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Research Article

ADDITIVE EFFECT OF BLACK PEPPER WITH MEBARID, AN AYURVEDIC ANTIDIARRHOEAL FORMULATION

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ABSTRACT

Aqueous Black pepper extract, Mebarid and Mebarid in combination with aqueous Black pepper extract were tested for antidiarrhoeal, antimotility and antisecretory activity. 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 Mebarid. These results suggest that ABPE produces additive effect with Mebarid in treating diarrhoea by increasing its antimotility, and antisecretory activity.

Keywords: Additive effect, Diarrhoea, Mebarid, Aqueous Black pepper extract.

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]. Diarrhoeal disease is a leading cause of mortality and morbidity in developing countries resulting in a major health care problem[3]. 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 as a vast majority of the people of the developing countries relies on herbal drugs for the management of diarrhoea[4, 5, 6].

Black pepper (*Piper nigrum* L. family Pipereraceae) is an aromatic pungent warming herb that lowers fever and improves digestion. Either powdered or its decoction is widely used in traditional Indian medicine[7]. The ancient Aryans considered it as a powerful remedy for various disorders of the anatomical system and prescribed it as an effective cure for diarrhoea, dyspepsia, malaria, delirium, tremors and hemorrhoids[8].

Mebarid syrup is an Ayurvedic formulation widely used for infantile diarrhoea & dysentery. It stops loose motions, prevents electrolyte losses and normalizes gastro-intestinal functions. Aim of the present study was to evaluate the additive effect of Black pepper with Mebarid in treating diarrhoea by studying the combined effect of Black pepper with Mebarid in castor oil and magnesium sulphate induced diarrhoea, intestinal transit and intestinal secretion in mice.

MATERIALS AND METHODS

Drugs

i) Mebarid – SG Phyto Pharma (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 Mebarid

Each 10 ml of Mebarid contains i) Ajmoda (100 mg), ii) Bael (100 mg), iii) Lodhara (100 mg), iv) Dadim (100 mg), v) Badishep (100 mg), vi) Daruhalad (100 mg), vii) Jaiphal (50 mg), viii) Sunth (50 mg), ix) Ativish (50 mg), x) Kuda (50 mg), xi) Sugar (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 ^{0}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 Mebarid 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 p.o., of ABPE was used. Mebarid 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 Mebarid was used.

Castor oil induced diarrhoea

Groups of six mice each were treated as outlined below:

Group 1 (Control group): Distilled water 10 ml/kg, p.o.,

Group 2 (Standard group): Loperamide 2 mg/kg, p.o.,

Group 3 (Test group): ABPE 300 mg/kg, p.o.,

Group 4 (Test group): Mebarid 2.5 ml/kg, p.o.,

Group 5 (Test group): ABPE 300 mg/kg, p.o. given with Mebarid 2.5 ml/kg, p.o.

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[10, 11].

Magnesium sulphate induced diarrhoea

A similar protocol as for castor oil induced diarrhoea was followed[12,13]. Magnesium sulphate 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, p.o.,

Group 2 (Standard group): Loperamide 2 mg/kg, p.o.,

Group 3 (Test group): ABPE 300 mg/kg, p.o.,

Group 4 (Test group): Mebarid 2.5 ml/kg, p.o.,

Group 5 (Test group): ABPE 300 mg/kg, p.o. given with Mebarid 2.5 ml/kg, p.o.

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, p.o.,

Group 3 (Standard group): Loperamide 2 mg/kg, p.o.,

Group 4 (Test group): ABPE 300 mg/kg, p.o.,

Group 5 (Test group): Mebarid 2.5 ml/kg, p.o.,

Group 6 (Test group): ABPE 300 mg/kg, p.o. given with Mebarid 2.5 ml/kg, p.o.

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[14, 15].

Small intestinal secretions

Effect of ABPE and Mebarid on intestinal secretion was indirectly studied by entero-pooling 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, p.o.,

Group 3 (Standard group): Loperamide 2 mg/kg, p.o.,

Group 4 (Test group): ABPE 300 mg/kg, p.o.,

Group 5 (Test group): Mebarid 2.5 ml/kg, p.o.,

Group 6 (Test group): ABPE 300 mg/kg, p.o. given with Mebarid 2.5 ml/kg, p.o.

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[16, 17].

