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Effects of Ethanolic Extract of Unripe fruit of *Aegle marmelos*(Bael) on Intestinal Fluid Transport and Motility in Rats.

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ABSTRACT

The present investigation was undertaken to study the effect of alcoholic extract of *Aegle marmelos* fruit (AME) on castor oil induced intraluminal fluid, electrolyte accumulation in jejunum and intestinal motility in rats. Enteropooling method was used to measure movement of fluid and electrolyte from 2ml of tyrode solution placed in jejunum (20 cm) of anesthetized rats in 30 min period. Intestinal transit of charcoal meal is used to assess intestinal motility in rats. In control rats there was net absorption of fluid and electrolyte (Na⁺,Cl⁻), whereas, fluid and electrolyte accumulation was observed in castor oil group compared to control. Pretreatment of rats with higher dose of AME (800 mg/kg,p.o.) significantly prevented castor oil induced fluid and electrolyte accumulation, whereas the lower dose (400 mg/kg) had no effect. Both doses of AME (400 and 800 mg/kg) significantly reduced the intestinal transit of charcoal meal compared to control rats. Our results provide experimental evidence and rationale for antidiarrhoeal effects of *Aegle marmelos* fruits.

INTRODUCTION

Secretory diarrhoea is common form of acute diarrhoea continues to be a major clinical problem has a major impact on morbidity and mortality worldwide. Secretory diarrhoea occurs as result of increased intestinal secretion or decreased intestinal absorption of fluid and electrolytes, but in some cases diarrhoea may result from a combination of these mechanisms [1, 2]. There has been a continuing search for drugs that might inhibit secretory process within the enterocytes. Further altered motility of gastrointestinal tract also leads to diarrhoea [2].

Aegle marmelos commonly known as Bael/Bilva belonging to the family Rutaceae has been reported to possess a number of medicinal properties used in indigenous system of Indian medicine [3]. Extensive studies have been reported on biological activities of various extracts of *Aegle marmelos* including antidiabetic [4], antiulcer [5], anticancer [6], antihyperlipidaemic [7], anti-spermatogenesis [8]. Previous report has demonstrated that fruit extract of *Aegle marmelos* is effective against castor oil induced diarrhoea in mice [9]. Further our preliminary study in laboratory has demonstrated that ethanolic extract of unripe fruit of *Aegle marmelos* (AME) is effective against various secretagogues induced diarrhoea in mice (unpublished data). To our knowledge the effects of AME on intestinal fluid transport is less reported.

Enteropooling technique that measures fluid and electrolyte movement across various segments of small intestine is widely employed to test the antidiarrhoeal effects of investigating agents on intestinal fluid transport in physiological or pathological state [10]. Castor oil stimulated intestinal secretion that results in the diarrhoea is commonly used in experimental antidiarrhoeal studies [11]. Further, the transit of charcoal meal along gastrointestinal tract after its oral administration is considered as measure of gut motility [12].

In present study we investigated the effect of AME on castor oil induced fluid and electrolytes secretion by enteropooling method in rat jejunum. We also investigated the effect of AME on intestinal motility by measuring intestinal transit of charcoal meal in rats.

MATERIALS AND METHODS

Chemicals

Castor oil IP (Oom Laboratories), Thiopental sodium (Neon Laboratories, Mumbai, India). Chemicals used for Tyrode and other solutions were of extra pure quality available from commercial sources.

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 [13]

Experimental animals

Wistar rats of either sex weighing 200-225g 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*. 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) [14].

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 [15].

