

RESEARCH AND REVIEWS: JOURNAL OF PHARMACOLOGY AND TOXICOLOGICAL STUDIES

Antidiarrhoeal Effects of Ethanolic Extract of Unripe fruit of *Aegle Marmelos* (Bael) in Mice.

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Research Article

Received: 13/01/2014
Revised: 26/02/2014
Accepted: 15/03/2014

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Keywords: *Aegle marmelos*,
Castor oil, Carbachol, 5-HT,
PGE₂, Antidiarrhoeal activity

ABSTRACT

This present study was undertaken to investigate antidiarrhoeal activity of ethanolic extract of unripe fruit of *Aegle marmelos* (EEAM) in castor oil, prostaglandin E₂ (PGE₂), 5-Hydroxytryptamine (5-HT), and carbachol induced diarrhoea in mice. The higher dose of EEAM (800mg/kg) completely prevented the diarrhoea whereas the lower doses (200 and 400mg/kg) significantly increased the time of induction of diarrhea, decreased the number and weight of wet faeces in castor oil induced diarrhoea. The higher doses of EEAM (400 and 800 mg/kg) significantly increased time of induction of diarrhoea, decreased the number and weight of wet faeces in PGE₂, 5-HT and carbachol induced diarrhoea, whereas lower doses had no effect on these parameters.

INTRODUCTION

Aegle marmelos commonly known as Bael/Bilva belonging to the family *Rutaceae* has been widely used in indigenous system of Indian medicine due to various medicinal properties [1] Extensive studies have been reported on biological activities of various extracts of *Aegle marmelos* including antidiabetic[2], antiulcer[3], anticancer[4], antihyperlipidaemic[5], antispermatogenesis [6], Antidiarrhoeal activity of *Aegle marmelos* fruit has been reported in castor oil induced diarrhea [7], Secretory diarrhoea is a common form of acute diarrhoea manifested as dehydration due to excessive fluid and electrolyte loss. Various toxins induced diarrhoea is mediated through release of varieties of endogenous secretagogues including 5-HT, PGE₂ and cholinergic pathway [8].Antidiarrhoeal activity of *Aegle marmelos* against various secretagogues induced diarrhoea is poorly reported. So, the present study was designed to evaluate antidiarrhoeal activity of ethanolic extract of *Aegle marmelos* in various secretagogues induced diarrhoea in mice.

MATERIALS AND METHODS

Chemicals

Castor oil IP (Oom Laboratories, Shimoga, Karnataka), Carbachol (Merck Mumbai), Prostaglandin E₂ and 5-Hydroxytryptamine (Cayman chemical company, Michigan, USA).

Plant Material

The unripe fruits of *Aegle marmelos* were collected from local areas of Bellary district, Karnataka, during July-September. The plant material was taxonomically identified and authenticated by Dr. Govindraj, HOD, Department of Botany, Smt. A.S.M. College for Women, Bellary, Karnataka, India.

Preparation of crude extract

Freshly collected unripe fruits of *Aegle marmelos* were thoroughly washed under running water to remove adherent impurities. Fruits were chopped and the pulp along with pericarp and seeds were subjected to shade drying at room temperature and coarsely powdered (#40). The powdered drug (100g) was macerated with 16 parts of ethanol (90%) for a week and filtered. The obtained extract was concentrated in a rotary vacuum evaporator under reduced pressure to obtain a reddish brown semi-solid mass. The percentage yield of the extract was 12.64 % w/w with respect to air dried plant material. The extract was stored at low temperature (4 to 8°C) for evaluation of phytochemical, toxicological and pharmacological studies.

Phytochemical Screening

In order to determine the presence of phytoconstituents, a preliminary phytochemical study of the extract was performed using specific reagents [9].

Experimental animals

Swiss albino mice of either sex weighing 25-30 g were procured from M/S Venkateshwara enterprises, Bangalore. They were housed in polypropylene cages and maintained under standard laboratory conditions (12:12 h light and dark cycles; temperature 25±2°C and relative humidity 55±10%). Animals were fed with standard diet and water *ad libitum*. Each experimental group consisted of eight animals housed in separate cages. Before the experimental study the animals were fasted overnight with free access to water.

The study protocol was approved by Institutional Animal Ethics Committee and experiments were performed in accordance with the current guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA)[10].

Acute toxicity (LD₅₀) study

Acute toxicity study of the extract was performed in overnight fasted albino mice by following fixed dose method as per OECD guidelines No.423. Mortality & toxic symptoms in the treated animals were observed continuously for the first 3 h after dosing, periodically during the first 24 h and then daily observation for a total period of 14 days [11].

