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Preliminary pharmacological evaluation of an Ayurvedic formulation Dasamularista

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SUMMARY

In this study the Ayurvedic formulation Dasamularista was studied for its preliminary pharmacological properties using laboratory mice. Dasamularista showed a decrease in food intake and stool formation, while the water content of stool and water intake was higher and the volume of the urine was less. Dasamularista in a slight extent reduced the intestinal motility. This constipating effect was further supported by the significant anti-diarrhoeal property of the formulation in castor oil induced dairrhoea. The tested formulation markedly increased the latent period of diarrhoea and reduced the purging index value. Dasamularista did not alter the acetic acid induced abdominal writhing. Significant reduction on the onset of sleeping time and increased duration of sleep was observed in pentobarbital induced sleeping time test.

Key words: Dasamularista; Ayurvedic; Acute metabolic; Gastrointestinal motility; Antidiarrhoeal; Acetic acid writhing; Pentobarbital sleeping

INTRODUCTION

Alternative medicines play a significant role in the health care delivery system in the developing countries including Bangladesh from the ancient times. Although tremendous progress has been taken place in the field of modern medicine, but the practice and use of traditional medicine is being continued throughout the developing countries even today. Because of unique geo-graphical location and favourable climatic condition for cultivation and growth of a wide variety of flora, having rich medicinal properties, the traditional medicine is intimately related to the culture of Bangladesh.

Bangladesh, being one of the few developing countries with a very large population living in the rural areas in the midst of extreme poverty, can hardly afford the expensive diagnostic and treatment facilities of modern medicine. Ayurvedic medicine can therefore be a convenient and economic way for providing with a better health care coverage to the mass people who are receiving minimal or no health care at all. It was estimated that in Bangladesh approximately 10 million patients who were suffering from different respiratory diseases, among them 7 million people were suffering from asthma. Cough is the most important clinical symptom of respiratory diseases. Sometimes cough is the only symptoms of major underlying illness, such as bronchial asthma, chronic bronchitis etc (Amin *et al*., 1999). There were also approximately 1.1 million people who were suffering from different neurological diseases (Khan, 1994). Among them epilepsy, insanity, insomnia, headache, debility are the most common.

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A survey on out-door patient attendance carried out at Noor Majid Ayurvedic Medical College covering the time period of January 1990 to December, 1997, which revealed a large number of people having various respiratory and neurological diseases were availing Ayurvedic medical service. This large number of population suffering from respiratory and neurological diseases especially asthma and epilepsy remain untreated and a good number of patients are being treated by unqualified practitioners due to mainly poverty, illiteracy and misconception (Ahsan, 1998; Sarkar, 1998).

Even in this time of advance medical therapy simple plant based treatments do produce positive pioneering, encouraged us to look for more in the plant derived preparations like Ayurvedic medicines. In this research work an Ayurvedic liquid formulation Dasamularista, widely used for various respiratory and neurological diseases, was studied for preliminary pharmacological activities utilizing laboratory mice.

MATERIALS AND METHODS

The formulation

Dasamularista was collected from the Sree Kundeshwari Aushadalay and was prepared according to the Bangladesh National Ayurvedic Formulary (Anonymous, 1992). The formulation contains 72 individual herbal ingredients, among which we have here listed the major ten plants (Table 1). The in-process and quality control for the preparation was strictly controlled and monitored by the experienced officials of Shree Kundeshwari Aushadalay.

Animals

Non-fasted male mice (Swiss-webstar strain, 20 - 25 g body weight) bred in the animal house of the Department of Pharmacy, Jahangirnagar University, were used for the experiments. The animals were provided with standard laboratory food and tap water *ad libitum* and maintained at natural day night cycle. The animals were divided in-groups of 6, with each group balanced for body weight. Control animals were administered with normal tap water. All the experiments were carried out at a single dose level of 10 ml/kg body weight.

Acute metabolic study

The effect of the Dasamularista formulation on acute metabolism was tested by using a "Nalgene Metabolic Cage". After the administration of the test preparation the food and water intake as well as urination and defecation was measured at 0, 4, 6, 8 and 24 h (Khan and Choudhuri, 1998).

