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Evaluation of antidiarrhoeal activity of extract of unripe fruit of *Aegle marmelos*

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Abstract

Aegle marmelos is a medium sized tree, widely distributed in Asia and Africa. The plant is widely used in the ayurvedic system of medicine. Traditionally the plant has been used for the treatment of various ailments such as pain, fever, inflammation, respiratory disorders, dysentery and diarrhoea. To justify its folklore, present study was undertaken to investigate the antidiarrhoeal activity of the ethenolic extract from the fruit of *Aegle marmelos*. Preliminary phytochemical screening, acute toxicity study and antidiarrhoeal activity were studied on castor oil induced diarrhoea at 400 mg and 800 mg/kg body weight. The preliminary phytochemical screening of the extract revealed presence of alkaloids, protein, tannins, sterols, phenolic compounds and saponins. Acute toxicity study revealed no visible signs of toxicity in mice even at the highest dose of 2000 mg/kg body weight. The doses of ethenolic extract of *A. marmelos* significantly decreased ($P < 0.05$) the total number of diarrhoeal faeces in mice. Percentage of inhibition of diarrhoeal faeces at 800mg/kg was comparable with standard drug Loperamide. The study concluded that *Aegle marmelos* fruit extract has the antidiarrhoeal activity in castor oil induced diarrhoea in mice.

Keywords: *Aegle marmelos*, diarrhea, loperamide, mice

Introduction

Plants and plant derived products have been utilised as a natural source of medicinal compounds since thousands of years. In last five decades, these plants have been extensively studied by advanced scientific techniques and reported for various medicinal properties. *A. marmelos* is a native plant of India belonging to Rutaceae family and commonly known as Bael in indigenous system of medicine. It is an important medicinal plant with several ethnomedicinal applications in traditional and folk medicinal systems. At present *Aegle marmelos* has become an important source of medicine for curing human and animal's diseases. All parts of *Aegle marmelos* are medicinally useful like leaves, fruits pulp, flowers, stem/ bark and roots etc. Fine powder of unripe fruits showed significant effect on intestinal parasites. It is also used as an astringent in dysentery, stomach-ache in diarrhoea, tonic, digestive, demulcent. Decoction of unripe fruits is astringent, useful in diarrhoea and chronic dysentery. Fresh half ripe fruit is a mild astringent, and has been used to cure dysentery, diarrhoea, hepatitis, tuberculosis and dyspepsia where as roots are reported to have anti-inflammatory and wound healing properties^[1,8,14]. Bael is one of the most important tree species used in various indigenous systems of medicine in India, China, Burma and Sri Lanka^[9]. The unripe fruit is said to be an excellent remedy for diarrhoea. According to Chopra *Aegle marmelos* is effective in chronic cases of diarrhoea due to presence of large quantities of mucilage, which act as a demulcent^[2].

The present study was undertaken to investigate the antidiarrheal activity of hydro-ethenolic extract from unripe fruit of *Aegle marmelos* to revalidate the antidiarrhoeal us

Material and Methods

The unripe fruits of *Aegle marmelos* were collected and identified and authenticated from expert botanist, Department of Botany, Shri Shivaji Science College, Akola (M.S). The shade dried fruits of *Aegle marmelos* were processed to get fine powder with the help of pulverizing machine. Freshly prepared powder was subjected to cold hydro-ethenolic extraction. The extract thus obtained was used for further studies.

Preparation of cold Extract and Determination of Per cent Extractability

The freshly prepared powder of fruits of *Aegle marmelos* (25 g) was immersed in hydro-ethenolic solution (40% distilled water + 60% ethanol) in a flask stoppered tightly with cotton

plug and kept on orbital shaker at room temperature for 48 hours at 150 rpm. The contents of the flask were filter through Whatman No. 1 filter paper. Final filtrate, so obtained was evaporated and per cent extractability was determined. The extract was store in airtight screw cap vials and kept in the desiccator until further use in this study.

Experimental Animals

The mice were procured from the recognized CPCSEA authorized laboratory animal house of Department of Veterinary Pharmacology and Toxicology, PGIVAS Akola and the experimental protocol was approved from (312/CPCSEA) IAEC of PGIVAS, Akola. All the animals were maintain under hygienic and standard manage mental condition in laboratory animal house of Department of Veterinary Pharmacology and Toxicology, PGIVAS Akola. All animals were given standard pelleted feed and *ad-lib* clean drinking water.

