



Evaluation of antidiarrhoeal potential of *Punica granatum* L. (Punicaceae) in Ayurvedic formulation

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ABSTRACT

Many Ayurvedic antidiarrhoeal formulations contain *Punica granatum* L. (Punicaceae) as one of the ingredient. Efficacy of *Punica granatum* L. in polyherbal antidiarrhoeal formulation was evaluated by comparing its antidiarrhoeal activity with Mebarid; an antidiarrhoeal Ayurvedic formulation. Antidiarrhoeal effect of an aqueous extract of rinds of fruits of *Punica granatum* (APG) and Mebarid was studied in castor oil induced diarrhoea, intestinal secretion and charcoal meal test in mice. APG significantly reduced diarrhoea and also produced antimotility and antisecretory activity in castor oil model. These results indicate that APG is an active ingredient of Mebarid in treating diarrhoea.

Key words: *Punica granatum* L., Mebarid, diarrhoea, antimotility, antisecretory.

INTRODUCTION

Punica granatum L. (Punicaceae) commonly known as Dalim (Marathi) and Anar (Hindi) is a shrub, usually with multiple stems, that commonly grows 1.8–4.6 m tall [1, 2]. The fruit is globose, 5–7.6 cm in diameter, and shiny reddish or yellowish green when mature. It is filled with crunchy seeds, each of which is encased in a juicy, somewhat acidic pulp that is itself enclosed in a membranous skin [3, 4]. Almost all parts of this plant are used in traditional medicine for the treatment of various ailments [5]. Bark and rind of the fruit are used in dysentery, diarrhea, piles, bronchitis, to reduce the risk of cardiovascular disease, and as an anthelmintic [6, 7].

Mebarid syrup is an Ayurvedic formulation used for infantile diarrhoea & dysentery. It is indicated in loose motions of specific & non-specific origin, amoebic & bacillary dysentery, gastroenteritis, entero-colitis, sprue, chronic intestinal problems. The current study was carried out to evaluate the antidiarrhoeal effect of aqueous extract of *Punica granatum* and its potential in polyherbal antidiarrhoeal formulation.

EXPERIMENTAL SECTION

Drugs

i) Mebarid – SG Phyto Pharma (P.) Ltd., ii) Castor oil (refined pure) – Paras Chemical Industries, iii) Activated Charcoal – E. Merck.

Plant material and preparation of the extract

Fruits of *Punica granatum* L. 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. The dried peels of fruits were coarsely powdered. The powdered peel (100 gm) was boiled in 2 L of distilled water for 15 min with continuous stirring. The

resultant solution was filtered through a filter paper. The filtrate was completely evaporated under reduced pressure. The various concentrations of the aqueous extract of *Punica granatum* L. (APG) were given 0.1 ml orally.

Composition of Mebarid

Each 10 ml of Mebarid contains i) Bael (100 mg), ii) Ajmoda (100 mg), iii) Lodhara (100 mg), iv) Dadim (100 mg), v) Badishep (100 mg), vi) Daruhlad (100 mg), vii) Jaiphal (50 mg), viii) Sunth (50 mg), ix) Ativish (50 mg), x) Kuda (50 mg), xi) Sugar (q.s.).

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

APG and Mebarid were studied for acute oral toxicity as per revised OECD guidelines number 423. APG was devoid of any toxicity up to 300 mg/kg in albino mice by oral route. Mebarid was devoid of any toxicity up to 20 ml/kg in albino mice by oral route. Hence for further studies doses of 25 to 100 mg/kg of APG and 10 ml/kg of Mebarid were used [8].

Castor oil induced diarrhea

The animals were divided into 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. Diarrhoea was induced by administering 0.2 ml of castor oil orally to mice. The control group received only distilled water (10 ml/kg, po); the positive control group received Mebarid (10 ml/kg, po); test group received APG at doses of 25, 50, 100 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 number of faecal output, and number of wet faeces [9, 10].

