

Evaluation of Antidiarrhoeal Activity of *Punica granatum* Peel and Seed Extracts in Experimental Animal Models

Tamanna Sharmin Tonny*, Kamrun Nahar, Golam Kibria, Sumsunnahar Shifa and Faruquzzaman

Department of Pharmacy, Primeasia University, Dhaka, Bangladesh

***Corresponding Author:** Tamanna Sharmin Tonny, Department of Pharmacy, Primeasia University, Dhaka, Bangladesh.

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Abstract

The present study was conducted to justify the traditional use of *Punica granatum* fruit in case of diarrhea. Methanol extracts of *Punica granatum* peel and seeds were used to evaluate the antidiarrhoeal activity against castor oil induced and magnesium sulfate induced diarrhoea in Mice models. The extracts produced significant antidiarrheal activities by reducing the percentage of defecation in both models when compared to the positive control, Loperamide in a dose dependent manner. At the highest dose (500 mg/kg) both the peel and seed extracts exerted significant protection against diarrhea in parallel to the standard drug. The results revealed rationalizes and established the proficiency of *Punica granatum* as a potent and safe organic antidiarrheal agent compared to the available synthetic drugs used to alleviate diarrhea.

Keywords: *Punica granatum*; Loperamide; Antidiarrheal Activity; Castor Oil Induced Diarrhea; Magnesium Sulfate Induced Diarrhea

Introduction

Punica granatum L. (Punicaceae) is known as pomegranate, originated in Iran, Afghanistan. The name pomegranate is derived from medieval Latin *pōmum* «apple» and *grānātum* «seeded» which translates to 'seeded apple'. The genus name of pomegranate is '*Punica*' was the Roman name for Carthage, is the place where the best pomegranate were grown. In Greek mythology, Pomegranate was considered as the sign of sanctity, fertility, and abundance [1].

P. granatum is a deciduous shrub with glossy leaves, 2 - 8 cm long, oblong or obovate. Its flowers are regular, solitary or in fascicles at apices, 4 - 6 cm petals lanceolate, 5 - 7 wrinkled and brilliant orange-red. Fruit is round berry, 5 - 12 cm interior compartmentalized with many pink-red sections of pulp-like tissue, each contains a seed grain [2]. This plant is cultivated in Spain, the United States, Iran, Turkey, India, Israel, China and countries along the north coast of Africa [3].

It is used in ethno medicines for the treatment of various ailment of gastrointestinal dysentery as it possesses astringent and stomachic property [4]. Almost all part of the plant i.e. Juice, seeds, leaves, flowers, bark and roots can be used for pharmacological effects. Pomegranate contains flavonoids of various types of which approximately 0.2% to 1.0% comes from fruit. The peel contains approximately 30% of all anthocyanidins found in pomegranate. The seeds contain isoflavones genistein, daidzein, genistin and daidzein as well as estrone, the metabolic derivative of estradiol. Isopelletierine, pelargonidin, pseudopelletierine and N-methylisopelletierine, anthocyanidins, ellagitannins, Gallic acid and Ellagic acid were found in pomegranate stems and roots [5].

Some of the traditional uses of pomegranate include anti-diarrheal, anthelmintic, lowering fever, treating diabetes, blood tonic, stopping the bleeding, and healing ulcers [6]. In India, pomegranate peels are dried and extracted in water which are used as astringents and germicides. The peel extract is used for the treatment of diarrhoea and inflammatory stomach. Different preparations of pomegranate

such as extracts from peels, flowers, and seeds as well as the juice show a significant anti-inflammatory activity in the gut. So, pomegranate seeds or juice is very salutary for the healing of diarrhea. Pomegranate seeds are rich source of polyphenols, ellagitannins and anthocyanins which are known to possess significant anti-ulcerant and anti-inflammatory activity. These compounds significantly diminish the growth of *Helicobacter pylori* which is accountable for diarrhoea [7].

In Bangladesh, Diarrhea is considered to be the second leading cause of death among children less than five years of age. According to icddr, each year diarrhea takes away 500,000 children lives less than five years of age. Worldwide, 1.7 billion cases of Diarrhea have been reported. Rotavirus is most common causes of diarrhea (approximately 40%) among children below five years of age. Other micro-organisms that are responsible for diarrhea include *Campylobacter*, *E. coli*, *nontyphoidal Salmonella* and *Shigella*, *Cryptosporidium*, *Entamoeba histolytica* and *Giardia lamblia* [8]. The present study was carried out to justify the anti-diarrheal effect of methanolic extract of *Punica granatum* using various standard models.

