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DIARRHEA PREVENTIVE POTENCY IN METHANOLIC LEAVES EXTRACT OF *ALBIZIA PROCERA* (ROXB.) BENTH. IN MICE

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Abstract

This project aimed to investigate the potency to reduce fecal intensity of methanol extract of *Albizia procera* (MEAP) in experimental diarrheal animal model. The methanol extract of *A. procera* (100 and 200 mg/kg dose) was administered orally in mice of different groups (five animals per group) in order to evaluate the activity of the extract against castor oil and $MgSO_4$ induced diarrhea model, Loperamide (5 mg/kg) was used as positive control or standard. The effect of the extract on $MgSO_4$ and castor oil-induced intestinal fluid accumulation (enteropooling) was assessed. Both the doses (100 and 200 mg/kg) of extracts showed substantial ($p < 0.01$) antidiarrheal activity in both diarrheal model. The most promising role was shown at 200 mg/kg dose and was comparable to that of the reference drug, loperamide (5 mg/kg). *Conclusion:* On the basis of the results showed by the extract of *A. procera*, this plant possesses significant antidiarrheal potentiality.

Keywords: *castor oil, defecation, diarrhea, intestinal movement*

Introduction

Natural phytochemicals have been reported to possess a wide range of biological activities including antidiarrheal, antioxidant, antimicrobial and anti-inflamatory etc. Properties [1]. Diarrhea is defined as an altered movement of bowel, which is characterized by a situation where an adult daily stools exceeds 300 g with 60 – 95 % of water [2]. Diarrhea can cause severe dehydration that can lead to death. In fact, it is considered as the second most common cause of infant mortality after pneumonia in developing countries, and it also a reason for the death of about 2 - 4 million children every year, especially in African countries [3-6].

Albizia procera (*A. procera*) (Family: Fabaceae) is a tree widely distributed from India and Myanmar through Southeast Asia to Papua New Guinea and Northern Australia. Locally leaves are said to be insecticidal [7], while barks are used for fish poison. The bark of this plant is also considered useful in pregnancy and stomachache and is given with salt as a medicine for water buffalo symptom [8]. The ethanolic extracts of bark showed significant anti-HIV-1 integrase activity [9]. The bark and leaf extracts of *A. procera* showed potent DPPH scavenging, analgesic, antibacterial, and antidepressant activity [10-12]. Considering the biomedical importance of methanolic extract of *A. procera*, the present study was carried out to provide the scientific value as well as investigate pharmacological potentiality against diarrheal animal model.

Materials and methods

Collection and extraction of plant materials

The plant's leaves were collected from Sitakunda area of Chittagong in between March to April in 2012 and were identified by a Botanist of University of Chittagong, Bangladesh. A voucher specimen with accession No. 3778 has also been deposited in Bangladesh National Herbarium, Chittagong, Bangladesh.

The leaves were kept in open to dry under shade, grinded to coarse powder, and extracted by dissolving in methanol at room temperature with occasional shaking. The extract was then filtered off through a cotton plug and finally through filter paper (Whatman filter). The filtrate was concentrated using vacuum rotary evaporator at 50°C. The calculated yields of the extract were 20 g. The extract was stored in a refrigerator for further use.

Animal

Swiss Albino mice (weighing 20 - 30 g) of both sex, were housed in standard metal cages. They were provided with food and water *ad libitum*, and were allowed a one-week acclimatization period prior to the study. The equipment, handling and sacrificing of the animals were done in accordance with the Institutional Animal Use and Care Committee (IAUCC) and European Council legislation 87/609/EEC for the protection of experimental animals [13].

Acute oral toxicity tests

Fifteen swiss albino mice (20 - 22 g) were randomly divided into five groups of three animals each. The mice were fed on mice pellets and water *ad libitum*. The animals were starved for 12 h prior to testing. Five doses of the extract (200 – 1000 mg/kg body weights) were administered by oral intubation to the animals. All animals were observed for 24 h and general symptoms of toxicity and mortality were recorded [14-15].