Gastrointestinal motility test with barium sulphate ($BaSO_4$) milk

This experiment was carried out by method

Table 1. List of major ten plants of Dasamularista

Plants	Family	Parts
Aegle marmelos Correa	Rutaceae	Root
Oroxylum indicum Linn.	Bignoniaceae	Root
Premna integrifolia Linn.	Verbenaceae	Root
Gmelina arborea Roxb.	Verbenaceae	Root
Stereospermum suaveolens D.C.	Bignoniaceae	Root
Solanum xanthocarpum Schrad	Solanaceae	Root
Solanum indicum Linn.	Solanaceae	Root
Uraria lagopoides DC.	Leguminosae	Root
Desmodium gangeticum DC.	Leguminosae	Root
Tribulus terrestris Linn.	Zygophyllaceae	Root

described by Chatterjee (1993). Barium sulphate milk (15% barium sulphate in 0.5% sodium carboxymethyl cellulose suspension) was given orally to the mice after 15 min of administration of the formulation or water to the groups. Each group of mice (n = 6) were sacrificed after 15 and 30 min of the administration of barium sulphate milk (10 ml/kg). The distances traversed by the barium sulphate milk were measured and expressed as a percentage of the total length of small intestine (from pylorus to the ileoceccal junction).

Castor oil induced antidiarrhoeal test

The method of Yegnanarayan *et al.* (1982) was followed. All the mice were screened initially by giving 1.0 ml of castor oil orally and only those showing diarrhoea were selected for further study. Test formulation pre-treatment was given orally 1 h before the mice were administered with the standard dose of 1.0 ml of castor oil. The animals were caged individually and examined for the presence of diarrhoea hourly for 6 h after the castor oil challenge. Diarrhoea was defined by the presence of fluidy materials in stool, which stained the absorbent paper placed beneath the cage. The number of respondents, the number of stools passed during the 6-hour period were noted for each mouse. Purging index (PI) was calculated as follows:

Purging index, PI = [% Respondents × Average number of stools] / Average latent period.

Acetic acid induced abdominal writhing assay

Muscular contraction induced by 0.6% solution of acetic acid (AA) (0.25 ml/mice). The test formulation was administered intraperitoneally to mice, 30 min before the acetic acid injection. After acetic acid administration, mice were placed in boxes. The number of muscular contraction was counted 15 min after injection and data represents the average number of the total number of writhes observed (Broadbear *et al.,* 1994). The percent protection was calculated as follows:

Percent protection = 100 - (treated mean / control mean) × 100.

Hypnotic action of pentobarbital

Pentobarbital induced sleeping time test was carried out according to the method devised (Tedeschi and Tedeschi, 1968; Williamson *et al.*, 1996). The test formulation was administered per oral 30 minutes before the administration of pentobarbital (50 mg/kg body weight, i.p.). The animals were observed for the onset and the duration of sleep, as evidenced by the observation of the loss of righting reflex.

Statistical analysis

Statistical analyses were performed by SPSS 10.0 for Windows. Independent samples *t*-test was done as the test of significance. Values were considered significantly different if $P < 0.05$. Data were expressed as Mean ± S.E.M.

RESULTS AND DISCUSSION

The Ayurvedic formulation Dasamularista consists of 72 ingredients (Anonymous, 1992) among which the major ten active ingredients are listed in Table 1. The name of the formulation *Dasamularista* also derived from this ten (*dasa*) roots (*mula*) of this composition. The present study was performed for preliminary pharmacological evaluation of this widely used formulation.

