Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2012, 4(4):2162-2166

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

Significance of Black pepper in Ayurvedic antidiarrhoeal formulation

Prashant B. Shamkuwar

Government College of Pharmacy, Thiba Palace, Ratnagiri (India)

ABSTRACT

Antidiarrhoeal effect of aqueous Black pepper extract, Enterocin, an Ayurvedic formulation and Enterocin in combination with aqueous Black pepper extract were subjected to pharmacological evaluation. Antidiarrhoeal effect was evaluated in castor oil and magnesium sulphate induced diarrhoea while antimotility and antisecretory effect was evaluated in charcoal meal test and castor oil induced intestinal secretions in mice. Aqueous Black pepper extract (ABPE) produced a significant increase in the antidiarrhoeal, antimotility, and antisecretory effect of Enterocin. These results suggest that ABPE produces additive effect with Enterocin in treating diarrhoea by increasing its antimotility, and antisecretory activity indicating its importance in ayurvedic antidiarrhoeal formulation.

Key words: Enterocin, aqueous Black pepper extract, diarrhoea, additive effect.

INTRODUCTION

Diarrhoea is a condition of passage of loose, watery stools with increased frequency. It is common in all age groups but children are more vulnerable. It is more common in poorly nourished children living in poor sanitary conditions [1]. Diarrhoea is one of the major health threats to populations in tropical and subtropical poor countries, responsible for about 5 million deaths annually, of which 2.5 million are children of less than 5 years [2]. Healers and patients in many communities still rely on locally available phytomedicines [3, 4]. World Health Organization in Diarrhoeal Disease Control Programme has given a special emphasis on the use of traditional medicines in the control and management of diarrhoea [5, 6].

Black pepper, scientifically called Piper nigrum L. (family- Pipereraceae), is one of the most popular spices used to increase the flavor of foods [7]. Black pepper helps in improving digestion, simultaneously it improves the appetite, prevents bacterial growth in the intestinal tract, acts as a carminative, helpful in fighting against diarrhoea. It is rich with antioxidant and antibacterial effects, which makes it beneficial for the digestive tract [8].

Enterocin is widely used Ayurvedic antidiarrhoeal pediatric syrup. The present study was aimed at the evaluation of possible additive antidiarrhoeal effect of Black pepper with Enterocin in established animal model.

EXPERIMENTAL SECTION

Drugs

i) Enterocin Syrup - 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.).

Plant material and preparation of the extract

Fruits of Black pepper (*Piper nigrum*, L. family Piperaceae) were purchased from local market. The botanical identification of the fruits was done by Dr. Dhabe, Herbarium incharge, Department of Botany, Dr. Babasaheb Ambedkar Marathwada University, Aurangabad (M.S.), India, where a voucher specimen has been deposited. After collection, the fruits were ground to coarse powder. 200 gm of the powdered fruit was boiled with 2 lit of distilled water in a conical flask for 30 min and the liquid was decanted. The resultant filtrate was evaporated to dryness in the oven at 40 °C. The dried aqueous Black pepper extract (ABPE) was reconstituted in distilled water [9].

Animals

"Swiss albino mice" of either sex, weighing; 20 - 25 gm obtained from VIPER, Pune (India), 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 Institutional Animal Ethical Committee of Government College of Pharmacy, Aurangabad, Maharashtra, India (GCPA/IAEC/2009/435, 04/05/2009), approved the study.

Experimental procedure for antidiarrhoeal activity

Acute toxicity

Initially the ABPE and Enterocin were studied for acute oral toxicity as per revised OECD guidelines number 423. ABPE was devoid of any toxicity up to 2000 mg/kg in albino mice by oral route. Hence for further studies dose of 300 mg/kg po, of ABPE was used. Enterocin was devoid of any toxicity up to 20 ml/kg in albino mice by oral route. Hence for further studies 2.5 ml/kg dose of Enterocin was used [10].