EVALUATION FOR ANTI-DIARRHOEAL ACTIVITY

Study of Intra Luminal Transport of Fluid and Electrolyte

Rats were divided into four groups of six animals each. Group I received vehicle (0.4 ml -2% Tween 80 p.o) and served as control. Group II, III and IV received castor oil (2 ml p.o.) and in addition Group III and IV received AME (400 and 800 mg/kg p.o. respectively) 1h before oral administration of castor oil. All the groups were prepared for Beubler enterpooling method^[10] with modifications. Briefly; after 90 min from administration of castor oil animals were anaesthetized with Thiopental sodium (40 mg /kg i.p.) and a midline incision was made, jejunum about 5 cms distal to the flexuraduodenojejunalis and 20 cms distally was cannulated with polythene catheters (No. 8).The jejunum was rinsed with warm sterile saline solution to remove the contents followed by blowing air with syringe. The distal end of the jejunum was closed by ligation. 2ml of pre warmed (37°C) Tyrode solution (composition g/l: NaCl-8.0, KCl-0.2, CaCl₂-0.2, MgCl₂-0.1, NaHCO₃-1.0, NaH₂PO₄-0.05, D-glucose-1.0) was instilled

in jejunum and catheter was withdrawn before tying the proximal end. After 30 min the jejunum was removed and the volume of the fluid content was noted. Animals were sacrificed by an overdose of Thiopental. The fluid and electrolyte transport were measured as difference between the initial and final volume in the loop.

Intestinal Transit of Charcoal Meal

Wistar rats of either sex (200-225g) were randomly divided into four groups of six rats each. Group I received vehicle (0.4 ml -2%Tween 80 p.o.), Group II and III were received orally 400 and 800 mg/kg body weight of AME respectively. Group IV received standard drug atropine (1mg/kg i.p.). After 1 h each animal was administered orally with 1 ml of charcoal meal (10% charcoal suspension in 5 % gum acacia). Thirty minutes later the rats were sacrificed and the distance travelled by charcoal from pylorus was measured and expressed as a percentage of total length of the intestine from the pylorus to caecum [12].

Statistical Analysis

Result are expressed as mean \pm SEM (n=6). Statistical difference between control and experimental values were analyzed by one-way analysis of variance (ANOVA), followed by Dunnet's t-test (Graph Pad software). P<0.05 were considered statistically significant.

RESULTS

Phytoconstituents

Preliminary phytochemical analysis of the ethanolic extract of *Aegle marmelos* revealed the presence of tannins, steroidal glycosides, flavonoids, alkaloids, coumarins and terpenoids.

Acute Toxicity Study

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, 400 and 800 mg/kg p.o extract doses were selected for screening of anti-diarrhoeal activity.

Study of Intra Luminal Transport of Fluid and Electrolyte

In control rats, there was net absorption of fluid (1.16 ± 0.092 ml). Sodium and chloride movement paralleled that of fluid (114 ± 3.48 mEq /L, Na⁺; 117.3 ± 3.07 mEq/L Cl⁻). Castor oil treatment led to fluid accumulation as indicated by significant increase in jejunal fluid volume (1.553 ± 0.055 ml) as compared to control. In these rats sodium and chloride levels were also significantly higher when compared to control (141.2 ± 5.3 mEq/L, Na⁺; 141.5 ± 2.4 mEq/L, Cl⁻). AME at higher dose, (800 mg/kg) reversed the castor oil induced fluid accumulation to absorption as indicated by significant decrease in jejunal fluid volume (1.225 ± 0.052 ml) compared to castor oil group. Sodium and chloride levels in Jejunal fluid were also significantly reduced compared to castor oil received group (117.33 ± 3.2 mEq/L, Na⁺; 126.6 ± 4.79 mEq / L Cl⁻). Lower dose of AME (400 mg/kg) had no effect on castor oil induced fluid accumulation and Cl⁻ secretion but significantly reduced the Na⁺ level (1.36 ± 0.047 ml; 128.66 ± 1.52 mEq/L, Na⁺; 140.33 ± 4.29 mEq/L, Cl⁻). Fluid and electrolytes accumulation in the Jejunum as shown in Figure-1, 2 and 3.

Intestinal Transit of Charcoal Meal

Pretreatment of rats with AME (400 and 800 mg/kg) significantly reduced the intestinal transit of charcoal meal (64.83 ± 3.96 and 54.4 ± 1.25 respectively) as compared to control (88.09 ± 3.36). Similarly, atropine treatment also significantly reduced intestinal transit of charcoal meal (32.03 ± 1.25) compared to control as shown in Figure-4.

