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Potential of *Myristica fragrans* (Myristicaceae) in Ayurvedic antidiarrhoeal formulation

Prashant B. Shamkuwar*, Deepak P. Pawar and Prashant B. Aswar.

Government College of Pharmacy, Thiba Palace, Ratnagiri 415612 (India).

ABSTRACT

In Maharashtra (India) Myristica fragrans (Myristicaceae) is used as antidiarrhoeal and antispasmodic in new born infants and children traditionally. Aqueous Myristica fragrans extract (MF) was tested for its antidiarrhoeal effect in castor oil induced diarrhoea in mice and antispasmodic effect on contractions induced by acetyl choline, nicotine and histamine in isolated guinea pig ileum. MF produced a significant and dose dependent antidiarrhoeal effect and also inhibited the acetyl choline, nicotine and histamine induced contractions of isolated guinea pig ileum. It can be concluded that MF possesses antidiarrhoeal and antispasmodic activity may be due to its anticholinergic and antihistaminic effect. Thus the study provided a scientific basis for the use of Myristica fragrans as an ingredient of traditional polyherbal formulations in the treatment of diarrhoea.

Key words: Aqueous *Myristica fragrans* extract, antidiarrhoeal, antispasmodic.

INTRODUCTION

Recurrent diarrhea is prevalent in developing countries, particularly in tropical regions. A natural based antidiarrheal home remedy can serve as an ideal health tool to limit diarrhea-related morbidity and mortality [1, 2].

Nutmeg is the dried seed kernel of *Myristica fragrans* belongs to the family Myristicaceae. It is commonly known as Jaiphal and Javitri in India [3, 4]. In the traditional Indian medical science of Ayurveda, nutmeg is one such plant said to possess antidiarrheal activity [5]. A study was therefore planned to assess the antidiarrhoeal and antispasmodic effect of Nutmeg.

MATERIALS AND METHODS

Drugs

i) Castor oil (refined pure) – Paras Chemical Industries, ii) Mebarid Syrup – SG Phyto Pharma (P) Ltd., iii) Acetylcholine - Sigma Chemicals Ltd. iv) Nicotine – Sigma Chemicals Ltd. v) Histamine - Sigma Chemicals Ltd.

Plant material and preparation of the extract

Seeds of Nutmeg (Myristica fragrans family Myristicaceae) 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 (Ref. No. Bot/4/1/2011). Grated nutmeg seed kernel (190 g) was soaked in 0.75 L of hot distilled water and left to stand for 72 h. This was thereafter filtered and the extract obtained. The extract was freeze-dried and kept frozen until used [6].

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

Aqueous *Myristica fragrans* extract (MF) was studied for acute oral toxicity as per revised OECD guidelines number 423. MF was devoid of any toxicity up to 2000 mg/kg in albino mice by oral route. Hence for further studies doses of 50 to 150 mg/kg of ASC 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): Mebarid (Ayurvedic formulation) 5 ml/kg, p.o.,
Group 3 (Test group): MF 50 mg/kg, p.o.,
Group 4 (Test group): MF 100 mg/kg, p.o.,
Group 5 (Test group): MF 150 mg/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 number of faecal output, and number of wet faeces [7].

Guinea pig ileum preparations.

Guinea pigs were killed by a cervical blow using an iron rod. The abdomen was opened and the caecum was identified. It was lifted forward and the ileum was found to be joined at its back. A piece of ileum was removed, cleaned of fat and adhering connective tissues such as mesenteries and was placed in a petri dish containing Tyrode solution. A thread was attached to the top to serve as a marker. The perfusion fluid in petri dish was aerated and debris inside the lumen was washed gently with pipette. The mesenteric membrane was trimmed for a length of ileum of approximately 2 cm. Two threads were tied to the upper and lower portion of the gut. The thread tied to the lower portion was attached to the hook of the air-delivery tube inside the bottom of the chamber, in a water jacketed organ bath containing 10 ml Tyrode solution (composition in mM: NaCl 136.89, KCl 2.68, $MgCl_2$ 1.05. $CaCl_2$ 1.36, NaH_2PO_4 0.32, $NaHCO_3$ 11.90 and glucose 5.55) and the thread tied to the upper portion of gut was attached to the force displacement transducer. Tissues were mounted under an initial load of 0.5 g and allowed to equilibrate for 30 min. before the addition of any drug. The experiments were performed at 37 °C and bubbled with a mixture of 95% oxygen and 5% carbon dioxide. Normal rhythmic motility was recorded on a student's physiograph (Bio-Device, Ambala – 134003) [8, 9]. The effect of MF (3 mg/ml) was tested on spontaneous contractions of guinea pig ileum induced by acetyl choline (1µM), nicotine (2 µg/ml) and histamine (1µg/ml). Each concentration tested was allowed a contact time of 1 min followed by washing three times. A resting period of 15 min was allowed before the next addition [10].

