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# Efficacy of *Aegle marmelos* L. Corr. (Rutaceae) in Ayurvedic antidiarrhoeal formulation

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**ABSTRACT**

*Aegle marmelos L. Corr. (Rutaceae) is an active ingredient of most of the ayurvedic antidiarrhoeal formulations. A study was undertaken to evaluate the effectiveness of Aegle marmelos in polyherbal antidiarrhoeal formulation by comparing its antidiarrhoeal and antispasmodic effect with Mebarid; an antidiarrhoeal Ayurvedic formulation. An aqueous extract of Aegle marmelos (AAM) was tested for its antidiarrhoeal and antispasmodic activities. Antidiarrhoeal effect of AAM was evaluated in castor oil induced diarrhea and intestinal secretion in mice and antispasmodic effect was evaluated by charcoal meal test in mice at a dose of 25, 50, 100 mg/kg. Antidiarrhoeal and antispasmodic effect of Mebarid was evaluated at the dose of 10 ml/kg. The % inhibition of diarrhoea was 31.81 %, 43.93 %, 54.54 % and 92.45 % for AAM 25, 50, 100 mg/kg (po) and Mebarid 10 ml/kg dose (po) respectively. The % inhibition of intestinal secretion was 29.30 %, 34.05 %, 46.73 %% and 81.58 % for AAM 25, 50, 100 mg/kg (po) and Mebarid 10 ml/kg dose (po) respectively. AAM 25, 50, 100 mg/kg (po) and Mebarid 10 ml/kg dose (po) produced 10.95 %, 21.65 %, 29.19 % and 47.15 % inhibition of intestinal transit respectively. These results suggest that AAM is an active ingredient of Mebarid in treating diarrhea by its antisecretory and antimotility effect.*

**Key words**: *Aegle marmelos*, antidiarrhoeal, antispasmodic.

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## INTRODUCTION

In developing countries, the majority of people almost exclusively use traditional medicines in treating all sorts of diseases, including diarrhea [1]. It would be interesting to search for plants with antidiarrhoeal activity that could be used against any type of diarrhoeal disease [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, 6].

*Aegle marmelos* (L.) Correa commonly known as Bael, belonging to the family Rutaceae, is used in different system of medicine, especially Ayurveda, Unani and Homeopathy. Every part of the plant has medicinal properties. The root is an important ingredient of the '*Dasmula*' (ten roots) recipe [7]. The decoction of the root and root bark is useful in intermittent fever, hypo-chondriasis, and palpitation of the heart [8]. The leaves and bark have been used in medicated enema. The leaves are also used in diabetes mellitus. The greatest medicinal value, however, has been attributed to its fruit and the unripe fruit is said to be an excellent remedy for diarrhoea and is especially useful in chronic diarrhoea [9, 10].

Mebarid is an ayurvedic formulation indicated in amoebic and bacillary dysentery and diarrhoea. The present study was therefore planned to assess the effectiveness of Aegle marmelos as a constituent of polyherbal antidiarhoeal formulation, Mebarid.

## MATERIALS AND METHODS

### 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 *Aegle marmelos* (L.) Correa (family Rutaceae) 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 fruits were coarsely powdered. The powdered fruit (500 gm) was taken in a round bottom flask and was extracted with water for 48 hr at room temperature. After 48 hr, the solution was filtered and the filtrate was concentrated in a rotary evaporator and the last trace was removed in vacuum. The various concentrations of the aqueous extract of *Aegle marmelos* (AAM) were given 0.1 ml orally [11].

### 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) Daruhalad (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

AAM and Mebarid were studied for acute oral toxicity as per revised OECD guidelines number 423. AAM 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 AAM and 10 ml/kg of Mebarid were used [12].

### 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. 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 AAM 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 weight of faecal output, total weight of wet faeces, total number of faecal output, and number of wet faeces [13].

### Small intestinal secretions

Effect of AAM on intestinal secretion was indirectly studied by enteropooling assay. The mice were divided into different groups and treated with AAM (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 [14].