To check the possible effects of the formulation Dasamularista, on the normal metabolic processes, acute metabolic study was carried out. In this test, five parameters of normal metabolic process, i.e. food intake, defecation, water content of stool, water intake, urination were noted (Table 2). Dasamularista showed negligible decrease on food intake and stool formation all through the 24 h study. Interestingly the water content of stool was statistically ($P < 0.049$) higher than the control on 0 - 24 h reading. But this was not surprising as the water intake was always higher than the control,

Table 2. Acute metabolic effect of Dasamularista

	Group	0 - 4 h	0 - 6 h	0 - 8 h	0 - 24 h
Food intake	CON	2.46 ± 0.70	3.49 ± 0.76	5.09 ± 1.14	15.35 ± 0.43
	DSM	2.01 ± 0.51(0.634)	3.09 ±0.68(0.731)	4.16 ±0.67(0.479)	13.72 ± 1.48(0.333)
Defecation	CON	0.86 ± 0.21	1.24 ± 0.14	1.85 ± 0.20	7.64 ± 0.21
	DSM	0.74 ± 0.15(0.665)	1.03 ±0.21(0.533)	1.40 ± 0.21(0.226)	7.38 ± 0.87(0.790)
Stool water content	CON	0.48 ± 0.10	0.70 ± 0.06	1.04 ± 0.11	4.25 ± 0.05
	DSM	0.42 ± 0.09(0.711)	0.72 ± 0.20(0.949)	0.92 ± 0.22(0.743)	5.18 ± 0.36(0.049)
Water intake	CON	2.86 ± 0.65	3.87 ±0.19	4.57 ± 0.52	14.57 ± 0.49
	DSM	3.43 ± 0.49(0.519)	4.74 ± 0.82(0.341)	4.74 ± 0.81(0.898)	15.10 ± 2.50(0.843)
Urination	CON	3.59 ± 0.37	3.77 ± 0.35	4.07 ± 0.39	8.29 ± 0.88
	DSM	1.16 ± 0.26(0.001)	2.10 ± 0.32(0.016)	2.52 ± 0.28(0.016)	7.44 ± 0.97(0.600)

Values are Mean ± S.E.M. (*P* value)
CON = Control (n = 3), DSM = Dasamularista (n = 6)

though none of the results were statistically significant. The volume of the urine was observed less in the experimental period (0 - 24 h) and was significant up to 8 h study.

The gastrointestinal motility test (Table 3) was carried out to find out whether the preparation has any effect on the motility of the gastrointestinal tract. Dasamularista slightly decreased the intestinal motility but the findings were not significant on both the time period after 15 and 30 min interval. The castor oil model incorporates both secretory and motility diarrhoea (Yegnanarayan and Shrotri, 1982). The liberation of ricinoleic acid from castor oil results in irritation and inflammation of the intestinal mucosa, leading to the release of prostaglandins, which stimulates motility and secretion (Pierce *et al.*, 1971). Ricinoleic acid was also reported to reduce active Na^+ and K^+ absorption with decreased Na^+, K^+ ATPase activity in the small intestine and colon (Gaginella and Phillips, 1975). In the castor oil study (Table 4) the preparation Dasamularista showed anti-diarrhoeal property. Dasamularista increased the latent period which were statistically very highly significant ($P < 0.001$). Considering the hour-wise stool pellet count of Dasamularista there was a decrease in the first four hours and the first two hours were statistically significant. After four hours the number of stool count was higher than control. This was because the number of respondents was less than the controls. The purging index value was observed very low than the control animals because of this less number of respondents. Our findings suggest that the extract can increase the absorption of water and electrolyte from the gastrointestinal tract, since the extract delays the intestinal transit in mice as compared to the control, allowing a greater time for absorption.

Table 3. Effect of Dasamularista on $BaSO_4$ induced gastrointestinal motility

Interval	Group	Total length	$BaSO_4$ length	% Traverse
15 min	CON	49.19 ± 1.66	32.06 ± 2.38	65.36 ± 4.67
	DSM	48.98 ± 1.90 (0.934)	26.00 ± 2.88 (0.127)	53.41 ± 6.19 (0.146)
30 min	CON	47.79 ± 1.01	33.58 ± 1.27	70.29 ± 2.23
	DSM	48.19 ± 1.64 (0.838)	32.00 ± 2.41 (0.572)	67.32 ± 5.69 (0.634)

Values are Mean ± S.E.M. (*P* value) in cm and n = 8
CON = Control, DSM = Dasamularista