Castor oil induced diarrhoea

Groups of six mice each were treated as outlined below:
Group 1 (Control group): Distilled water 10 ml/kg, po,
Group 2 (Standard group): Loperamide 2 mg/kg, po,
Group 3 (Test group): ABPE 300 mg/kg, po,
Group 4 (Test group): Enterocin 2.5 ml/kg, po,
Group 5 (Test group): ABPE 300 mg/kg, po given with Enterocin 2.5 ml/kg, po.

After 30 min, castor oil (0.2 ml/mouse) was administered to each mouse. The animals were then placed under separate glass funnels, with the floor lined with blotting paper, for observation for 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 [11, 12].

Magnesium sulphate induced diarrhoea

A similar protocol as for castor oil induced diarrhoea was followed [13, 14]. Magnesium sulfate was given in the dose of 2 g/kg to the animals 30 min after pre-treatment with:
Groups of six mice each were treated as outlined below:
Group 1 (Control group): Distilled water 10 ml/kg, po,
Group 2 (Standard group): Loperamide 2 mg/kg, po,
Group 3 (Test group): ABPE 300 mg/kg, po,
Group 4 (Test group): Enterocin 2.5 ml/kg, po,
Group 5 (Test group): ABPE 300 mg/kg, po given with Enterocin 2.5 ml/kg, po.

Gastrointestinal motility by charcoal meal

Six mice were allotted to different groups. Treatment was then carried out as outlined below:
Group 1 (Normal group): Distilled water 10 ml/kg, p.o.,

Group 2 (Control group): Distilled water 10 ml/kg, po,
Group 3 (Standard group): Loperamide 2 mg/kg, po,
Group 4 (Test group): ABPE 300 mg/kg, po,
Group 5 (Test group): Enterocin 2.5 ml/kg, po,
Group 6 (Test group): ABPE 300 mg/kg, po given with Enterocin 2.5 ml/kg, po.

After 30 min treatment, each animal was given castor oil (0.2 ml/mouse, p.o.) except Group 1 (Normal Group). Each animal was given orally 0.2 ml of charcoal meal (3% charcoal in 5 % gum acacia), 30 min after castor oil administration. Animals were sacrificed 30 min after administration of charcoal meal and the small intestine immediately isolated. Peristaltic index for each mouse was expressed as percentage of the distance travelled by the charcoal meal relative to the total length of the small intestine[15, 16].

Small intestinal secretions

Effect of ABPE and Enterocin on intestinal secretion was indirectly studied by enteropooling assay. Six mice were allotted to different groups. Treatment was then carried out as outlined below:
Group 1 (Normal group): Distilled water 10 ml/kg, p.o.,
Group 2 (Control group): Distilled water 10 ml/kg, po,
Group 3 (Standard group): Loperamide 2 mg/kg, po,
Group 4 (Test group): ABPE 300 mg/kg, po,
Group 5 (Test group): Enterocin 2.5 ml/kg, po,
Group 6 (Test group): ABPE 300 mg/kg, po given with Enterocin 2.5 ml/kg, po.

Castor oil (0.2 ml/mouse) was administered to each mouse except Group 1 (Normal Group) after 30 min of above treatment. The mice were sacrificed 30 min after castor oil administration and the 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 [17, 18].

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 Black pepper with Enterocin on castor oil induced diarrhoea in mice.

ABPE showed the 53.09% inhibition of diarrhoea. Enterocin (2.5 ml/kg) showed the 53.09% inhibition of diarrhoea. Enterocin (2.5 ml/kg) with ABPE (300 mg/kg) showed 83.36% inhibition of diarrhoea while loperamide at dose of 2 mg/kg showed 92.45% inhibition of diarrhoea as shown in Table 1.

Castor oil is an effective laxative. It decreases fluid absorption, increases secretion in the small intestine and colon, and affects smooth muscle contractility in the intestine [19]. Several mechanisms have been previously proposed to induce the diarrhoeal effect of castor oil. However, it is well documented that castor oil produces diarrhoea due to its most active component recinoleic acid by a hypersecretory response [20]. ABPE has enhanced the antidiarrhoeal effect of Enterocin in the castor oil induced diarrhoea.

