

Evaluation of antidiarrhoeal effect of Black pepper (*Piper nigrum* L.)

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ABSTRACT

Aqueous black pepper extract (75, 150, 300 mg/kg, po) was tested for its antidiarrhoeal, antimotility and antisecretory activity in mice. The methods of castor oil and magnesium sulphate induced diarrhoea were used to evaluate antidiarrhoeal activity, while charcoal meal test and castor oil induced intestinal secretions were used for testing antimotility and antisecretory activity in mice. Aqueous Black pepper extract (ABPE) produced a significant and dose dependent antidiarrhoeal, antimotility, and antisecretory effect. Preliminary phytochemical screening of ABPE showed the presence of carbohydrates, and alkaloids. It can be concluded that ABPE possesses antidiarrhoeal effect may be due to its antimotility and antisecretory effect. Antimotility and antisecretory effect of Black pepper may be due to the presence of carbohydrates and alkaloids.

Key words: Aqueous black pepper extract; diarrhoea; intestinal transit; intestinal secretion.

INTRODUCTION

Diarrheal disease is a leading cause of mortality and morbidity, especially among children in developing countries resulting in a major health care problem [1]. In view of this, the World Health Organization has initiated Diarrhoeal Disease Control Program to study traditional medical practices and other related aspects [2]. A range of medicinal plants with antidiarrhoeal property have been widely used by traditional healers [3, 4]. However, the therapeutic potentials of some of these medicines have not been scientifically evaluated [5].

Black pepper is the dried unripe fruit of perennial climbing *Piper nigrum* L. family Piperaceae. It is an aromatic pungent warming herb that lowers fever and improves digestion. Either powdered or its decoction is widely used in traditional Indian medicine [6]. It is used in ayurvedic medicine to stimulate the digestive system and used for the treatment of diarrhoea, nausea, lack of appetite, and other dyspeptic complaints [7]. The aim of the present investigation was to evaluate the antidiarrhoeal potential of black pepper in Ayurveda.

MATERIALS AND METHODS

Drugs

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

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 [8].

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/2011/235, 11/03/2011), approved the study.

Experimental procedure for antidiarrhoeal activity**Acute toxicity**

Initially the ABPE was 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 doses of 75 to 300 mg/kg po, of aqueous black pepper extracts were used [9].

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 [10]. The control group received only distilled water (10 ml/kg, po); the positive control group received loperamide (2 mg/kg, po); test group received ABPE at doses of 75, 150, 300 mg/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.

Magnesium sulphate induced diarrhea

A similar protocol as for castor oil induced diarrhoea was followed. 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, ABPE at doses of 75, 150, 300 mg/kg, po, to test group [11].

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 ABPE at doses of 75, 150, 300 mg/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 [12].

Small intestinal secretions

Effect of ABPE on intestinal secretion was indirectly studied by enteropooling assay. The mice were divided into different groups and treated with ABPE (75, 150, 300 mg/kg, po), distilled water (10 ml/kg, po) and standard chlorpromazine (30 mg/kg, ip) 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 [13].

Preliminary phytochemical screening

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

Statistics

The results of all experiments were reported as mean \pm 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 ABPE 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 ABPE 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. ABPE showed dose dependent inhibition of castor oil induced diarrhoea in albino mice. This effect was significant at 300 mg/kg in comparison to control group, however, this activity was less as compared to loperamide as shown in Table 1.

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

Group	Dose (/kg)	Onset of diarrhoea (min)	Total weight of stool (g)	Weight of wet stools (g)	Total numbers of stools	Number of wet stools	% Inhibition
Control		53 \pm 2.11	0.372 \pm 0.010	0.35 \pm 0.010	13.33 \pm 0.33	11.00 \pm 0.36	
ABPE	75 mg	67 \pm 2.04	0.321 \pm 0.009	0.293 \pm 0.009	11.66 \pm 0.49	9.33 \pm 0.55	15.18
ABPE	150 mg	73 \pm 3.26	0.270 \pm 0.007	0.250 \pm 0.008	9.66 \pm 0.66	7.83 \pm 0.47	28.81
ABPE	300 mg	85 \pm 3.60	0.176 \pm 0.007	0.152 \pm 0.007	6.00 \pm 0.25	5.16 \pm 0.16	53.09
Loperamide	2 mg	223 \pm 5.16	0.036 \pm 0.002	0.030 \pm 0.003	1.00 \pm 0.25	0.83 \pm 0.16	92.45

Values are mean \pm standard error of mean.

Each value represents average of six determinations.

P < 0.05 vs. control, student's 't' test.

