faecal output, total weight of wet faeces, total number of faecal output, and number of wet faeces [8, 10].

Magnesium sulphate induced diarrhea

A similar protocol as for castor oil induced diarrhoea was followed [11]. Magnesium sulphate was given in the dose of 2 g/kg, po, to the animals 30 min after pre-treatment with distilled water (10 ml/kg, po,) to the control group, loperamide (2 mg/kg, po) to the positive control group, Enterocin at doses of 2.5, 5, 10 ml/kg, po, to test group.

Gastrointestinal motility by charcoal meal

The animals were divided in to control, positive and test groups of six mice each. Each animal was given orally 0.2 ml of charcoal meal (3% charcoal in 5 % gum acacia). The test groups received the Enterocin at doses of 2.5, 5, 10 ml/kg, po, body weight immediately after charcoal meal administration. The positive control group received atropine sulphate (5 mg/kg, ip), while the control group received distilled water (10 ml/kg, po). After 30 min., the animals were sacrificed and the movement of charcoal from pylorus to caecum was measured. The peristaltic index, which is the distance travelled by charcoal meal to the total length of small intestine expressed in terms of percentage [1].

Small intestinal secretions

Effect of Enterocin on intestinal secretion was indirectly studied by enteropooling assay. The mice were divided in to different groups and treated with distilled water (10 ml/kg, po, control group), Enterocin (2.5, 5, 10 ml/kg, po, test group), and chlorpromazine (30 mg/kg, ip, positive control group) before the oral administration of castor oil 0.2 ml per mouse. These mice were sacrificed 30 min later and entire small intestine from each animal was weighed and their group average was calculated. The difference in the weight of intestine in control and castor oil treated group was considered as the castor oil induced accumulation of intestinal fluid [12].

Preliminary phytochemical analysis

Chemical tests were carried out on Enterocin using standard procedures, to identify its major groups of chemical constituents [13, 14, 15, 16].

Statistics

The results of all experiments were reported as mean ± S.E.M. Statistical analysis was carried out using Student's '*t*'-test. A level of significance of $P < 0.05$ was regarded as statistically significant.

RESULTS AND DISCUSSION

Effect of Enterocin on castor oil induced diarrhoea

In the course of observation for 4 h. after castor oil administration, all the mice in control group produced copious diarrhoea. Pretreatment of mice with the different doses of Enterocin caused a significant dose dependent decrease in the frequency of purging (reduction of number of wet stools and total no of stools) and, weight of wet stools as shown in Table 1.

The ricinoleic acid, the active ingredient of castor oil is liberated from the action of lipases on castor oil. The ricinoleic acid produces irritating and inflammatory actions on the intestinal mucosa leading to the release of prostaglandins. This condition induces an increase in the permeability of the mucosal cells and changes in electrolyte transport, which results in a hypersecretory response (decreasing Na^+ and K^+ absorption), stimulating peristaltic activity and diarrhoea [17]. Thus the castor oil induced diarrhoea demonstrates secretory diarrhea, since recinolic acid induces diarrhoea by a hypersecretory response [18, 19]. Since the Enterocin successfully inhibited the castor oil induced diarrhoea, it can be assumed that the antidiarrhoeal action was exerted by antisecretory mechanism. This was also evident from the reduction of total number of wet faeces in the test groups in the experiment.

Table 1: Effect of Enterocin on castor oil induced diarrhoea in mice.

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
Enterocin	2.5 ml	81 ± 2.78	0.185 ± 0.006	0.172 ± 0.008	6.16 ± 0.40	5.16 ± 0.30	53.09
Enterocin	5 ml	111 ± 3.64	0.103 ± 0.007	0.095 ± 0.007	3.33 ± 0.33	2.83 ± 0.65	74.27
Enterocin	10 ml	163 ± 4.40	0.051 ± 0.005	0.042 ± 0.005	1.66 ± 0.211	1.33 ± 0.33	87.90
Loperamide	2 mg	223 ± 5.16	0.036 ± 0.002	0.030 ± 0.003	1.0 0± 0.25	0.83 ± 0.16	92.45

Values are mean ± standard error of mean. Each value represents average of six determinations. P < 0.05 vs. control, student's 't' test.