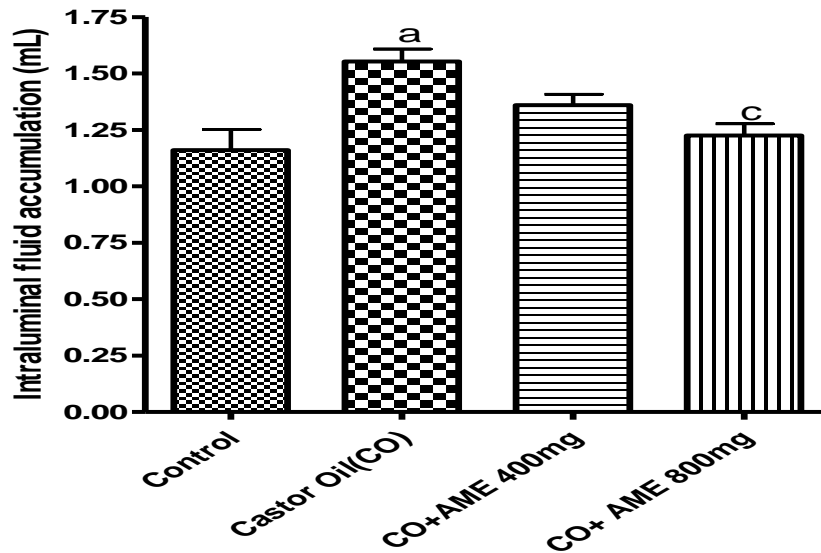


Figure 1: Effect of *Aegle Marmelos* fruit extract (AME) on castor oil (2ml p.o) induced elevated intraluminal fluid accumulation. Data are expressed as Mean \pm SEM for six experiments. ^ap < 0.01 when compared to control, ^cp < 0.01 when compared to castor oil.

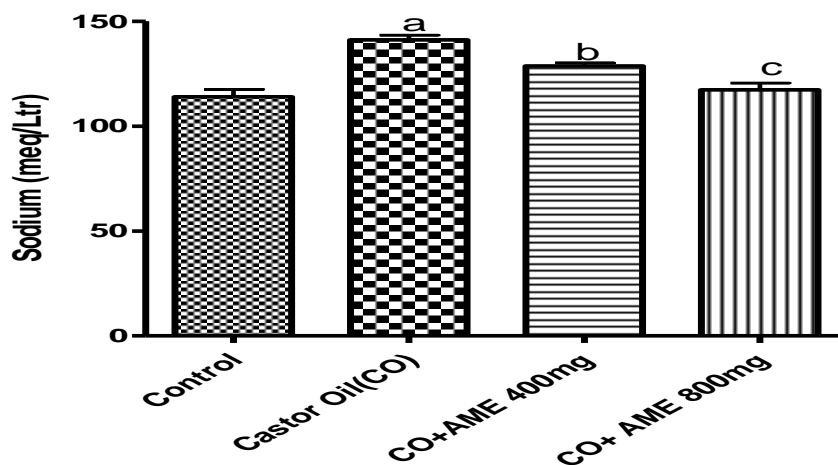


Figure 2: Effects of *Aegle Marmelos* fruit extract (AME) on castor oil (2ml p.o) induced elevated sodium level. Data are expressed as Mean \pm SEM for six experiments. ^ap < 0.01 when compared to control, ^bp < 0.05 & ^cp < 0.01 when compared to castor oil.