EVALUATION FOR ANTI-DIARRHOEAL ACTIVITY

Castor oil induced diarrhea

The method reported by Awouters et.al [12] with modifications has been used in the present study. Swiss albino mice of either sex (24-30 g) were screened initially by giving 0.3ml of castor oil orally and only those showing diarrhoea were selected for the final experiment. Mice were deprived of food overnight before the experiment but had free access to water. The animals were divided into control and test groups containing eight mice in each group.

Each animal was placed in an individual cage having mesh grid with blotting paper placed on the floor of the cage. Diarrhoea was induced by oral administration of castor oil (0.3ml p.o). The different groups of animals received either vehicle or ethanolic extract of *Aegle marmelos* (200, 400 and 800 mg/kg) 30 min prior to castor oil administration. During observation period of 4 hours, the onset of diarrhoea, the number and weight of wet stools excreted by the animals were recorded and used for assessing the antidiarrhoeal activity.

PGE₂, 5-HT, and carbachol induced diarrhea

Different groups of mice were administered with PGE₂ (0.2mg/kg i.p.), 5-HT (1mg/kg i.p) or carbachol (0.5 mg/kg i.p.) to induce diarrhoea [13, 14, 15]. Diarrhoea was observed at around 7, 8, or 14 minutes after dosing PGE₂, 5-HT or carbachol respectively. The EEAM (200,400,800mg/kg) or vehicle was given orally 1h before the dosing of each diarrhoeogenic agent. To assess antidiarrhoeal activity, the time of induction of diarrhea, the total number and weight of wet faeces were recorded for a period 2 h.

Statistical analysis

The results are expressed as mean ± SEM (n=8). Statistical difference between control and experimental values were analyzed by one-way analysis of variance (ANOVA), followed by Dunnett's t-test (Graph Pad software). P values < 0.05 were considered significant.

RESULTS AND DISCUSSION

Preliminary phytochemical analysis of the ethanolic extract of *Aegle marmelos* revealed the presence of tannins, steroidal glycosides, flavonoids, alkaloids, coumarins and terpenoids. Acute toxicity studies were carried out to evaluate toxicity and to determine the minimum lethal dose of the test extract using Swiss albino mice. Fixed dose method of OECD Guideline No.423 was adopted for toxicity studies. It was found that no mortality and changes in the behavior were observed up to dose 2000 mg/kg body wt. Therefore, 200, 400 and 800 mg/kg p.o extract doses were selected for screening of antidiarrhoeal activity.

The EEAM at dose 800mg/kg produced a marked antidiarrhoeal effect with no incidence of diarrhoea in any of the animals in the group (results not shown).The other two doses of extract (200mg and 400mg/kg) significantly prolonged the time of induction of diarrhoea along with reduced number and total weight of wet faeces during 4 hours of observation period (Table 1)

Table 1: Effect of ethanolic extract of *Aegle marmelos* (EEAM) on castor oil induced diarrhoea in mice

Group	Treatment	Dose mg/kg p.o.	On set of diarrhoea in minutes	Total number of wet faeces	Total weight of wet faeces (mg)
I	Control [Tween 80(2%)]	0.4 ml	94.50 ± 5.08	8.88 ± 0.479	448 ± 16.38
II	EEAM	200	145.25 ± 06.69**	6.13 ± 0.295**	302 ± 14.07*
III	EEAM	400	174.25 ± 05.14**	4.00 ± 0.189**	180 ± 7.26**
IV	EEAM	800	-	-	-

Values are expressed as mean ± SEM, (n=8); *P<0.05, **P<0.01, as compared to control and castor oil treated (Dunnett's t-test)

The higher test doses (400 and 800mg/kg) of EEAM significantly prolonged the time of induction of diarrhoea along with decreased number and total weight of faeces during the 2 h of observation period in all three secretagogues induced diarrhoea, whereas lower dose (200mg/kg) did not alter any of the parameters in all three secretagogues induced diarrhoea (Table 2). Secretory diarrhoea occurs as a result of increased intestinal secretion or decreased intestinal absorption of fluid and electrolytes or a combination of these mechanisms^[16]. It is well accepted that various enterotoxins induced diarrhoea and inflammatory diarrhoea generally believed to be mediated through altering the intestinal absorption as well as increased intestinal secretion. It is well established that castor oil releases ricinoleic acid on oral administration which induces changes in mucosal fluid and electrolyte transport resulting in hypersecretory response and diarrhea^[17, 18]. Further, ricinoleic acid induced water and electrolyte secretion in the small intestine is mediated through release of PGE₂. In the present study EEAM not only prevented castor oil induced diarrhoea, it also reduced the severity of PGE₂ induced diarrhoea. (Table 3). Therefore it can be speculated that *Aegle marmelos* may alter PGE₂ induced secretory process and thus prevent the diarrhoea.