Phytochemical Analysis

Phytochemical analysis (qualitative) of fruits of *Aegle marmelos* extracts in twelve different solvent viz. acetic acid, acetone, benzene, chloroform, distilled water, ethyl acetate, ethanol, hexane, hydro-ethanol, methanol, petroleum ether, and xylene were carried out for the presence of the active phytochemical constituents as per various test such as Test for Alkaloids (Dragendroff's reagent, Mayer's reagent (Potassium mercuric iodide reagent) and Wagner's reagent), Test for reducing sugars (Fehling's solution test and Benedict's reagent), Test for glycosides (Benedict's reagent and Fehling's reagent), Test for Tannins (Ferric chloride test and Lead acetate test), Test for sterol (Salkowski's reaction), Test for Anthraquinones (Borntrager's test), Test for flavonoids, Test for proteins (Biuret test and Xanthoprotein test), Test for amino acids (Ninhydrine test), Test for Saponins (Foam test) and Test of Phenolics^[11].

Acute Toxicity Study

Acute toxicity study was performed according to the OECD-423 guide lines. Swiss albino mice (20 – 25 g) of either sex were used. The animals were administered with 500 mg, 1000 mg and 2000 mg/kg of extract of unripe fruits of *Aegle marmelos* orally (p.o). The animals were observed for 24 h, then for further 14 days for deaths and manifestation of general signs and symptoms of toxicity.

Antidiarrheal activity

Castor oil induced diarrhoea

Castor oil induced diarrhoea model was used.^[7] Twenty four albino mice (20-25 g) of either sex were divided into 4 groups of six animals each. The animals were fasted for 18 hrs before conducting the study. The first group (positive control group) received vehicle (0.2 ml normal saline) orally. The second group (standard control group) received Loperamide hydrochloride (3 mg/kg) orally as a standard drug. The third and fourth group (test groups) received extract of unripe fruits of *Aegle marmelos*, at 400 mg and 800 mg/kg body weight orally, respectively. One hour after treatment, each mice were received 1ml of castor oil orally. All the animals were observed for defecation for the next 4hr. The presences of characteristic wet and dry diarrhoeal droppings were noted down on non-wetting paper sheet of uniform weight for period of 4 hr. The per cent inhibition of defecation was calculated as a function of the castor oil control as per the following formula.^[7]

$$\% \text{ Inhibition} = (\text{control} - \text{test}) \times 100\% / \text{control}.$$

Statistical analysis

The data obtained was analysed statistically by using Web Based Agricultural Statistics Software Package (WASP), ICAR Research Complex for Goa, Ela, Old Goa, Goa. 403 402. India.

Results and Discussions

In Phytochemical (qualitative) analysis of hydroethanolic extract of unripe fruit of *Aegle marmelos* was evaluated in twelve different solvents extracts to estimate the various active phytoconstituents.^[11] The average of yield of extract was 2.1 gm from 25 gm of powder of fruits of *Aegle marmelos*. The per cent extractability of extract was 8.1%. The extract was blackish-brown in colour and sticky in consistency, bitter-salty in taste and pleasant in odour. The phytochemical analysis of hydroethanolic extract of *Aegle marmelos* revealed presence of alkaloids, protein, tannins, sterols, phenolic compounds and saponins. In related study^[4, 6, 13] phytochemical analysis of ethanolic leaf and fruit extract of *A. marmelos* reported several compounds of confirmed biological activity such as presence of tannins, flavonoids, saponins and alkaloids. The aqueous and methanolic extracts of seeds of *A. marmeloes* contain alkaloids, carbohydrates, proteins, glycosides and phenolics qualitatively.^[12] In another study^[10] anthraquinone, glycosides, catechins, fixed oils, saponins, alkaloids, flavonoids and proteins were found in aqueous extract of leaves of *Aegle marmelos*.

