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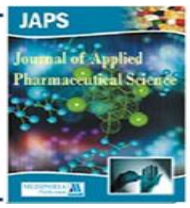

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Evaluation of Antidiarrhoeal activity of extract from leaves of *Aegle marmelos*

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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 diarrhea. To justify its folklore, present study was undertaken to investigate the antidiarrhoeal activity of the aqueous extract from the leaves of Aegle marmelos. Preliminary phytochemical screening, acute toxicity study and antidiarrhoeal activity were studied on castor induced diarrhea, Magnesium sulphate induced diarrhea, and gastric transit time at 50, 100and 200mg/kg body weight. The preliminary phytochemical screening of the extract results with the presence of anthraquinone glycosides, catechins, fixed oils and saponins etc., LD_{50} >2000mg/kg. The doses of aqueous extract of A.marmelos significantly decreased ($P< 0.05$) the total number of diarrhoeal faeces. Percentage of inhibition of diarrhoeal faeces at 200mg/kg was comparable with standard drug Loperamide. Conclusively, Aegle marmelos leaf extract has the antidiarrhoeal activity in experimental rats.

Keywords: *Aegle marmelos,* Diarrhea, Magnesium sulphate, Loperamide.

INTRODUCTION

Diarrhea presents as a disease or as symptoms of some disease conditions. It is associated with viral, bacteria, fungi infection, food poisoning and other disease conditions. Uncontrolled diarrhea is dangerous as it can lead to loss of body fluid results in electrolyte imbalance. Excessive loss of body fluid results in severe dehydration and death. In developing countries, diarrhea continues to be one of the leading causes of mortality and morbidity in children less than 3 years old. According to World Health Report, diarrhea is cause of 3.3% of all deaths. Worldwide distribution of diarrhea accounts for more than 5 – 8 million deaths each year in children. The incidence of diarrhoeal disease still remains high despite the effort by many government and international organizations to reduce it. Use of traditional medicines to combat the consequences of diarrhea has been employed by WHO in its Diarrhea Control Programme (Shariff *et al.*, 2010, Sunilson *et al.*, 2009, Chitme *et al.*, 2004, Syder *et al.*, 1982, Lutterodt *et al.*, 1982, WHO, 2004). Despite immense technological advancement in modern medicine, many people in the developing countries still rely on the healing practices and medicinal plants for their daily health care needs (Ojewole *et al.*, 2004). Therefore, the world Health Organization encouraged studies for the treatment and prevention of diarrhoeal diseases depending on traditional medical practices (Atta *et al.*, 2004).

Aegle marmelos Correa ex Roxb (Family- Rutaceae), commonly known as Bael tree, is a deciduous tree, 7-8m in height with trifoliate aromatic leaves and bisexual flowers, indigenous to India, Myanmar and Sri Lanka, often planted in the Vicinity of Shiva temple (CSIR, 1985, Chopra *et al.*, 1982). It grows wild all over the sub Himalayan forests, Central India, its west coast and in dry hilly places. Fresh half ripe fruit is a mild astringent, and has been used to cure dysentery , diarrhea , hepatitis, tuberculosis and dyspepsia where as roots are reported to have anti-inflammatory and wound healing properties (Trease *et al.*, 2003, Arul *et al.*, 2005, Kirtikar *et al.*, 1935). Bael is one of the most important tree species used in various indigenous systems of medicine in India, China, Burma and Sri Lanka (Kritikar *et al.*, 1984). Out of more than 66 ethno botanical uses of Bael, 48 are exclusively for medicinal purposes. All most all parts are used in preparing medicine. The leaves of the plant are reported to possess anti-cancer, anti hyperglycaemic, anti-inflammatory, antipyretic, and analgesic, anti diabetic, anti-spermatogenic, anti bacterial, anti diarrhoeal and chemo preventive activities (Jagetia *et al.*, 2005, Ponnachan *et al.*, 1993, Singh *et al.*, 2000, Arul *et al.*, 2005). The leaves amongst other properties indicated predominant antipyretic and antidiarrhoeal properties. Leaf: Abscess, backache, eye complaints, abdominal disorders, vomiting, cut and wounds, ulcer, dropsy, beriberi, weakness of heart, cholera, diarrhea, cardio tonic, blood sugar, injuries caused by animals, nervous disorders, hair tonic, acute bronchitis, child birth (Kritikar *et al.*, 1984, The Wealth of India, 1989, Jain *et al.*, 1991, Gaur *et al.*, 1999, Veerappan *et al.*, 2000, George *et al.*, 2003).

Chemical constituents: Various phytoconstituents have been isolated from the various parts of the Aegle marmelos, which may be categorized as (Maity *et al.*, 2009).

Table; Phytoconstituents of *Aegle marmelos.*

S. No.	Part	Phytoconstituents
1	Leaf	Skimmianine, Aegeline, Lupeol, Cineol, Citral, Citronella, Cuminaldehyde, Eugenol, Marmesinine.
2	Bark	Skimmianine, Fagarine, Marmin.
3	Fruit	Marmelosin, Luvangetin, Aurapten, Psoralen, Marmelide, Tannin.