Table 2: Effect of ethanolic extract of *Aegle marmelos* (EEAM) on 5-HT induced diarrhoea in mice

Group	Treatment	Dose mg/kg p.o.	On set of Diarrhoea in minutes	Total number of wet faeces	Total weight of wet faeces (mg)
I	Control [Tween 80(2%)]	0.4 ml	7.50± 2.11	4.62 ± 0.18	89.87± 9.62
II	EEAM	200	9.50± 0.50	3.75 ± 0.16	83.75± 10.92
III	EEAM	400	12.25 ± 1.04*	2.25 ± 0.16**	50.00± 8.68**
IV	EEAM	800	14.62±0.73**	2.12 ± 0.22**	22.37±1.61**

Values are expressed as mean ± SEM, (n=8);*P<0.05, **P<0.01, as compared to control and serotonin treated (Dunnett's t-test)

Table3: Effect of ethanolic extract of *Aegle marmelos* (EEAM) on Prostaglandin-E₂ (PGE₂) induced diarrhoea in mice.

Group	Treatment	Dose mg/kg p.o.	On set of diarrhoea in minutes	Total number of wet faeces	Total weight of wet faeces (mg)
I	Control [Tween 80(2%)]	0.4 ml	6.62 ± 0.26	5.50 ± 0.42	145.50 ± 26.65
II	EEAM	200	8.13 ± 0.55	4.87 ± 0.44	101.00 ± 18.63
III	EEAM	400	10.25 ± 0.31**	3.25 ± 0.41**	64.00 ± 24.17*
IV	EEAM	800	11.50 ± 0.57**	3.00 ± 0.32**	47.12 ± 11.68**

Values are expressed as mean ± SEM, (n=8);*P<0.05, **P<0.01, as compared to control and PGE₂ treated (Dunnett's t-test)

Table 4: Effect of ethanolic extract of *Aegle marmelos* (EEAM) on carbachol induced diarrhoea in mice

Group	Treatment	Dose mg/kg p.o.	On set of diarrhoea in minutes	Total number of wet faeces	Total weight of wet faeces (mg)
I	Control [Tween 80(2%)]	0.4 ml	14.00 ± 1.58	4.12 ± 0.39	95.50 ± 15.15
II	EEAM	200	17.75 ± 0.99	3.87 ± 0.12	70.12 ± 11.58
III	EEAM	400	22.75 ± 1.20**	3.00 ± 0.18**	41.50 ± 8.69**
IV	EEAM	800	27.00 ± 1.48**	2.50 ± 0.18**	33.00 ± 5.39**

Values are expressed as mean ± SEM, (n=8); **P<0.01, as compared to control and carbachol treated (Dunnett's t-test)

In the present study EEAM prevented severity of 5-HT induced diarrhoea by prolonging the time of induction of diarrhoea along with decreasing the number and weight of wet faeces induced by 5-HT. (Table 4). Similar effects of EEAM in the present study were also observed in carbachol induced diarrhoea. It is reported that 5-HT has acetylcholine like effects on enterocytes [19] and also stimulates white cells in lamina propria to release prostaglandins [20]. A number of intracellular mediators like cyclic AMP, cGMP, Ca²⁺ contribute in regulation of water and electrolyte movement across intestinal luminal membrane. Secretory /antiabsorptive stimuli of 5-HT,PGs and Acetylcholine in intestinal tract is mediated through increase in intracellular Ca²⁺ and activation of PKC. The results of the present study indicate that the extract may exert its effect by modulating Ca²⁺ activated secretory/antiabsorptive stimuli. However, further studies required to establish mechanism involving antidiarrhoeal effect.

The flavanoids and terpenoid derivatives are known for inhibiting release of autocoids and prostaglandins and thereby inhibit motility and secretion induced by castor oil [24]. The presence of flavonoids and terpenoids in the EEAM may contribute to antidiarrhoeal effect observed in the present study, however further studies involving isolation of active principles of EEAM and evaluation of their antidiarrhoeal activity may support these findings.

CONCLUSION

In conclusion our results demonstrate that EEAM is effective against various secretagogues induced diarrhoea and provide rationale for its traditional use as an antidiarrhoeal agent.

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