### 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 AAM 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 [15].

**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 AAM 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 AAM 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. AAM showed 31.81 %, 43.93 %, 54.54 % inhibition of diarrhoea at doses of 25 mg/kg, 50 mg/kg and 100 mg/kg while Mebarid at dose of 10 ml/kg showed 92.45 % inhibition of diarrhoea as shown in Table 1.

**Table1: Effect of AAM 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±2.11 | 0.372±0.010 | 0.35±0.010 | 13.33±0.33 | 11.00±0.36 | |
| AAM | 25 mg | 74±3.54 | 0.283±0.008 | 0.255±0.009 | 9.33±0.55 | 7.50±0.42 | 31.81 |
| AAM | 50 mg | 81±4.06 | 0.231±0.007 | 0.208±0.007 | 7.66±0.42 | 6.16±0.40 | 43.93 |
| AAM | 100 mg | 88±3.17 | 0.179±0.007 | 0.163±0.006 | 5.83±0.30 | 5.00±0.25 | 54.54 |
| Mebarid | 10 ml | 179±5.27 | 0.049±0.003 | 0.037±0.002 | 1.16±0.16 | 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 AAM on small intestinal secretion**

AAM reduced the castor oil induced intraluminal accumulation of fluid by 29.30 %, 34.05 % and 46.73 % at doses of 25 mg/kg, 50 mg/kg and 100 mg/kg respectively while Mebarid at dose of 10 ml/kg showed 81.58 % inhibition of castor oil induced intraluminal accumulation of fluid as shown in Table 2.

**Table 2: Effect of AAM 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 | |
| AAM | 25 mg | 1480±38 | 357±28 | 29.30 |
| AAM | 50 mg | 1456±17 | 333±21 | 34.05 |
| AAM | 100 mg | 1392±26 | 269±15 | 46.73 |
| 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.*

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

| Group | Dose (/kg) | Percent intestinal transit | % Inhibition |
|---|---|---|---|
| Normal | | 73.3±1.60 | |
| Control | | 81.33±2.13 | |
| AAM | 25 mg | 65.27±2.46 | 10.95 |
| AAM | 50 mg | 57.43±2.29 | 21.65 |
| AAM | 100 mg | 51.92±2.14 | 29.19 |
| 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.*

**Effect of AAM on small intestinal transit**

The results revealed that AAM inhibited the gastrointestinal transit of charcoal in mice by 10.95 %, 21.65 % and 29.19 % at doses of 25 mg/kg, 50 mg/kg and 100 mg/kg respectively while Mebarid at dose of 10 ml/kg showed 47.15 % inhibition of gastrointestinal transit as shown in Table 3.

## DISCUSSION

Castor oil is an effective laxative. It decreases fluid absorption, increases secretion in the small intestine and colon, and affects smooth muscle contractility in the intestine [16]. Several mechanisms have been previously proposed to induce the diarrhoeal effect of castor oil. However, it is well documented that castor oil produces diarrhoea due to its most active component recinoleic acid by a hypersecretory response [17]. As AAM 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.

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. Thus diarrhoea occurs when the bowels secrete more electrolytes and water than they absorb [18]. AAM has decreased the castor oil induced intestinal fluid accumulation, proving its role in the intraluminal fluid blocking activity of Mebarid.

Gastrointestinal motility describes the contraction of the muscles that mix and propel contents in the gastrointestinal tract [19]. Charcoal meal test in mice is a method used to study the effect of drugs on the motility of intestine [20]. AAM was found to be the inhibitor of intestinal motility, suggesting its contribution in the antispasmodic effect of Mebarid.

## CONCLUSION

These results revealed that *Aegle marmelos* L. Corr. is an active ingredient of Mebarid, an Ayurvedic polyherbal formulation in treating the diarrhoea. It has produced its antidiarrhoeal effect through decreasing intestinal secretions and intraluminal fluid accumulation and antispasmodic effect by inhibiting the intestinal motility.

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