The present study was undertaken to investigate the antidiarrhoeal of the aqueous extract from the leaves of *Aegle marmelos* to justify its folklore use in diarrhea.

MATERIAL AND METHODS

Drug and chemicals

Loperamide, Castor oil, Normal saline, Magnesium sulphate and Char coal (1%) suspension were used.

Plant material

Aegle marmelos leaf powder was supplied by Grovel Drugs and Chemicals Private Limited. (Medak District, Andhra Pradesh, India).

Preparation of Extract

About 500gms of leaf powder was dissolved in 5 liters of distilled water and boiled for 5 hrs to get 50gms of the paste. About 100mg of the paste was dissolved in 0.5 ml of water just prior to the administration. The voucher specimen has been preserved for further verification.

Preliminary phytochemical screening

The preliminary phytochemical screening of the extract was carried out in order to ascertain the presence of its constituents (Evans, 2000).

Experimental animals

Albino wistar rats of either sex weighing 150-250g were used in pharmacology and toxicology studies. The inbred animals were taken from NIN, Hyderabad. The animals were maintained in a well ventilated room with at 12:12 hr light dark cycle in propylene cages and maintained at 22±1°c with humidity at 55± 5 %. They were fed balanced rodents pellet diet from (Agro Corporation Private Limited, Bangalore, India). The experiment protocol was approved by the IAEC (Institutional Ethics Committee) of CPCSEA (Reg.no.798/03/C/CPCSEA-2003) of NRI Medical College, Chinakakani. The animal bed in the cages was renewing thrice a week to ensure hygienic condition and maximum comfort of animals.

Acute toxicity study

2000 mg/kg of aqueous extract was administered orally to three female rats. The general signs and symptoms of toxicity, intake of food and water and mortality were recorded for a period of two days and then for a period of 14 days (OECD, 2002). It was observed that the test extract was not lethal to rats even at 2000mg/kg dose.

Castor oil induced diarrhea

Castor oil induced diarrhea model was carried out using the method described by Shoba and Thomas (2001). The animals were screened initially by giving 1ml of castor oil and those showing diarrhea were selected for the final experiment .Twenty five albino rats were randomly divided in to five equal group (n=5) divided in to control group, standard group and test groups. The control group received vehicle (1ml/rat). The standard group received loperamide at the dose of 3mg/kg orally (Rao *et al.*, 2006).

The test group received aqueous extract of Aegle marmelos leaves 50, 100, 200mg/kg orally. Each animal were placed in individual cage, the floor of which was lined with bloating paper. The floor lining was changed for every hour. Diarrhea was induced by oral administration of 1.0 ml castor oil to each rat. 1hour after the above treatment during an observation period of 4hours, the total numbers of faeces excreted by the animals were recorded. A numeric score based on the stool consistency was assigned as follows: normal stool=1, semi solid stool= 2 and watery stool =3. The number of diarrhoeal faeces and percentage of Inhibition of diarrhoeal faeces were calculated (Sunil *et al.*, 2001, Teke *et al.*, 2007 and Mani *et al.*, 2011).Percentage inhibition was calculated as follows.

$$PI = \frac{\text{Mean defecation (control-treated group) x100}}{\text{Mean defecation of control group}}$$

Magnesium sulphate induced diarrhea

A similar protocol as for castor oil induced diarrhea was followed. Diarrhea was induced by oral administration of magnesium sulphate at the dose of 2gm/kg to the animals 1 hr after the vehicle (1ml/rat) to the control group, Loperamide (3mg/kg) to the standard group and to the extract treated groups. All the administrations were carried out through oral route (Doherty *et al.*, 1981).

Gastro intestinal transit time

Rats were fasted for 24hr and divided in to five groups of five rats each. Each animal was given 1ml of 1% charcoal suspension orally 60 min after an oral dose of the test drug, standard and vehicle. Group I was administered 0.5ml distilled water, and Group II received Loperamide 3mg/kg, Group III, IV and V received extract at the dose of 50mg/kg, 100mg/kg and 200mg/kg body weight respectively. The faecal bolus was expelled were collected. Each faecal bolus was pressed on a white sheet of paper examine the presence of char coal meal. The time for the appearance of the 1st faecal bolus with char coal meal was recorded.

Statistical analysis

The results were expressed as mean ± SEM and analyzed statistically to find out significance difference between control groups against each test group separately. The value of P< 0.05 was considered statistically significant.

RESULTS

Preliminary phytochemical screening

The preliminary phytochemical screening showed the presence of alkaloids, glycosides, flavonoids and proteins.

Acute toxicity study

Oral administration of AEAM produced no visible signs of toxicity in the animals at the 2000mg/kg body weight of the rats. No mortalities were recorded. In addition, no toxic symptoms were observed and neither food nor water intake was found to be reduced during the period.

Table. 1: Effect of Aqueous Extract of Leaves of *Aegle marmelos* on Castor oil (1ml) Induced diarrhea in Rats.