Table 4. Effect of Dasamularista on castor oil induced diarrhoea

Group	Hour	Mean latent period	Number of stool pellets	Purging index
CON	1	36.25 ± 4.56	4.12 ± 0.74	11.36
	2		4.13 ± 0.95	11.39
	3		1.63 ± 0.46	4.49
	4		1.00 ± 0.38	2.75
	5		0.88 ± 0.40	2.43
	6		0.75 ± 0.25	2.07
DSM	1	298.75 ± 41.52 (0.001)	0.00 ± 0.00 (0.001)[c]	-
	2		1.38 ± 0.71 (0.036)[a]	0.092
	3		0.63 ± 0.32 (0.097)	0.042
	4		0.88 ± 0.52 (0.848)	0.059
	5		2.00 ± 0.85 (0.599)	0.133
	6		2.38 ±1.19 (0.204)	0.371

[a] = P <0.05, [c] = P <0.001
Values are Mean ± S.E.M. (P value) and n = 6
CON = Control, DSM = Dasamularista

Thus the inhibitory effect of the propulsive movement of small intestine can explain the antidiarrhoeal activity in our experimental models. Among the ten main ingredients the *Aegle marmelos* (Shoba and Thomas, 2001) and *Gmelina arborea* (Abdulkarim *et al*., 2005) have showed the anti-diarrhoeal activity in castor oil induced diarrhoea, which might be responsible for the current antidiarrhoeal activity of the formulation.

Intraperitonial administration of acetic acid causes algesia by liberating noxious endogenous substances including serotonin, histamine, prostaglandin, bradykinin and substance *P* that sensitize pain nerve endings (Collier *et al*., 1968; Raj, 1996). Of the prostanoids, mainly prostacyclin (PGI_2) has been held responsible for the causation of pain following acetic acid administration (Murata *et al*., 1997). The abdominal writhes were almost unchanged in the Dasamularista group only 7% protection was observed compared to control, it did not significantly protect the animals from acetic acid induced abdominal writhings (Table 5). Although the analgesic and anti-inflammatory activity has been reported (Skachkova *et al*., 1974; Barik *et al*., 1992; Agarwal *et al*., 1994; Ali *et al*., 1998; Rahman *et al*., 2003; Rathi *et al*., 2004; Veerappan *et al*., 2005) for some of the constituents of Dasamularista, the formulation did not showed the activity against acetic acid experiment. As we know plant contains diverse of molecules and may act in a variety of way, antagonistic effect may be responsible for the absence of this activity.

The depressive effects of Dasamularista in the CNS was also evaluated by the increased pentobarbital induced sleeping time. Decrease in sleep latency and increase in sleeping time are classically related to CNS depressant drugs (Williamson *et al*., 1996). Our results showed that Dasamularista (Table 6) quickened the onset of sleeping time (P < 0.011) and increased the duration of sleeping time (P < 0.001) which suggests a depressant activity of CNS. The CNS depressant activity of the formulation may be related to the effect of *Desmodium gangeticum* and *Tribulus terrestris*, which have

Table 5. Acetic acid induced abdominal writhing test of Dasamularista

Group	Number of writhes	% Protection
CON	12.30 ± 2.35	-
DSM	11.40 ± 1.59 (0.755)	7.32

Values are Mean ± S.E.M. (P value) and n = 10
CON = Control , DSM = Dasamularista

Table 6. Effect of Dasamularista on pentobarbital induced sleeping time test

Group	Onset of sleep	Duration of sleep
CON	10.80 ± 2.10	32.40 ± 6.22
DSM	4.00 ± 0.52 (0.011)[b]	79.30 ± 6.70 (0.001)[c]

[b] = $P < 0.01$, [c] = $P < 0.001$
Values are Mean ± S.E.M. (P value) in min and n = 10
CON = Control, DSM = Dasamularista

previously showed CNS depressant activity (Bourke *et al*., 1992; Jabbar *et al*., 2001).

CONCLUSION

The results suggest Dasamularista reduced the intestinal motility in our experimental models and have antidiarrhoeal effect. Dasamularista did not alter the acetic acid induced abdominal writhing, but the significant reduction on the onset of sleeping time and increased duration of sleep indicates the formulation may have effect on the central nervous system. Further studies are suggested with individual ingredients and as a formulation.

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