Effect of Enterocin on magnesium sulphate induced diarrhoea

All the mice in control group produced diarrhoea after magnesium sulphate administration during the observation period of 4 h. Pretreatment of mice with the different doses of Enterocin caused a significant dose dependent decrease in the frequency of purging (reduction of number of wet stools and total no of stools) and, weight of wet stools as shown in Table 2.

Table 2: Effect of Enterocin on magnesium sulphate induced diarrhoea in mice

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		41 ± 2.06	0.32 ± 0.01	0.291 ± 0.009	11.50 ± 0.42	8.16 ± 0.30	
Enterocin	2.5 ml	79 ± 2.45	0.143 ± 0.011	0.127 ± 0.008	4.83 ± 0.30	3.50 ± 0.36	57.1
Enterocin	5 ml	115 ± 3.63	0.085 ± 0.008	0.073 ± 0.008	2.83 ± 0.30	2.00 ± 0.36	75.49
Enterocin	10 ml	190 ± 2.89	0.039 ± 0.007	0.031 ± 0.006	1.33 ± 0.21	0.833 ± 0.30	89.79
Loperamide	2 mg	207 ± 6.58	0.030 ± 0.004	0.027 ± 0.006	0.83 ± 0.16	0.66 ± 0.21	91.11

Values are mean ± standard error of mean. Each value represents average of six determinations. P < 0.05 vs. control, student's 't' test.

Magnesium sulphate produces the diarrhoea by osmotic properties, preventing reabsorption of water ions, leading to increase in the volume of the intestinal content. It promotes the liberation of cholecytokinin from the duodenal mucosa, which increases the secretion and motility of small intestine and thereby prevents the reabsorption of sodium chloride and water [11, 20]. Enterocin found to reduce the diarrhoeic condition in this model. Enterocin 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.

Effect of Enterocin on small intestinal transit

GI motility describes the contraction of the muscles that mix and propel contents in the gastrointestinal tract [21]. Charcoal meal test in mice is a method used to study the effect of drugs on the motility of intestine [22]. In present study Enterocin was found to be the inhibitor of intestinal motility as shown in Table 3.

Table 3: Effect of Enterocin on intestinal transit in mice

Group	Dose (/kg)	Percent intestinal transit	% Inhibition
Normal		73.30 ± 1.60	
Control		81.33 ± 2.13	
Enterocin	2.5 ml	60.74 ± 2.46	17.12
Enterocin	5 ml	51.10 ± 1.76	30.28
Enterocin	10 ml	42.82 ± 1.25	41.57
Atropine sulphate	5 mg	32.29 ± 1.02	55.94

Values are mean ± standard error of mean. Each value represents average of six determinations. P < 0.05 vs. control, student's 't' test.

Effect of Enterocin on small intestinal secretion

Diarrhoea occurs when the bowels secrete more electrolytes and water than they absorb [23]. Castor oil produces permeability changes in the intestinal mucosa membranes to water and electrolytes resulting in fluid and watery luminal content that flows rapidly through small and large intestines [24, 25]. Enterocin inhibited the castor oil induced intestinal fluid accumulation as shown in Table 4.

Table 4: Effect of Enterocin on intraluminal fluid accumulation in mice

Experimental Group	Dose (/kg)	weight of small intestine mg	Castor oil induced intraluminal fluid (mg)	% Inhibition
Normal		1123 ± 25		
Control		1628 ± 23	505 ± 40	
Enterocin	2.5 ml	1386 ± 31	263 ±14	47.92
Enterocin	5 ml	1293 ± 22	170 ± 13	66.33
Enterocin	10 ml	1238 ± 36	115 ± 16	77.22
Chlorpromazine	30 mg	1176 ± 24	53 ± 8	89.50

Values are mean ± standard error of mean. Each value represents average of six determinations. P < 0.05 vs. control, student's 't' test.

Phytochemical screening of Enterocin

The phytochemical analysis of the Enterocin showed the presence of carbohydrates, steroids, triterpenoids, alkaloids, flavonoids and tannins as major constituents.