Materials and Methods

Collection and identification of plant material

The fresh and matured fruits were collected during the month of September 2016 from open market, Mahakhali, Dhaka and authenticated for identification from the Bangladesh National herbarium, Dhaka. The acknowledgement slip was entrusted on Bangladesh National Herbarium and the accession number was 43471.

Preparation and extraction of plant material

The peel and seeds of elected *Punica granatum* was dried at room temperature maintaining lucid and salubrious circumstance. The dried plant materials were comminuted into coarse powder using electrical grinder. 150 gm powdered peel and seeds were soaked separately in 95% Methanol in clean flat bottomed containers for seven days with maintenance of regular shaking. After filtration with cotton and filter paper the filtrate was being concentrated using rotary evaporator and stored at - 70°C for further use.

Animals used for experimentation

The Swiss Albino mice were collected from Department of pharmacy Jahangirnagar University, Savar, Dhaka. The selected mice weighed around 22 - 25 gm and showed diarrhoea after administering castor oil and MgSO₄ Heptahydrate respectively for antidiarrhoeal activity exploration of peel and seed extracts. The selected mice were divided into four groups containing five mice in each group for both castor oil induced method as well as MgSO₄ induced method. They were kept in individual cages, being marked individually in their tail according to weight and were provided with standard food collected from Jahangirnagar University as well as with fresh water. Before 12 hours of commencement of the experiment, the mice were kept at fasting state. Suitable environmental conditions as well as alternate 12 hours of light and dark cycle were maintained at the laboratory. The cages were cleaned every day to ensure a clean environment.

Castor oil induced method to explore the peel extract

After 30 minutes of oral administration of 10 ml/kg body weight of 0.9 % saline solution to the mice of control group, Loperamide Hydrochloride (Imotil) as standard at 3 mg/kg b.w. to the mice of positive control group, *Punica granatum* peel extract at 250 mg/kg b.w. to the mice of test group and *Punica granatum* peel extract at 500 mg/kg b.w. to the mice of another test group, 0.5 ml castor oil was given orally to all of the mice of each group. The mice were transferred to premarked cages according to group lining with blotting paper. The blotting paper was changed with 1 hour interval. The Exploration was continued for 3 hours after giving castor oil. Total number of fecal episodes was tabulated.

Castor oil induced method to explore the seed extract

After 30 minutes of oral administration of 10 ml/kg body weight of 0.9 % saline solution to the mice of control group, Loperamide Hydrochloride (Imotil) as standard at 3 mg/kg b.w. to the mice of positive control group, *Punica granatum* seed extract at 250 mg/kg b.w. to the mice of test group and *Punica granatum* seed extract at 500 mg/kg b.w. to the mice of another test group, 0.5 ml castor oil was given orally to all of the mice of each group. The mice were transferred to premarked cages according to group lining with blotting paper. The blotting paper was changed with 1 hour interval. The Exploration was continued for 3 hours after giving castor oil. Total number of fecal

episodes was tabulated.

MgSO₄ induced method to explore the peel extract

After 30 minutes of oral administration of 10 ml/kg body weight of 0.9 % saline solution to the mice of control group, Loperamide Hydrochloride (Imotil) as standard at 3 mg/kg b.w. to the mice of positive control group, *Punica granatum* peel extract at 250 mg/kg b.w. to the mice of test group and *Punica granatum* peel extract at 500 mg/kg b.w. to the mice of another test group, 0.5 ml castor oil was given orally to all of the mice of each group. The mice were transferred to premarked cages according to group lining with blotting paper. The blotting paper was changed with 1 hour interval. The Exploration was continued for 3 hours after giving castor oil. Total number of fecal episodes was tabulated.

MgSO₄ induced method to explore the seed extract

After 30 minutes of oral administration of 10 ml/kg body weight of 0.9% saline solution to the mice of control group, *Punica granatum* seed extract at 250 mg/kg b.w. to the mice of test group and *Punica granatum* seed extract at 500 mg/kg b.w. to the mice of another test group, 0.5 ml castor oil was given orally to all of the mice of each group. The mice were transferred to premarked cages according to group, Loperamide Hydrochloride (Imotil) as standard at 3 mg/kg b.w. to the mice of positive group lining with blotting paper. The blotting paper was changed with 1 hour interval. The Exploration was continued for 3 hours after giving castor oil. Total number of fecal episodes was tabulated.