Castor oil-induced diarrhea

Castor oil induced diarrhea model was carried out using the method described by Shoba and Thomas (2001) [16]. The animals were screened initially by giving 1 ml 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 (1 ml/rat). The standard group received loperamide at the dose of 5 mg/kg orally [17]. The test group received aqueous extract of *A. procera* leaves 100 and 200 mg/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. 1 hour after the above treatment during an observation period of 4 hours, 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 [18-20]. Percentage inhibition was calculated as follows:

$$PI = \frac{Mean\ defecation\ (control - treated\ group)}{Mean\ defecation\ control\ group}\ X\ 100$$

$MgSO_4$ induced diarrhea

A similar protocol as for castor oil induced diarrhea was also followed for this model as well. Diarrhea was induced by oral administration of magnesium sulphate ($MgSO_4$) at the dose of 2 gm/kg to animals. 1 hr following diarrhea induction each group received their treatment, vehicle (1 ml/mice) to the control group, Loperamide (5 mg/kg) to the standard group, and to the extract treated groups. All the administrations were carried out through oral route [21].

Statistics

The results were expressed as mean ± SEM and analyzed statistically to find out significance difference between control groups against each test group separately. Graph-Pad Prism 6 was used in this regard to complete the data analysis. We did One-way ANOVA along with Dunnet test comparison. The value of *P*<0.05 was considered statistically significant.

Results

Acute toxicity test

No visible a sign of toxicity was reported on oral administration of MEAP in the animals at the dose between 200-1000 mg/kg body weight of the rats, no mortalities were recorded either. However, further observation for next 48 h was also continued for any behavioral change.

Castor oil induced diarrhea

The doses of MEAP significantly decreased (P<0.05) the total number of diarrhea faces produced by administration of castor oil (3.00 ± 0.58 at the dose of 200 mg/kg) as compared to castor oil treated control group (7.67 ± 1.45) and comparable to the standard drug. The percentage of inhibition of castor oil induced diarrhea in MEAP treated rats was 60.89 % at the dose of 200 mg/kg body weight of the rats and presented in table 1 and figure 1.

$MgSO_4$ induced diarrhea

The doses of MEAP significantly decreased (P<0.05) the total number of diarrhoeal faeces produced by administration of $MgSO_4$ (2.33 ± 0.33 at the dose of 200 mg/kg) as compared to $MgSO_4$ treated control group (6.67 ± 1.45) and comparable to the standard drug. The percentage of inhibition of castor oil induced diarrhea in MEAP treated mice was 65.07% at the dose of 200 mg/kg body weight of the mice and presented in table 2 and figure 2.

Discussion

Diarrhea is usually considered a result of altered motility and fluid accumulation within the intestinal tract. Antidiarrheal drugs either acts by reducing gastrointestinal motility or the secretions. Castor oil induces diarrhea due to the active metabolite, recinolic acid [22]; its increases the peristaltic movement in small intestine leading to changes in the electrolyte permeability of the intestinal mucosal membrane. The actual pathway of castor oil action is through provoking the prostaglandin biosynthesis [23- 24]. Prostaglandin contributes in the control of various pathophysiological functions in gastrointestinal tract [25] inhibition of prostaglandin synthesis can reduce GI motility, thus, reduce castor oil-induced diarrhea [26].

Ricinolic acid causes diarrhea that is liberated by the action of lipases on castor oil. This stimulates peristaltic activity in the small intestine, and also stimulates the endogenous prostaglandins [27]. Castor oil induced diarrhea can be called actually secretory and motility diarrhea [28] as it cause different gastric secretion and improve motility. The observations from this study imply that the antidiarrheal effect may be a contribution of 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. Antidiarrheal effect of medicinal plants were found to be due to the presence of some common phytochemicals like tannins, alkaloids, saponins, flavonoids, steroids and terpenoids [29]. Previous studies conducted on this plant have found presence of alkaloids, saponins, flavonoids, and steroids.

