Available online at www.pelagiaresearchlibrary.com

Pelagia Research Library

Asian Journal of Plant Science and Research, 2012, 2 (3):284-289

ISSN : 2249-7412
CODEN (USA): AJPSKY

Effect of Black pepper on antidiarrhoeal activity of an Ayurvedic formulation: Kutajarishta

Prashant B. Shamkuwar*[a] and Sadhana R. Shahi[b]

[a]*Government College of Pharmacy, Thiba Palace, Ratnagiri (India)*
[b]*Government College of Pharmacy, Vedant Road, Aurangabad (India)*

ABSTRACT

Aqueous Black pepper extract, Kutajarishta and Kutajarishta along with aqueous Black pepper extract were tested for 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 increase in the antidiarrhoeal, antimotility, and antisecretory effect of Kutajarishta. It can be concluded that ABPE produces additive effect with Kutajarishta in treating diarrhoea by enhancing its antimotility, and antisecretory activity.

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

INTRODUCTION

Diarrhoea is defined as passage of loose, watery stools with increased frequency [1]. Diarrhoeal disease is a leading cause of mortality and morbidity in developing countries resulting in a major health care problem [2]. 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. In view of this, World Health Organization has initiated Diarrhoeal Disease Control Program to study traditional medical practices and other related aspects [3, 4].

Black Pepper (*Piper nigrum* L. family Pipereraceae) is a wonderful spice with a rich history and many uses in the kitchen and in the medicine. Either powdered or its decoction is widely used in traditional Indian medicine [5]. 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 [6].

Kutajarishta is self generated alcohol containing ayurvedic preparation. It is extensively being used in disorders like Atisar and Pravahika (diarrhoea and dysentery) by ayurvedic physicians [7]. Present study was conducted to investigate the additive effect of Black pepper with Kutajarishta in treating diarrhoea by evaluating combined effect of Black pepper with Kutajarishta in castor oil and magnesium sulphate induced diarrhoea, intestinal propulsive movement and intestinal fluid accumulation in mice.

MATERIALS AND METHODS

Drugs

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

Composition of Kutajarishta

Each 10 ml of Kutajarishta contains i) Kutaja (3 gm.), ii) Draksa (1.5 gm), iii) Madhuka Puspa (300 mg), iv) Gambhari (300 mg), v) Dhataki (600 mg), vi) Guda (3 gm), vii) Asav Base Q.S.

Plant material and preparation of the extract

Fruits of Black pepper (*Piper nigrum*, 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 and Kutajarishta 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. Kutajarishta 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 Kutajarishta was used [9].

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): Kutajarishta 2.5 ml/kg, po,
Group 5 (Test group): ABPE 300 mg/kg, po given with Kutajarishta 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 [10, 11].

Magnesium sulphate induced diarrhoea

A similar protocol as for castor oil induced diarrhoea was followed (Afroz et al., 2006). Magnesium sulfate was given in the dose of 2 g/kg to the animals 30 min after pre-treatment with [12, 13]:
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): Kutajarishta 2.5 ml/kg, po,
Group 5 (Test group): ABPE 300 mg/kg, po given with Kutajarishta 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): Kutajarishta 2.5 ml/kg, po,
Group 6 (Test group): ABPE 300 mg/kg, po given with Kutajarishta 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 [14, 15].

Small intestinal secretions

Effect of ABPE and Kutajarishta 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): Kutajarishta 2.5 ml/kg, po,
Group 6 (Test group): ABPE 300 mg/kg, po given with Kutajarishta 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 [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 Kutajarishta on castor oil induced diarrhoea in mice.

ABPE showed the 53.09% inhibition of diarrhoea. Kutajarishta (2.5 ml/kg) showed the 48.54% inhibition of diarrhoea. Kutajarishta (2.5 ml/kg) with ABPE (300 mg/kg) showed 78.78% inhibition of diarrhoea while loperamide at dose of 2 mg/kg showed 92.45 % inhibition of diarrhoea as shown in Table 1.