In acute oral toxicity studies, extracts of unripe fruit of *Aegle marmelos* was administered at 500, 1000 and 2000 mg/kg body weight to mice. Acute toxicity study revealed no visible signs of toxicity in mice within and after 24 hrs at any of the doses administered. There were no lethality or mortality observed even at the highest dose of 2000 mg/kg body weight (Table 1). The absence of toxicity symptoms suggest that hydroethanolic extracts of unripe fruits of *Aegle marmelos* was non-toxic and well tolerated at the doses employed in this study.

In present study, two doses of extracts of unripe fruits of *Aegle marmelos* viz 400 mg/Kg and 800 mg/Kg body weight orally were used. Loperamide at dose rate of 3 mg/kg was used as reference standard drug. Control group mice were treated with normal saline.

In castor oil induced diarrhoea, the mice of vehicle control group showed diarrhoea after 30 min and continued up to observation period of 4 hours with signs of watery and frequent defecation. The mice treated with polyherbal extract (400 mg/kg b.w. and 800 mg/kg b.w.) and loperamides (3mg/kg b.w.) produced significant ($p < 0.01$) reduction in the frequency of defecation in castor oil induced diarrhoea. Both the doses of extract of unripe fruits of *Aegle marmelos* significantly decreased ($p < 0.01$) the total number of wet faeces produced by administration of castor oil. The mean per cent inhibition of frequency of defecation by extract of unripe fruits of *Aegle marmelos* at 400 mg/kg and 800 mg/kg was found to be 67.44% and 70.93%, respectively. While per cent inhibition of frequency of defecation by loperamide at 3 mg/kg was 86.00% (Table 2). Thus, extract of unripe fruits of *Aegle marmelos* showed potent antidiarrhoeal activity in castor oil induced diarrhoea in mice. Both the doses of extract (400mg/kg and 800mg/kg) did not differ significantly with each other.

Castor oil induced diarrhoea due to its active ingredient ricinoleic acid, which is liberated as a result of action of

lipases on castor oil in the duodenum. The ricinoleic acid is poorly absorbed in small intestine and results in irritation and inflammation of the intestinal mucosa which leads to release of autocoids and prostaglandins which stimulates motility and alters electrolyte permeability of the intestinal mucosa leading to hypersecretions and diarrhoea.^[10]

In the present study extract of unripe fruits of *Aegle marmelos* significantly inhibited castor oil induced diarrhoea. It was evident by reduction in total number of wet faeces produced by extract of unripe fruits of *Aegle marmelos* treated animals. The antidiarrhoeal action exhibited by extract of unripe fruits of *Aegle marmelos* might be due to active phytoconstituents

such as alkaloids, flavonoids, tannins and saponins present in the extract. The observations suggest that the antidiarrhoeal effect of the extract may be due to inhibition of prostaglandin synthesis.^[3] Earlier studies showed that antidiarrhoeal and antidiarrhoeal properties of medicinal plants were due to alkaloids, flavonoids, tannins, sterol, saponins, terpenes and reducing sugars.^[5, 7]

Further studies are required to confirm the underlying mechanism of the observed activity of the plant. The study concluded that hydro-ethanolic extracts of unripe fruits of *Aegle marmelos*, possesses potent antidiarrhoeal activity and may be evaluated in clinical cases of diarrhea in livestock.

Table 1: Acute toxicity study of *Aegle marmelos* on Swiss albino mice

Sr. No.	Extract used in study	Dose I	Dose II	Dose III	Number of death after 24 hrs	Number of death after 14 days
1	<i>Aegle marmelos</i>	500 mg	1000 mg	2000mg/kg	0	0

Table 2: Effect of extract of unripe fruits of *Aegle marmelos* on Castor oil induced diarrhea in mice

Groups	Treatment	Dose	Total no of frequency in 4 hrs	% Inhibition
I	Control	N.S	8.22± 0.065**	0
II	Loperamide	3 mg/kg	1.15±0.022	86.0
III	extract of unripe fruits of <i>Aegle marmelos</i>	400 mg/kg	2.8±0.066	67.44
IV	extract of unripe fruits of <i>Aegle marmelos</i>	800 mg/kg	2.5±0.060	70.93

Values are expressed in mean ±S.E. (n=6), Student's t-test, **P<0.01, when compared to control

Conflict of Interest: All authors declare no conflict of interest.

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