Wet stool is one of the common characteristics of diarrhea, which is usually caused by altered motility and fluid accumulation in the intestinal lumen. $MgSO_4$ is an osmotic laxative; it induces diarrhea by increasing the volume of intestinal content and

prevents water reabsorption. In the present study, diarrhea characterized by intestinal fluid accumulation was developed in mice after the administration of $MgSO_4$. Obvious watery stool

in the colon, increased fecal water content, and increased defecation were observed in vehicle-treated mice. While in MEAP-treated mice we noticed that MEAP attenuated the severity of diarrhea by reducing the degree of watery stool in the colon, the fecal water content, and in a dose-dependent manner. The results suggest that MEAP has an antidiarrheal activity in $MgSO_4$ -induced diarrhea in mice partly via reducing the colonic water secretion induced by $MgSO_4$.

The antidiarrheal activity of the plant extract was comparable to the standard drug, loperamide; effectively antagonizes the diarrheal activity induced either by castor oil [30], increased biosynthesis of prostaglandins [31] or cholera toxin [32]. Loperamide, other than regulating the gastrointestinal tract, is also prominent in slowing down transit time of intestinal content, reduces flow rate through colon, and consequently affect the colonic motility [33-35]. The significant inhibition of the castor oil and $MgSO_4$-induced enter pooling in mice suggests that *A. procera* leaf extract produces relief in diarrhea either by spasmolytic pathway or due to antisecretory activity in diarrheal animal model.

In conclusion, the present study revealed that *Albizia procera* contains pharmacologically active substances effective for management of diarrhea. Further studies are required to fully investigate the mechanisms responsible for this observed antidiarrheal activity.

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Table 1: Effect of Methanolic leaves extract of *A. procera* on castor oil induced diarrhea in mice

Group	Treatment	No. of fecation in 4 hr	% of fecal inhibition
I (Control)	CO + NS	7.67 ± 1.45	0
II (Standard)	CO + Loperamide (5 mg/kg)	2.33 ± 0.67 **	69.62
III (MEAP_200 mg/kg	CO + MEAP 200 mg/kg	3.00 ± 0.58 *	60.89
IV (MEAP_100 mg/kg)	CO + MEAP 100 mg/kg	4.00 ± 0.58 *	47.85

**p<0.01 *p<0.05; CO- castor oil, NS- normal saline, MEAP- methanolic extract of *A. procera*; values presented here as Mean ± SEM (n=5), One-way ANOVA was performed to define the significant limit.

Table 2: Effect of Methanolic leaves extract of *A. procera* on $MgSO_4$ induced diarrhea in mice

Group	Treatment	No. of fecation in 4 hr	% of fecal inhibition
I (Control)	MS + NS	6.67 ± 1.45	0
II (Standard)	MS + Loperamide (5mg/kg)	1.67 ± 0.67 **	74.96
III (MEAP_200 mg/kg	MS + MEAP 200 mg/kg	2.33 ± 0.33 *	65.07
IV (MEAP_100 mg/kg)	MS + MEAP 100 mg/kg	2.67 ± 0.67 *	59.97

**p<0.01 *p<0.05; MS- $MgSO_4$, NS- normal saline, MEAP- methanolic extract of *A. procera*; values presented here as Mean ± SEM (n=5), One-way ANOVA was performed to define the significant limit.