Table 1: Effect of Kutajarishta in combination with aqueous Black pepper extract 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 ± 0.01	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
Kutajarishta	2.5 ml	74 ± 2.18	0.205 ± 0.005	0.181 ± 0.007	7.00 ± 0.25	5.66 ± 0.42	48.54
Kutajarishta + ABPE	2.5 ml +300 mg	130 ± 4.69	0.087 ± 0.006	0.081 ± 0.004	2.83 ± 0.30	2.33 ± 0.21	78.78
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 Kutajarishta on magnesium sulphate induced diarrhoea in mice.

ABPE produced the 55.14% inhibition of diarrhoea. Kutajarishta (2.5 ml/kg) produced the 50.98% inhibition of diarrhoea. Kutajarishta (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.

Table 2: Effect of Kutajarishta in combination with aqueous Black pepper extract 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	
ABPE	300 mg	81 ± 3.29	0.142 ± 0.006	0.133 ± 0.006	5.00 ± 0.44	3.66 ± 0.33	55.14
Kutajarishta	2.5 ml	74 ± 3.01	0.153 ± 0.006	0.131 ± 0.005	5.33 ± 0.21	4.00 ± 0.33	50.98
Kutajarishta + ABPE	2.5 ml +300 mg	165 ± 3.44	0.052 ± 0.003	0.042 ± 0.003	1.66 ± 0.211	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 Kutajarishta on small intestinal transit in mice.

ABPE (300 mg/kg) inhibited the gastrointestinal transit of charcoal in mice by 30.35%. Kutajarishta (2.5 ml/kg) inhibited the gastrointestinal transit of charcoal in mice by 15.87%. Kutajarishta (2.5 ml/kg) with ABPE (300 mg/kg) inhibited the gastrointestinal transit of charcoal in mice by 37.49% while atropine sulphate at dose of 5 mg/kg showed 55.94 % inhibition of gastrointestinal transit as shown in Table 3.

Table 3: Effect of Kutajarishta in combination with aqueous Black pepper extract on 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
Kutajarishta	2.5 ml	61.66 ± 2.30	15.87
Kutajarishta + ABPE	2.5 ml+ 300 mg	45.81 ± 2.02	37.49
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.

Effect of Black pepper with Kutajarishta on small intestinal secretion in mice.

ABPE (300 mg/kg) inhibited the castor oil induced intraluminal accumulation of fluid by 54.45%. Kutajarishta (2.5 ml/kg) inhibited the castor oil induced intraluminal accumulation of fluid by 45.54%. Kutajarishta (2.5 ml/kg) with ABPE (300 mg/kg) inhibited the castor oil induced intraluminal accumulation of fluid by 68.91% 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.

Table 4: Effect of Kutajarishta in combination with aqueous Black pepper extract 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
Kutajarishta	2.5 ml	1398 ± 38	275 ± 22	45.54
Kutajarishta + ABPE	2.5 ml+ 300 mg	1280 ± 25	157 ± 11	68.91
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.

DISCUSSION

Castor oil induces diarrhoea by causing increased secretion of fluid and electrolytes into the lumen of the bowel by intestinal mucosa, resulting in fluid accumulation and a watery luminal content that flows rapidly through the small and large intestines [18]. This is brought about by the irritant effect of ricinoleic acid liberated by pancreatic lipases, which hydrolyse the oil derived from the seeds of *Ricinus communis* [19]. ABPE has shown to enhance the inhibitory effect of Kutajarishta 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]. Kutajarishta along with ABPE have reduced the diarrhoeic condition in this model than the Kutajarishta alone.

Gastrointestinal motility describes the contraction of the muscles that mix and propel contents in the gastrointestinal tract. 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 Kutajarishta on intestinal motility.

Diarrhoea occurs when the bowels secrete more electrolytes and water than they absorb. 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]. Kutajarishta in combination with ABPE has significantly inhibited the castor oil induced intestinal fluid accumulation.