Small intestinal secretions

Effect of APG on intestinal secretion was indirectly studied by enteropooling assay. The mice were divided into different groups and treated with APG (25, 50, 100 mg/kg, po), distilled water (10 ml/kg, po) and Mebarid (10 ml/kg, po) 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 [11, 12].

Gastrointestinal motility by charcoal meal

The animals were divided into 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 APG at doses of 25, 50, 100 mg/kg, po, body weight immediately after charcoal meal administration. The positive control group received Mebarid (10 ml/kg, po), 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 [13].

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 AND DISCUSSION

Effect of APG 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 APG caused a significant dose dependent decrease in the frequency of purging (reduction of number of wet stools and total no of stools). APG showed dose dependent inhibition of castor oil induced diarrhoea in albino mice. This effect was significant at 100 mg/kg in comparison to control group, however, this activity was less as compared to Mebarid as shown in Table 1.

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 [14]. Thus the castor oil induced diarrhoea demonstrates secretory diarrhea, since ricinoleic acid induces diarrhoea by a hypersecretory response [15]. As APG has effectively inhibited the castor oil induced diarrhoea, it can be assumed that its antidiarrhoeal action was exerted by antisecretory mechanism, indicating its involvement in the antidiarrhoeal effect of Mebarid.

Table1: Effect of APG on castor oil induced diarrhoea in mice

Group	Dose (/kg)	Onset of diarrhoea (min)	Total numbers of stools	Number of wet stools	% Inhibition
Control		53±2.11	13.33±0.33	11.0±0.366	
APG	25 mg	69±3.12	9.66±0.42	7.83±0.300	28.81
APG	50 mg	75±4.17	8.50±0.42	7.00±0.366	36.36
APG	100 mg	83±4.23	6.33±0.33	5.33±0.211	51.54
Mebarid	10 ml	179±5.27	1.16±0.16	0.83±0.163	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 APG on small intestinal secretion

APG, dose dependently reduced the castor oil induced intraluminal accumulation of fluid. Maximum effect was produced at 100 mg/kg in comparison to control group, however, this activity was less as compared to Mebarid (Table 2).

Diarrhoea occurs when the bowels secrete more electrolytes and water than they absorb [16]. APG has decreased the castor oil induced intestinal secretion, proving its role in the intraluminal fluid blocking activity of Mebarid.

Table 2: Effect of APG on castor oil induced intraluminal fluid accumulation in mice

Experimental Group	Dose (mg/kg)	Weight of small intestine (mg)	Castor oil induced intraluminal fluid (mg)	% Inhibition
Control		1628±23	505±40	
APG	25 mg	1484±29	361±32	28.51
APG	50 mg	1465±27	342±25	32.27
APG	100 mg	1430±30	307±17	39.20
Mebarid	10 ml	1216±25	93±11	81.58

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 APG on small intestinal transit

The results revealed that APG inhibited the castor oil induced gastrointestinal transit of charcoal in mice by dose dependent manner. Maximum effect was produced at 100 mg/kg in comparison to control group, however, this activity was less as compared to Mebarid as shown in Table 3.

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 [17]. APG was found to be the inhibitor of intestinal motility, suggesting its contribution in the antispasmodic effect of Mebarid.

Table 3: Effect of APG on castor oil induced intestinal transit in mice

Group	Dose (/kg)	% intestinal transit	% Inhibition
Control		81.33±2.13	
APG	25 mg	63.85±2.37	12.92
APG	50 mg	59.12±2.48	19.34
APG	100 mg	54.46±2.28	25.70
Mebarid	10 ml	38.75±1.13	47.15

Values are mean ± standard error of mean. Each value represents average of six determinations. $P < 0.05$ vs. control, student's 't' test.

CONCLUSION

These results indicated that *Punica granatum* L. produced its antidiarrhoeal effect through decreasing intestinal secretions and by inhibiting the intestinal motility, suggesting its importance in antidiarrhoeal Ayurvedic formulation, Mebarid.

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