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

Effect of Black pepper with Mebarid on castor oil induced diarrhoea in mice

ABPE showed the 53.09% inhibition of diarrhoea. Mebarid (2.5 ml/kg) showed the 56.09% inhibition of diarrhoea. Mebarid (2.5 ml/kg) with ABPE (300 mg/kg) showed 86.36% inhibition of diarrhoea while loperamide at dose of 2 mg/kg showed 92.45% inhibition of diarrhoea as shown in Table 1.

Effect of Black pepper with Mebarid on magnesium sulphate induced diarrhoea in mice.

ABPE produced the 55.14% inhibition of diarrhoea. Mebarid (2.5 ml/kg) produced the 59.19% inhibition of diarrhoea. Mebarid (2.5 ml/kg) with ABPE (300 mg/kg) produced 89.82% inhibition of diarrhoea while loperamide at dose of 2 mg/kg showed 91.11 % inhibition of diarrhoea as shown in Table 2.

Effect of Black pepper with Mebarid on small intestinal transit in mice

ABPE (300 mg/kg) inhibited the gastrointestinal transit of charcoal in mice by 30.35%. Mebarid (2.5 ml/kg) inhibited the gastrointestinal transit of charcoal in mice by 20.48%. Mebarid (2.5 ml/kg) with ABPE (300 mg/kg) inhibited the gastrointestinal transit of charcoal in mice by 43.20% while atropine sulphate at dose of 5 mg/kg showed 55.94 % inhibition of gastrointestinal transit as shown in Table 3.

Table 1: Effect of Black pepper with Mebarid 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
Mebarid	2.5 ml	83 ± 2.09	0.177 ± 0.006	0.16 ± 0.006	6.16 ± 0.30	4.83 ± 0.30	56.09
Mebarid + ABPE	2.5 ml 300 mg	157 ± 4.61	0.059 ± 0.004	0.045 ± 0.004	1.83 ± 0.30	1.5 ± 0.22	86.36
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.

Table 2: Effect of Black pepper with Mebarid 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
Mebarid	2.5 ml	86 ± 3.31	0.135 ± 0.006	0.123 ± 0.005	4.66 ± 0.33	3.33 ± 0.49	59.19
Mebarid + ABPE	2.5 ml 300 mg	207 ± 4.76	0.039 ± 0.003	0.031 ± 0.002	1.16 ± 0.21	0.83 ± 0.16	89.82
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.

Table 3: Effect of Black pepper with Mebarid on small 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
Mebarid	2.5 ml	58.28 ± 1.73	20.48
Mebarid +	2.5 ml	41.63 ±1.27	43.20
ABPE	300 mg		
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 Black pepper with Mebarid on small intestinal secretion 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
Mebarid	2.5 ml	1372 ± 22	249 ± 17	50.69
Mebarid +	2.5 ml	1238 ± 32	115 ± 18	77.22
ABPE	300 mg			
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 Mebarid on small intestinal secretion in mice

ABPE (300 mg/kg) inhibited the castor oil induced intraluminal accumulation of fluid by 54.45%. Mebarid (2.5 ml/kg) inhibited the castor oil induced intraluminal accumulation of fluid by 50.69%. Mebarid (2.5 ml/kg) with ABPE (300 mg/kg) inhibited the castor oil induced intraluminal accumulation of fluid by 77.22% 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.

DISCUSSION

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[18]. Thus the castor oil induced diarrhoea demonstrates secretory diarrhoea, since ricinoleic acid induces diarrhoea by a hypersecretory response[19]. ABPE has produced increase in the antidiarrhoeal effect of Mebarid in the castor oil induced diarrhoea.

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[20, 21]. ABPE has shown to enhance the inhibitory effect of Mebarid in the magnesium sulphate induced diarrhoea.

Charcoal meal test in mice is a method used to study the effect of drugs on the motility of intestine[22]. In present study ABPE has increased the inhibitory effect of Mebarid on intestinal motility.

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[23, 24]. Mebarid in combination with ABPE has significantly inhibited the castor oil induced intestinal fluid accumulation.

CONCLUSION

Black pepper produces additive effect with Mebarid in treating diarrhoea by enhancing antisecretory and antimotility effect of Mebarid. Thus Black pepper can be used as an active ingredient of Ayurvedic formulation used in diarrhoea.

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