CONCLUSION

These results indicate that Enterocin possesses antidiarrhoeal effect may be due to its antimotility and antiseretory effect. Antimotility and antisecretory effect of Enterocin may be due to the presence of different phytochemicals.

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REFERENCES

[1] OO Adeyemi; AJ Akindele. *Journal of Ethanopharmacology*, **2008,** 116, 407 – 412.
[2] MJG Farthing. International Journal of Antimicrobial Agents. **2000**, 14: 65 – 69.
[3] H Jing; G Wen-Yuan; L Ning-Sheng; L Chang-Xiao. *Journal of Ethanopharmacology*, **2009,** 115, 450 – 455.
[4] MM Suleiman; CA Dzenda; CA Sani. *Journal of Ethanopharmacology*, **2008,** 116, 125 – 130.
[5] DH Tambekar; SB Dahikar. *J. Chem. Pharm. Res.*, **2010**, 2(5): 494-501.
[6] HOC Mbagwu; OO Adeyemi. *Journal of Ethanopharmacology*, **2008,** 116, 16 – 20.
[7] AG Gilman; TW Rall; AS Nies. Agents affecting gastrointestinal water flux and motility. In: Goodman and Gilman's Pharmacological basis of Therapeutics, 9th ed., **1996,** 917 – 927.
[8] OO Adeyemi; AJ Akindele; EA Ogunleye. *Journal of Ethanopharmacology*, **2009,** 123, 459 – 463.

[9] FG Shoba; M Thomas. *Journal of Ethnopharmacology*, **2001,** 76, 73 – 76.
[10] PK Mukhergi; K Scha; T Murugesan; SC Mandal; M Pal; BP Scha. *Journal of Ethanopharmacology*, **1998,** 60, 85 – 89.
[11] S Afroz; M Alamgir; MTH Khan; S Jabbar; N Nahar; MSK Choudhari. *Journal of Ethanopharmacology*, **2006,** 105, 125 – 130.
[12]AM Mujumdar; AV Misar; AS Upadhye. *Journal of Ethanopharmacology*, **2005,** 102, 213 – 216.
[13] R Senthamarai; TSV Kirubha; S. Gayathri. *J. Chem. Pharm. Res.*, **2011**, 3(6):829-838.
[14] M Ramila Devi; A. Manoharan. *J. Chem. Pharm. Res.*, **2011**, 3(6):166-172.
[15] C Das; S Dash; D C Sahoo; A K Sahu; R Hota; D Rout. *J. Chem. Pharm. Res*., **2011**, 3(5):15-19.
[16] S Suganya; V Irudayaraj; M Johnson. *J. Chem. Pharm. Res*., **2011**, 3(1):721-731
[17] ASS Rouf; MS Islam; MT Rahman. *Journal of Ethanopharmacology*, **2003,** 84, 307 – 310.
[18] SJ Uddin; JA Shilpi; SMS Alam; M Alamgir; MT Rahman, SD Sarker. *Journal of Ethanopharmacology*, **2005,** 101, 139 – 143.
[19] SJ Uddin; K Mondal; JA Shilpi; MT Rahman; SD Sarker. *Fitoterapia*, **2006,** 77, 134 – 136.
[20] B Parimala; R Boominath; SC Mandal. *Phytomedicine*, **2002**, 9, 739 – 742.
[21] NR Pillai. *International Journal of Pharmacognosy*, **1992,** 30, 161 – 168.
[22] P. Mathur; K Saroha; N Syan; S Verma; S Nanda; V Valecha. *Der Pharmacia Sinica*, **2011**, 2 (1): 161-169
[23] M Uchida; Y Kato; K Matsueda; R Shoda; A Muaoka; S Yamato. *The Japnese Journal of Pharmacology*, **2000,** 82, 168 – 170.
[24] AM Mujumdar; AS Upadhye; AV Misar. *Journal of Ethanopharmacology*, **2000,** 70, 183 – 187.
[25] V Claudia; C Fernando; E Baldomero; B Elizabeth; C Samuel. *Journal of Ethanopharmacology*, **2009**, 126, 455 – 458.