Table 1: Effect of Enterocin in combination with ABPE 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
Control		53 ± 2.11	0.372 ± 0.011	0.35 ± 0.010	13.33 ± 0.33	11.00 ± 0.36	
ABPE	300 mg	85 ± 3.60	0.176 ± 0.007	0.152 ± 0.007	6.0 ± 0.25	5.16 ± 0.16	53.09
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 + ABPE	2.5 ml 300 mg	133 ± 5.6	0.079 ± 0.003	0.066 ± 0.006	2.5 ± 0.22	1.83 ± 0.30	83.36
Loperamide	2 mg	223±5.16	0.036 ± 0.002	0.030 ± 0.003	1.00 ± 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 Black pepper with Enterocin on magnesium sulphate induced diarrhoea in mice.
ABPE produced the 55.14% inhibition of diarrhoea. Enterocin (2.5 ml/kg) produced the 57.1% inhibition of diarrhoea. Enterocin (2.5 ml/kg) with ABPE (300 mg/kg) produced 85.78% inhibition of diarrhoea while loperamide at dose of 2 mg/kg showed 91.11 % inhibition of diarrhoea as shown in Table 2.

Magnesium sulphate induces diarrhoea by increasing the volume of intestinal content through prevention of reabsorption of water [21, 18]. 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 [22, 19]. ABPE has shown to increase the inhibitory effect of Enterocin in the magnesium sulphate induced diarrhoea.

Table 2: Effect of Enterocin in combination with ABPE 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 ± 0.010	0.291± 0.009	11.50 ± 0.42	8.16 ± 0.30	
ABPE	300 mg	81 ± 3.29	0.142 ± 0.006	0.133 ± 0.006	5.00 ± 0.44	3.66 ± 0.33	55.14
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 + ABPE	2.5 ml 300 mg	153 ± 3.76	0.053 ± 0.004	0.038 ± 0.003	1.83 ± 0.16	1.16 ± 0.16	85.78
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.

Effect of Black pepper with Enterocin on small intestinal transit in mice.
ABPE (300 mg/kg) inhibited the gastrointestinal transit of charcoal in mice by 30.35%. Enterocin (2.5 ml/kg) inhibited the gastrointestinal transit of charcoal in mice by 17.12%. Enterocin (2.5 ml/kg) with ABPE (300 mg/kg) inhibited the gastrointestinal transit of charcoal in mice by 41.49% while atropine sulphate at dose of 5 mg/kg showed 55.94 % inhibition of gastrointestinal transit as shown in Table 3.

Gastrointestinal motility describes the contraction of the muscles that mix and propel contents in the gastrointestinal tract [18, 23]. Charcoal meal test in mice is a method used to study the effect of drugs on the motility of intestine [19, 20]. ABPE has enhanced the antimotility effect of Enterocin.

Table 3: Effect of Enterocin in combination with ABPE on castor oil induced intestinal transit in mice

Group	Dose (/kg)	Percent intestinal transit	% Inhibition
Normal		73.30 ± 1.60	
Control		81.33 ± 2.13	
ABPE	300 mg	51.04 ± 1.31	30.35
Enterocin	2.5 ml	60.74 ± 2.46	17.12
Enterocin + ABPE	2.5 ml 300 mg	42.88 ± 1.04	41.49
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.

Table 4: Effect of Enterocin in combination with ABPE on castor oil induced 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	
ABPE	300 mg	1353 ± 35	230 ± 20	54.45
Enterocin	2.5 ml	1386 ± 31	263 ± 14	47.92
Enterocin + ABPE	2.5 ml 300 mg	1254 ± 18	131 ± 15	74.05
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.

Effect of Black pepper with Enterocin on small intestinal secretion in mice.
ABPE (300 mg/kg) inhibited the castor oil induced intraluminal accumulation of fluid by 54.45%. Enterocin (2.5 ml/kg) inhibited the castor oil induced intraluminal accumulation of fluid by 47.92%. Enterocin (2.5 ml/kg) with ABPE (300 mg/kg) inhibited the castor oil induced intraluminal accumulation of fluid by 74.05% while chlorpromazine hydrochloride at dose of 30 mg/kg showed 89.50 % inhibition of castor oil induced intraluminal accumulation of fluid as shown in Table 4.