CONCLUSION

These results indicate that ABPE produces additive effect with Kutajarishta in treating diarrhoea by enhancing antisecretory and antimotility effect of Kutajarishta. Thus it provides a scientific basis for the use of Black pepper in antidiarrhoeal Ayurvedic formulation like Kutajarishta.

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

REFERENCES

[1] M.J.G. Farthing, *International Journal of Antimicrobial Agents*, **2000**, 14: 65-69.
[2] O.O. Adeyemi, A.J. Akindele, *Journal of Ethanopharmacology*, **2008**, 116, 407-412.
[3] S. Afroz, M, Alamgir, M.T.H. Khan, S. Jabbar, N. Nahar, M.S.K. Choudhari, *Journal of Ethanopharmacology*, **2006**, 105, 125-130.
[4] A.M. Mujumdar, A.S. Upadhye, A.V. Misar, *Journal of Ethanopharmacology*, **2000**, 70, 183-187.
[5] A. Singh, A.R. Rao, *Cancer letters*, **1993**, 72, 5-9.
[6] B. Vladimir, M. Muhammad, P. Lakshmi, *J. Nutr. Biochem*, **2000**, 11, 109 – 113.
[7] S.K. Dixit, P.C. Sen, D. Joshi, *Ancient Science of Life*, **1988**, 2, 100-102
Extraction
[8] S.S. Agarwal, M. Paridhavi, Herbal drug technology, Universities Press (India) Private Limited, Hyderabad, 1st ed., **2007**, 625 – 627.
Acute
[9] P.G. Pillaia, P. Suresha, G. Mishrab, M. Annapurna, *European Journal of Experimental Biology*, **2011**, 1 (3):236-245.
[10] P.K. Mukhergi, K. Scha, T. Murugesan, S.C. Mandal, M. Pal, B.P. Scha, *Journal of Ethanopharmacology*, **1998,** 60, 85 – 89.
[11] P.B. Shamkuwar, S.R. Shahi, D.P. Pawar, *European Journal of Experimental Biology*, **2012**, 2 (1), 194-198
[12] S. Afroz, M. Alamgir, M.T.H. Khan, S. Jabbar, N. Nahar, M.S.K. Choudhari, *Journal of Ethanopharmacology*, **2006,** 105, 125 – 130.
[13] P.B. Shamkuwar, S.R. Shahi, *Der Pharmacia Lettre*, **2012**, 4 (1), 217-221
[14] O.O. Adeyemi, A.J. Akindele, *Journal of Ethanopharmacology*, **2008,** 116, 407 – 412.
[15] P.B. Shamkuwar, S.R. Shahi, *Der Pharmacia Sinica* **2012**, 3 (1), 71-75.
[16] A.M. Mujumdar, A.V. Misar, A.S. Upadhye, *Journal of Ethanopharmacology*, **2005,** 102, 213 – 216.
[17] P.B. Shamkuwar, S.R. Shahi, *Journal of Chemical and Pharmaceutical Research*, **2012,** 4 (1), 460-464.
[18] S.J. Uddin, K. Mondal, J.A. Shilpi, M.T. Rahman, S.D. Sarker, *Fitoterapia*, **2006**, 77, 134-136.
[19] B. Parimala, R. Boominath, S.C. Mandal, *Phytomedicine*, **2002**, 9, 739-742.
[20] N.R. Pillai, *International Journal of Pharmacognosy*, **1992**, 30, 161-168.
[21] A.S.S. Rouf, M.S. Islam, M.T. Rahman, *Journal of Ethanopharmacology*, **2003,** 84: 307-310.
[22] A.M. Mujumdar, A.S. Upadhye, A.V. Misar, *Journal of Ethanopharmacology*, **2000**, 70, 183-187.

[23] M. Uchida, Y. Kato, K. Matsueda, R. Shoda, A. Muaoka, S. Yamato, *The Japnese Journal of Pharmacology*, **2000**, 82, 168-170.
[24] P.B. Shamkuwar, S.R. Shahi, S.T. Jadhav, *Asian Journal of Plant Science and Research*, **2012**, 2 (1), 48-53