Acute toxicity test

Acute toxicity test was performed on albino mice weighing about 22 - 25g. The crude extract was given in the doses 250, 500 and 1000 mg/kg of body weight to three groups of mice, each group containing 5 mice. The behaviors were observed for 2, 4, 6, 8, 24 and 48h. The number of survived animals after 24 and 48h of experimentation were also recorded. A normal control group was also run at parallel, who received normal saline (10 mg/kg). All the mice received normal diet and tap water *ad libitum* [9].

Statistical analysis

The results of the antidiarrheal activity of peel and seed extracts in castor oil induction and magnesium sulfate induction models were represented as mean ± SEM (n = 5) and the statistical analysis of the results was carried out using One-way ANOVA, followed by Dunnett test. Whereas values of p < 0.05 and P < 0.01 were considered statistically significant.

Results and Discussion

The treatment groups containing the methanolic extracts and standard drug reduced the number of faecal dropping greatly when compared with the untreated control group.

Castor oil induced diarrhea

The effects produced by methanol peel and seed fractions of *Punica granatum* in castor oil induced diarrheas is shown below by table1 and 2 respectively.

Treatment	Dose (p.o.)	No of faecal droppings	% Inhibition of defecation
Castor oil (Control)	0.5 ml/mouse	3.60 ± 0.5099	-
Loperamide	3 mg/kg	1.20 ± 0.3742 ^{a**}	66.67
<i>P. granatum</i> peel	250 mg/kg	2.40 ± 0.5099 ^{a ns}	33.33
<i>P. granatum</i> peel	500 mg/kg	1.60 ± 0.5099 ^{a*}	55.56

Table 1: % inhibition of defecation of *Punica granatum* peel as well as Loperamide standard in castor oil induced method.

The values are represented as mean ± S.E.M (n = 5) and statistical significance between treated and control groups was analyzed using of One-way ANOVA, followed by Dunnett's test. P < 0.05* and 0.01** were considered statistically significant, ns: Not Significant. a: When compared with Castor oil control group.

Treatment	Dose (p.o.)	No of faecal droppings	% Inhibition of defecation
Castor oil (Control)	0.5 ml/mouse	3.60 ± 0.5099	-
Loperamide	3 mg/kg	1.20 ± 0.3742 ^{a**}	66.67
<i>P. granatum</i> seed	250 mg/kg	2.20 ± 0.2000 ^{a*}	38.89
<i>P. granatum</i> seed	500 mg/kg	1.80 ± 0.3742 ^{a*}	50.00

Table 2: % inhibition of defecation of *Punica granatum* seed as well as Loperamide standard in castor oil induced method.

The values are represented as mean ± S.E.M (n = 5) and statistical significance between treated and control groups was analyzed using of One-way ANOVA, followed by Dunnett’s test. P < 0.05* and 0.01** were considered statistically significant, ns: Not Significant; a: When compared with Castor oil control group.

At 250 mg/kg dose, in castor oil induced diarrhea, the *Punica granatum* peel extract reduced defaecation by nonsignificant 33.33%, whereas in case of the seed extract 38.89% reduction was seen. The results were dose dependent, therefore the best results were produced at 500 mg/kg of the methanol extracts. In case of 500 mg/kg dose, methanolic peel extract of *P. granatum* showed significant 55.56% and seed extract 50.00% reduction in the percentage of defaecation, whereas Loperamide (3 mg/kg) showed better 66.67% protection. In terms of protection from Diarrhea after 4h, the 250 mg/kg dose of *P. granatum* peel fraction protected 1 out 5 mice and the seed fraction protected 0 out of 5 mice while at the 500 mg/kg of dose, both peel and seed fractions protected 2 out of 5 Mice. The standard drug Loperamide at 3 mg/kg dose, provided protection from Diarrhea for 3 out of 5 animals, whereas for negative control groups, all the animals were Diarrhea affected.

MgSO₄ induced diarrhea

The antidiarrheal effects produced by methanol peel and seed fractions of *Punica granatum* in magnesium sulfate induced diarrhea is shown below by table 3 and 4 respectively.