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 MF 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 MF caused a significant dose dependent decrease in the number of wet stools and total no of stools. MF showed dose dependent inhibition of castor oil induced diarrhoea in albino mice. This effect was significant at 150 mg/kg in comparison to control group, however, this activity was less as compared to Mebarid (Table 1).

Table 1 Effect of MF on castor oil induced diarrhoea in mice

Group	Dose (/kg)	Onset of diarrhoea (min)	Total numbers of stools	Number of wet stools	% Inhibition
Control		52±1.90	13.66±0.21	11.50±0.42	
MF	50 mg	61±3.40****	11.5±0.42****	9.50±0.62*	25.42
MF	100 mg	77±2.82****	9.5±0.56****	7.66±0.61**	31.80
MF	150 mg	90±1.96****	6.16±0.30****	5.00±0.25****	42.85
Mebarid	5 ml	173±4.33****	1.16±0.16****	1.00±0.25****	90.47

*Values are mean± S.E.M. (n=6). *p < 0.05, **p < 0.02, ***p < 0.01, ****p < 0.001 vs. control; Student's t-test.*

Effect of MF on stimulant effect of Acetyl choline, Nicotine, and Histamine in isolated guinea pig ileum.

MF inhibited spontaneous contractions of guinea pig ileum. Application of acetylcholine, nicotine and histamine to the bathing medium of the isolated guinea pig ileum increased the contractions of the tissue. MF (3 mg/ml) reduced the contractile effect of acetyl choline (1µM) and histamine (1µg/ml) on isolated guinea pig ileum. However, the contractile effect of nicotine (2 µg/ml) on the tissue was not inhibited by the MF (3 mg/ml) (Table 2).

Table 2 Effect of MF with Acetyl choline, Nicotine and Histamine on guinea pig ileum.

Group	Dose (/ml)	Effects on tissue	Contraction (gm)
Normal			1.05 ± 0.03
MF	3 mg	Relaxation	0.41 ± 0.03****
Acetyl choline	1 µM	Contraction	2.41 ± 0.04****
MF + Acetyl choline	3 mg 1 µM	Relaxation	1.08 ± 0.03****
Nicotine	2 µg	Contraction	1.76 ± 0.04**
MF + Nicotine	3 mg 2 µg	No Relaxation	1.75 ± 0.05***
Histamine	1 µg	Contraction	2.16 ± 0.06*
MF + Histamine	3 mg 1 µg	Relaxation	1.21 ± 0.02**

*Values are mean± S.E.M. (n=6). *p < 0.05, **p < 0.02, ***p < 0.01, ****p < 0.001 vs. normal; Student's t-test.*

DISCUSSION

Antidiarrhoeal effect of MF

The castor oil-induced diarrhoea demonstrates secretory diarrhoea, since recinolic acid, the active ingredient of castor oil, induces diarrhoea by a hypersecretory response [11, 12]. Since the Aqueous *Myristica fragrans* extract successfully inhibited the castor oil-induced diarrhoea, it can be assumed that the antidiarrhoeal action was exerted by antisecretory mechanism.

Antispasmodic effect of MF

The property of reducing intestinal contractions is demonstrated by most antidiarrhoeal agents, and this helps in preventing excessive loss of fluid and ingesta [13, 14]. The Aqueous *Myristica fragrans* extract inhibited the spontaneous contractions of the isolated guinea pig ileum. The contractile effects of acetylcholine and histamine on isolated guinea pig ileum were attenuated or abolished by the extract. However, the contractile effect of nicotine on isolated guinea pig ileum was not inhibited by the extract. It indicates that antispasmodic activity of Aqueous *Myristica fragrans* extract may be through muscarinic and histamine receptors but not through nicotinic receptors.

CONCLUSION

Aqueous *Myristica fragrans* extract have antidiarrhoeal and antispasmodic activity in the pharmacological models used, and thus their use in traditional medicine is justified.

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