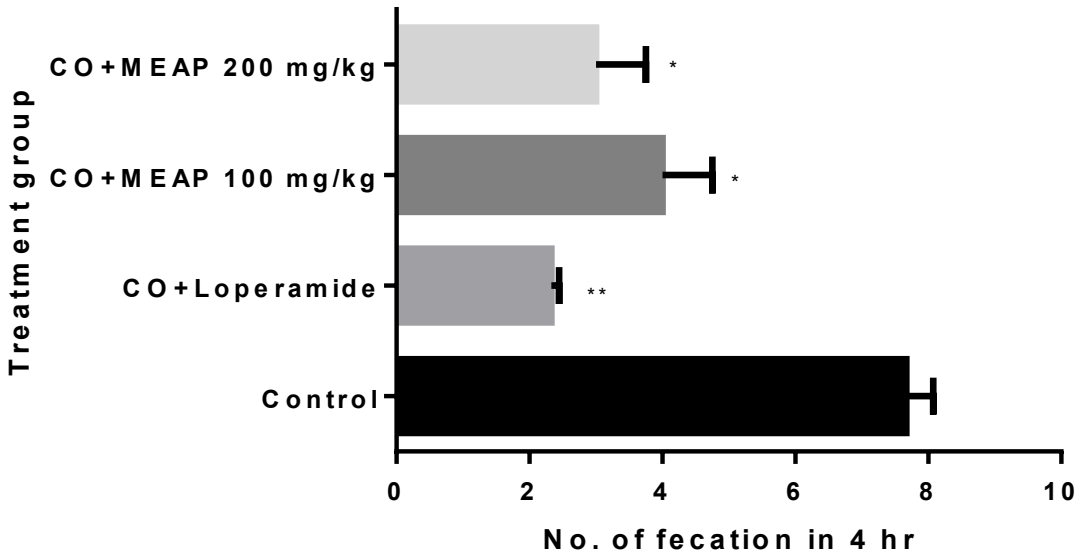

Figure 1: Anti-diarrheal effect of *A. procera* in diarrhea induced animal model. Here, significant values are asterisked, **p<0.01, and *p<0.05; CO- castor oil, MEAP- methanolic extract of *A. procera*.

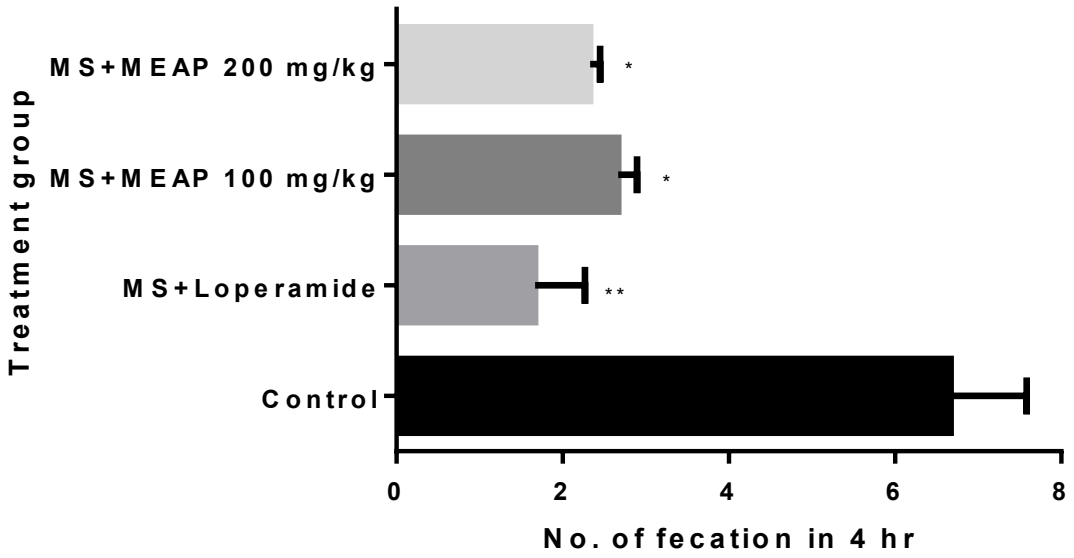

Figure 2: Anti-diarrheal effect of *A. procera* in diarrhea induced animal model. Here, significant values are asterisked, $^{**}p<0.01$, and $^{*}p<0.05$; MS- $MgSO_4$, MEAP- methanolic extract of *A. procera*.