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 [18, 19, 23]. Enterocin and ABPE combination has shown remarkable inhibition of the castor oil induced intestinal fluid accumulation.

CONCLUSION

The results suggest that antidiarrhoeal effect of Enterocin was remarkabely increased by Black pepper may be due to increasing antisecretory and antimotility effect. Thus Black pepper can be used as an active ingredient of Ayurvedic formulation used in diarrhoea.

Acknowledgement
The authors are grateful to the Principal, Government College of Pharmacy, Aurangabad and CEO, VIPER, (Ale) Pune for providing research facilities.

REFERENCES

[1] R Mujumdar; S Bhatachrya; A Mujumdar; AK Pattanaik; PM Tiwary; S Chaudhary. *Phytotherapy Research*, **2006**, 20, 82-84.
[2] M Heinrich; B Heneka; A Ankil; H Rimpler; O Sticher; T Kostiza. *Journal of Pharmacy and Pharmacology*, **2005**, 57, 1081-1085.
[3] OO Adeyemi; AJ Akindele. *Journal of Ethanopharmacology*, **2008,** 116, 407-412.
[4] S Afroz; M Alamgir; MTH Khan; S Jabbar; N Nahar; MSK Choudhari. *Journal of Ethanopharmacology*, **2006**, 105, 125-130.
[5] TR Chanu; V Pai; R Chakraborty; B Raju; R Lobo; M Ballal. *J. Chem. Pharm. Res.*, **2011**, 3 (6), 961-967.
[6] S Kumar; H Choudhary; C Seniya. *J. Chem. Pharm. Res.*, **2011**, 3(4), 854-860.
[7] JS Pruthi. Spices and condiments. 5th ed., National book trust, New Delhi, **1998**, 198-200.
[8] YF Bai; H Xu. *Acta Pharmacologica Sinica*, **2000**, 21, 357-359.
[9] SS Agarwal; M Paridhavi. Herbal drug technology. 1st ed.,Universities Press (India) Private Limited, Hyderabad, **2007**, 625-627.
[10] G Manvi; P Garg. *J. Chem. Pharm. Res.*, **2011**, 3(6), 99-109.
[11] PK Mukhergi; K Scha; T Murugesan; SC Mandal; M Pal; BP Scha. *Journal of Ethanopharmacology*, **1998,** 60, 85 – 89.
[12] PB Shamkuwar; SR Shahi; DP Pawar. *European Journal of Experimental Biology*, **2012**, 2 (1), 194-198
[13] R Rouf; JU Shaikh; AS Jamil; M Alamgir. *Journal of Ethanopharmacology*, **2007**, 109, 539-542.
[14] PB Shamkuwar; SR Shahi. *Der Pharmacia Lettre*, **2012**, 4 (1), 217-221
[15] OO Adeyemi; AJ Akindele. *Journal of Ethanopharmacology*, **2008**, 116, 407-412.
[16] PB Shamkuwar; SR Shahi. *Der Pharmacia Sinica* **2012**, 3 (1), 71-75.
[17] PB Shamkuwar; DP Pawar. *J. Chem. Pharm. Res.*, **2012**, 4 (3), 1489-1492.
[18] PB Shamkuwar; SR Shahi. *J. Chem. Pharm. Res.*, **2012**, 4 (1), 460-464.
[19] PB Shamkuwar; SR Shahi; ST Jadhav. *Asian Journal of Plant Science and Research*, **2012**, 2, 48-53.
[20] MA Zavala; S Perez; C Perez; R Vargas; RM Perez. *Journal of Ethanopharmacology*, **1998**, 61, 41-47.
[21] J Galvez; A Zarzuelo; ME Crespo; MD Lorente; MA Ocete; J Jimenez. *Plant Medica*, **1993**, 59, 333-336.
[22] C Fernando; A Ramon; P Halley. *Journal of Ethanopharmacology*, **2010**, 128, 49-51.
[23] TS Gaginella; SF Philips. *Digestive Diseases*, **1975**, 20, 1171 – 1177.