Groups	Treatment	No of faecal droppings in 4hours	%Inhibition of defecation
I (Control)	Castor oil (1ml p .o)+ Normal saline (1ml/p.o)	10.6 ± 0.8	0
II (Standard)	Castor oil (1ml p .o)+ Loperamide 3mg/kg	1.2± 0.33**	88.6
III	Castor oil (1ml/p.o)+ AEAML 50mg/kg	8.2 ± 0.43**	22.6
IV	Castor oil (1ml/p.o)+ AEAML 100mg/kg	5.2 ± 0.33**	50.9
V	Castor oil (1ml/p.o)+ AEAML 200mg/kg	4.8 ± 0.52**	54.7

Values are presented as Mean ± SEM, (n=5); ** p<0.05, Dun net's t-test as compared to Control. AEAM: Aqueou s extract of *Aegle marmelos* leaves.

Castor oil induced diarrhea

he doses of AEAML significantly decreased (P<0.05) the total number of diarrhoeal faeces produced by administration of castor oil (4.8 ± 0.52 at the dose of 200mg/kg) as compared to castor oil treated control group (10.6 ± 0.8) and comparable to the standard drug. The percentage of inhibition of castor oil induced diarrhea in AEAML treated rats was 54.7 % at the dose of 200mg/kg body weight of the rats and presented in table 1.

Magnesium sulphate induced diarrhea

The doses of AEAML significantly decreased (P<0.05) the total number of diarrhoeal faeces produced by administration of $Mgso_4$ (2.4±0.35 at the dose of 200mg/kg) as compared to castor oil treated control group (6.4±0.45) and comparable to the standard drug. The percentage of inhibition of castor oil induced diarrhea in AEAML treated rats was 62.5% at the dose of 200mg/kg body weight of the rats and presented in table 2.

Table. 2: Effect of Aqueous Extract of Leaves of *Aegle marmelos* leaves on Mgso4 Induced diarrhea in Rats.

Groups	Treatment	No of faecal droppings in 4hours	%Inhibition of defecation
I (Control)	Mgso4 (2g/kg p .o)+ Normal saline (1ml/p.o)	6.4±0.45	0
II (Standard)	Mgso4 (2g/kg p .o)+ Loperamide 3mg/kg	1.2±0.33**	81.2
III	Mgso4 (2g/kg p .o)+ AEAML 50mg/kg	4.2±0.43**	34.3
IV	Mgso4 (2g/kg p .o)+ AEAML 100mg/kg	3.6±0.53**	43.7
V	Mgso4 (2g/kg p .o)+ AEAML 200mg/kg	2.4±0.35**	62.5

Values are presented as Mean ± SEM, (n=5); ** p<0.05, Dun net's t-test as compared to Control. AEAML: Aqueous Extract of *Aegle marmelos* leaves.

Gastro intestinal transit time

In the gastro intestinal transit test, the extract at the doses of 50, 100 and 200 mg/kg retarded the gastro intestinal transit of char coal meal in rats where a significant (p<0.05) retardation of intestinal transit was observed at the doses of 50, 100 and 200 mg/kg dose when compared to control (Table3).

Table. 3: Effect of Aqueous extract of leaves of *Aegle marmelos* on Char coal suspension stimulated gastrointestinal transit time.

Groups	Treatment	Time(minutes) for the appearance of 1st faecal bolus with char coal suspension
I Control	Distilled water(1ml)	49 ±1.87
II Standard	Loperamide (3mg/kg)	152 ± 1.22 **
III	AEAML 50mg/kg	52 ±1.70 **
IV	AEAML 100mg/kg	65.8 ±1.42 **
V	AEAML 200mg/kg	97.8 ±3.18 **

Values are presented as Mean ± SEM, (n=5); ** p<0.05, Dunnet's t-test as compared to Control. AEAML: Aqueous extract of *Aegle marmelos* leaves.

DISCUSSION

In this study, aqueous leaf extract of *A. marmelos* exhibited significant antidiarrhoeal activity in the models tested.

Castor oil causes diarrhea due to its active metabolite, ricinolic acid, which is liberated as a result of action of lipases on castor oil. This stimulates peristaltic activity in the small intestine, leading to changes in the electrolyte permeability of the intestinal mucosa. It also stimulates the endogenous prostaglandins (Galvez *et al.*, 1993, Yoshio et al 1999). Castor oil elicits secretory and motility diarrhea (Rouf *et al*., 2003). Inhibitors of prostaglandin synthesis are known to delay diarrhea induced with castor oil (Sunil *et al.*, 2001). The observations suggest that the antidiarrhoeal effect of the extract may be due to inhibition of prostaglandin synthesis. The extract also exhibited a significant inhibition of the small intestine propulsive movement the effect was comparable to that of the standard drug Loperamide, used in the study. Anti diarrhoeal and anti dysenteric of medicinal plants were found to be due to the presence of tannins, alkaloids, saponins, flavonoids, steroids and terpenoids (Havagiray et al ., 2004). Further studies are required to confirm the underlying mechanism of the observed activity of the plant.

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