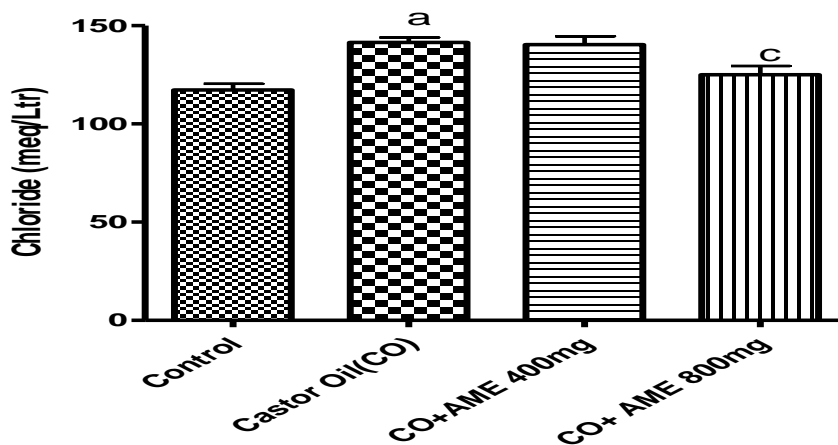


Figure 3: Effects of *Aegle Marmelos* fruit extract (AME) on castor oil (2ml p.o) induced elevated chloride level. Data are expressed as Mean \pm SEM for six experiments. ^ap < 0.01 when compared to control, ^cp < 0.05 when compared to castor oil

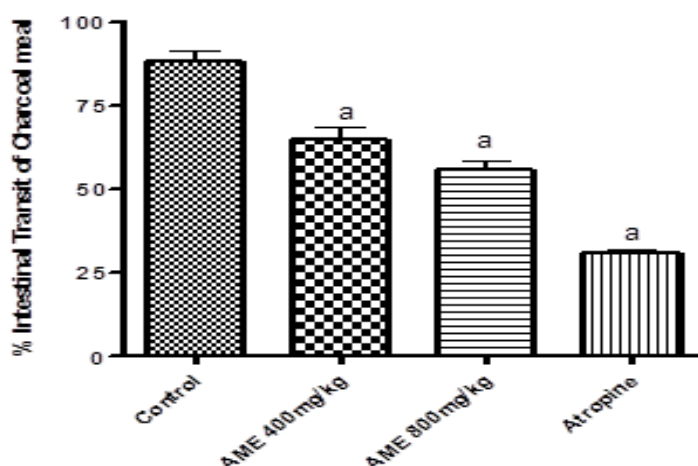


Figure 4: Effect of *Aegle marmelos* fruit extract on intestinal transit of charcoal meal. Data are expressed as Mean \pm SEM for six experiments. ^ap < 0.01 when compared to control.

DISCUSSION

In the present study, we have shown that AME prevents castor oil induced fluid accumulation in rat Jejunum. Further, the plant extract also inhibits charcoal meal transit in rats. Castor oil and its active ingredient ricinoleic acid change the transport of water and electrolytes to net hyper secretory response that results into diarrhea [16, 17]. Consistent with these reports, in the present study we observed that castor oil induced the fluid and electrolytes (Na⁺, Cl⁻) accumulation in Jejunum in 30 min period. In human intestine chloride serves as primary ion driving secretion of water into lumen and sodium serves key for regulating water absorption into lumen [18]. Hence, we used this 30 min period to assess effect of AME on castor oil induced fluid and electrolyte accumulation.

Pretreatment of rats with AME prevented the castor oil induced fluid accumulation along with decreased levels of Na⁺ and Cl⁻ in intraluminal fluid. These observations indicate that AME has modulatory effect on castor oil induced changes in intestinal lumen that affects fluid and electrolyte transport.

Diarrhoea is also caused by changes in gastrointestinal motility that results into enhanced movement of intestinal contents. Drugs like atropine and loperamide are known to reduce the intestinal motility and are clinically used in treatment of diarrhoea [19]. We observed that AME treatment in normal rats reduced the intestinal transit (54.4%) which was little lower than that of atropine (32%). These observations suggest that AME has inhibitory effect on intestinal motility. Based on our observations it appears that anti-diarrhoeal effect of AME observed in the previous study could be due to modulatory effect on intestinal transport of fluid and electrolytes and also intestinal motility.

CONCLUSION

Our results demonstrate that AME prevents castor oil induced intestinal accumulation of fluid and electrolytes. Further, it also reduces intestinal transit in normal rats. These observations provide experimental evidence that support anti-diarrhoeal effect of *Aegle marmelos*.

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