Effect of ABPE 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 ABPE 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. ABPE showed dose dependent inhibition of magnesium sulphate induced diarrhoea in albino mice. This effect was significant at 300 mg/kg in comparison to control group, however, this activity was less potent as compared to loperamide (Table 2).

Table 2. Effect of 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 \pm 2.06	0.32 \pm 0.01	0.291 \pm 0.009	11.50 \pm 0.42	8.16 \pm 0.30	
ABPE	75 mg	60 \pm 2.20	0.263 \pm 0.007	0.240 \pm 0.007	9.3 \pm 0.33	6.66 \pm 0.66	18.38
ABPE	150 mg	65 \pm 3.09	0.230 \pm 0.006	0.205 \pm 0.006	8.16 \pm 0.56	5.66 \pm 0.49	30.63
ABPE	300 mg	81 \pm 3.29	0.142 \pm 0.006	0.133 \pm 0.006	5.00 \pm 0.44	3.66 \pm 0.33	55.14
Loperamide	2 mg	207 \pm 6.58	0.030 \pm 0.004	0.027 \pm 0.006	0.83 \pm 0.16	0.66 \pm 0.21	91.11

Values are mean \pm standard error of mean.

Each value represents average of six determinations.

P < 0.05 vs. control, student's 't' test.

Effect of ABPE on small intestinal transit

The results revealed that ABPE inhibited the castor oil induced gastrointestinal transit of charcoal in mice by dose dependent manner. Maximum effect was produced at 300 mg/kg in comparison to control group, however, this activity was less as compared to atropine sulphate as shown in Table 3.

Table 3. Effect of 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	75 mg	67.74 ± 2.28	7.57
ABPE	150 mg	61.45 ± 1.93	16.16
ABPE	300 mg	51.04 ± 1.31	30.35
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 ABPE on small intestinal secretion

ABPE, dose dependently reduced the castor oil induced intraluminal accumulation of fluid. Maximum effect was produced at 300 mg/kg in comparison to control group, however, this activity was less as compared to chlorpromazine as shown in Table 4.

Table 4. Effect of 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	75 mg	1464 ± 33	341 ± 23	32.47
ABPE	150 mg	1388 ± 31	265 ± 21	47.52
ABPE	300 mg	1353 ± 35	230 ± 20	54.45
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 analysis of ABPE

The phytochemical analysis of the ABPE showed the presence of carbohydrates and alkaloids.

DISCUSSION

Usually diarrhoea is considered as a consequence of altered motility and fluid accumulation in intestinal tract [17]. In the present study the ABPE exhibited significant antidiarrhoeal activity against castor oil, and magnesium sulphate induced diarrhoea. ABPE also produced antimotility and antisecretory effect.

Castor oil is an effective laxative. It decreases the fluid absorption, increases secretions in small intestine and colon, and affects smooth muscle contractility in the intestine. Castor oil produces diarrhoea due to its active component ricinoleic acid [18]. The precise mechanism of action of castor oil is through elevated prostaglandin biosynthesis. Prostaglandin contributes to the patho-physiological functions in gastrointestinal tract [19]. ABPE reduced the castor oil induced diarrhoea may be through the inhibition of prostaglandin biosynthesis.

Magnesium sulphate increases the volume of the intestinal content by preventing the reabsorption of water and sodium chloride. It also promotes the liberation of cholecystokinin from duodenal mucosa, which increases the secretion and motility of small intestine. [20]. ABPE found to reduce the diarrhoeic condition in this model. ABPE may have increased the absorption of water and electrolyte from the gastrointestinal tract.

Charcoal meal test in mice is a method used to study the effect of drugs on the motility of intestine [21, 22]. In present study ABPE was found to be the inhibitor of intestinal motility indicating its antispasmodic effect.

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]. ABPE inhibited the castor oil induced intestinal fluid accumulation. Preliminary phytochemical analysis revealed the presence of carbohydrates and alkaloids as major constituents.

CONCLUSION

These results indicate that ABPE produces antidiarrhoeal effect through its antisecretory and antimotility effect. The delay in the gastrointestinal transit prompted by the ABPE might have contributed to their antidiarrhoeal activity by allowing a greater time for absorption. Preliminary phytochemical analysis showed the presence of carbohydrates and alkaloids as major constituents which may be responsible for the antisecretory and antimotility effect of ABPE. Thus it provides a scientific basis for the use of black pepper in diarrhoea. Further studies are required to isolate the active components in the crude extract.

Acknowledgement

The authors are grateful to the Principal, Government College of Pharmacy, Aurangabad, for providing research facilities.

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