Treatment	Dose (p.o.)	No of faecal droppings	% Inhibition of defecation
Mg SO ₄ (Control)	0.5 ml/mouse	3.00 ± 0.3162	-
Loperamide	3 mg/kg	1.20 ± 0.2000 ^{a**}	60.00
<i>P. granatum</i> peel	250 mg/kg	1.60 ± 0.2449 ^{a**}	46.67
<i>P. granatum</i> peel	500 mg/kg	1.40 ± 0.2449 ^{a**}	53.33

Table 3: % Inhibition of defecation of *Punica granatum* peel as well as Loperamide standard in MgSO₄ induced method.

The values are represented as mean ± S.E.M (n = 5) and statistical significance between treated and control groups was analyzed using of One way ANOVA, followed by Dunnett’s test. P < 0.05* and 0.01** were considered statistically significant, ns: Not Significant. a: When compared with Mg SO₄ control group.

Treatment	Dose (p.o.)	No of faecal droppings	% Inhibition of defecation
Mg SO ₄ (Control)	0.5 ml/mouse	3.00 ± 0.3162	-
Loperamide	3 mg/kg	1.20 ± 0.2000 ^{a**}	60.00
<i>P. granatum</i> seed	250 mg/kg	1.80 ± 0.2000 ^{a**}	40.00
<i>P. granatum</i> seed	500 mg/kg	1.40 ± 0.2449 ^{a**}	53.33

Table 4: % Inhibition of defecation of *Punica granatum* seed as well as Loperamide standard in MgSO₄ induced method.

The values are represented as mean ± S.E.M, (n = 5) and statistical significance between treated and control groups was analyzed using of One way ANOVA, followed by Dunnett’s test. P < 0.05* and 0.01** were considered statistically significant, ns: Not Significant; a: When compared with Mg SO₄ control group.

In MgSO₄ induced Diarrhea, at 250 mg/kg, the peel and seed fractions of *P. granatum*, produced good inhibitory action on Diarrheal faecal droppings marked by 46.67% and 40.00% respectively. The Methanol peel and seed fractions at 500 mg/kg produced consistent and significant 53.33% reduction of diarrheal episodes, whereas the standard drug loperamide at 3 mg/kg produced 60.00% inhibition of diarrheal defaecation. After 4h of induction, at 250 mg/kg dose, the peel fraction of *P. granatum* protected 2 out of 5 mice while the seed fraction could protect 1 out of 5 mice from intense Diarrhea. For 500 mg/kg of dose, both peel and seed fractions protected well 3 out 5 mice simultaneously Loperamide at 3 mg/kg protected 3 out of 5 animals from Diarrhea, whereas in the negative control groups, all 5 mice were affected by Diarrhea.

The active ingredient of castor oil, r ricinoleic acid produces a hypersecretory response in the body which induces diarrhea. This secretory diarrhea induced by Castor oil is attributed to the changes in intestinal mucosal membrane permeability to water and electrolytes [10].

In this present study in case of Castor oil induced Diarrhea, from the observation of inhibition of percentage of defecation and visual wet content, it can be said that the methanol extract of peel and seeds, specially at the dose of 500 mg/kg produced significant reduction of secretory diarrhea compared to the standard drug, Loperamide. Additionally, Magnesium sulfate induces diarrhea by preventing reabsorption of water therefore increasing the volume of intestinal content. It facilitates the liberation of Cholecystokinin from the duodenal mucosa, which increase the secretion and motility response of small intestine causing the loss of reabsorption of water and Sodium chloride [11].

As the extracts lingered the gastrointestinal transit in mice compared to negative control groups, thereby it might have anti motility activity. Although both the 250 mg/kg and 500 mg/kg doses of peel and seed extracts produced good therapeutic effect in a dose dependent manner, the peel fraction at 500 mg/kg produced excellent anti- diarrheal action compared to the protection showed by the standard drug.

Acute toxicity test

During the period of acute toxicity test, no single mouse showed any type of abnormal behavior. They took their food, water and rest in the appropriate manner, which indicates the safety of the plant extracts at given dose. No mortality was recorded even at the high doses of the plant extracts.

Conclusion

The results observed in the present study revealed that the methanol extract produced protection of animals against both castor oil and magnesium sulfate induced diarrhea in a dose dependent manner. This study commends that the antidiarrheal effect exerted by the methanol extract of *Punica granatum* peel and seeds are may be due to reduction of intestinal secretory response and inhibition of intestinal motility. Additionally, no potential adverse effects produced from the toxicity test, ensures its' safe and economical use against the common yet deadly disease, diarrhea when compared to the synthetic commercial drugs. Therefore, it can be concluded that the traditional use of *Punica granatum* as ethno medicinal antidiarrheal agent is justified.

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