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Antidiarrhoeal activity of rind of *Punica granatum*

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ABSTRACT

Punica granatum (Family: Punicaceae) is a widely consumed fruit in Bangladesh locally known as Anar or Bedana. Traditionally the plant is used to treat diarrhoea, dysentery, hemorrhage, tuberculosis and chronic periodontitis. The present study was undertaken in order to justify and authenticate the traditional use of rind of *P. granatum* fruit in diarrhoea. Methanolic extract of the rind of the fruit was tested in castor oil- and magnesium sulfate-induced diarrhoea in mice at the doses of 200 and 400 mg/kg body weight. The results of the present study revealed significant antidiarrhoeal activity ($p<0.001$) in both animal models. The extract inhibited 31.25% defaecation at the dose of 200 mg/kg and 53.75% at the dose of 400 mg/kg in castor oil-induced diarrhoea while standard drug loperamide inhibited 71.25% defaecation. In magnesium sulfate-induced diarrhoea the inhibition of defaecation was 45.71 and 57.14% at the doses of 200 and 400 mg/kg respectively while loperamide inhibited 64.29% defaecation. The antidiarrhoeal effect of the extract was concentration dependent in both castor oil-induced diarrhoea and magnesium sulfate-induced diarrhoea. Phytochemical screening of the extract revealed the presence of flavonoids and alkaloids that may play key role in its antidiarrhoeal activity. The results of the present study validate the folkloric use of the rind of *P. granatum* in the treatment of diarrhoea.

Key Words: *Punica granatum,* antidiarrhoeal activity, castor oil-induced diarrhoea, magnesium sulfate-induced diarrhoea.

INTRODUCTION

Punica granatum Linn. (Pomegranate) is a plant of punicaceae family locally known as Anar. *P. granatum* is a fruit of great antiquity and is known to have been cultivated in the Middle East more than 5,000 years ago. The plant is found all over India and Bangladesh. The fruit of this plant is used as food and as a diet in convalescence after diarrhoea. It is used in Siddha, Ayurvedha and Unani medicine especially for the treatment of gastrointestinal (GI) diseases. Dried, pulverized flower buds are employed as a remedy for bronchitis. Traditionally, it is also used in diarrhoea, dysentery and stomatitis due to its strong astringent property (Ghani, 2003).

Different types of chemical compounds have been isolated from different parts of the plant. Rind of *P. granatum* is enriched with beta-carotene, potassium, phosphorous and calcium. Previous research on *P. granatum* revealed various chemical constituents like ellagic acid, ellagitannins, punicic acid, flavonoids, anthocyanidins, anthocyanins, and estrogenic flavonoids and flavones (Jurenka, 2008). *In vitro* assays of different extracts of *P. granatum* fruit in three different cancer cell lines exhibited anticancer activity (Akhlaghi and Band, 2009). Seed oil of *P. granatum* also has anti-inflammatory activity (Caceres *et al*., 1987). Alcoholic and aqueous extract of. *P. granatum* rind possesses antioxidant activity (Rajan *et al*., 2011). Flavonoid-rich polyphenol fractions from the *P. granatum* fruit exert anti-proliferative, anti-invasive, anti-eicosanoid, and pro-apoptotic actions in breast and prostate cancer cells and anti-angiogenic activities *in vitro* and *in vivo* (Kawaii and Lansky, 2004). Ethyl acetate fraction *of P. granatum* rind extract revealed the presence of pyrogallol, 5-hydroxymethylfurfural, D-allose, 2-methoxy-1, 4-benzenediol and 2, 3 dimethylfumaric acid (Sangeetha and Vijayalakshmi, 2011).

Leaves, seeds, roots and bark have displayed hypotensive, antispasmodic and anthelmintic activity in bioassay**.** Methanolic extract of fruit rind

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exhibited significant antibacterial activity (Chaturvedula *et al.*, 2011) because of tannin content. Extracts of the bark, leaves, immature fruit and fruit rind have been given to halt diarrhoea, dysentery and hemorrhages (Said, 1996, Ghani, 2003). This study aimed to investigate the antidiarrhoeal activity of methanol extract of rind of *P. granatum* using castor oil-induced and magnesium sulfate-induced diarrhoeal models in mice.

MATERIALS AND METHODS

Extraction of plant material

Mature fruits of *P. granatum* were collected from Mohakhali Kacha Bazar, Dhaka, Bangladesh in the month of July 2012. The plants were identified by the taxonomist of the Bangladesh National Herbarium (Accession No. 37848) where a voucher specimen has been deposited for future reference. The rind of the collected fruits was separated and then sun dried for two days. Then it was dried in oven at a reduced temperature (at 55°C) to make it suitable for the grinding process. Powdered materials were soaked in methanol for seven days with occasional stirring. The supernatant liquid was then decanted and filtered through a plug of cotton. The process was repeated for complete extraction. The extract was then dried and this crude extract was used for the investigations.

Animals

Swiss albino mice (22-25 g) were obtained from the Animal Resources Branch of the International Center for Diarrhoeal Disease and Research, Bangladesh (ICDDR, B). The animals were maintained at constant room temperature (25.0±2.0°C), humidity 55-65% and 12h light: 12h dark cycle. The animals were fed with standard diet (ICDDR, B formulated) and had free access to water *ad libitum*.

Phytochemical screening

The extract was tested for detection of phytochemicals such as carbohydrates, alkaloids, glycosides, anthraquinone glycosides, glucosides, tannins, saponins, flavonoids and steroids following standard procedures (Ghani, 2003).

Antidiarrhoeal activity test in castor oil-induced diarrhoea

The experimental animals were all screened at first with 0.5 ml of castor oil and animals showing diarrhoea were selected for the experiment. The animals were divided into four groups accordingly as control, positive control and two test groups (PGR) on the basis of the difference of doses of the extract. In each of the groups five mice were taken. At the beginning of the experiment the control group was given vehicle (normal saline) at a dose of 10 ml/kg orally. The positive control group received Loperamide at a dose of 3 mg/kg orally. The test groups orally received the extract of *P. granatum* rind at the doses of 200 and 400 mg/kg body weight. Then the animals were placed into cages individually. The floor and walls of the cage was lined with blotting paper. The floor lining was changed every hour. Diarrhoea was induced by oral administration of 0.5 ml castor oil to each mouse, 30 min after the above treatments. During an observation period of 4 h, the total numbers of faeces excreted by the animals were recorded (Shoba and Thomas, 2001).

Antidiarrhoeal activity test in magnesium sulfate-induced diarrhoea

A similar protocol as for castor oil-induced diarrhoea was followed for magnesium sulfate-induced diarrhoea. Diarrhoea was induced by oral administration of magnesium sulfate at the dose of 2 g/kg to the animals 30 min after pre-treatment with vehicle (normal saline, 10 ml/kg, p.o.) to the control group, loperamide (3 mg/kg) to the positive control group, the methanol extract of *P. granatum* at the doses of 200 and 400 mg/kg to the test groups. All the administrations were carried out through oral route. The observation was performed following the same procedure as that of castor oil-induced diarrhoeal activity test (Doherty, 1981).

RESULTS

Effect on castor oil-induced diarrhoea

In castor oil-induced diarrhoeal model, the methanol extract of the rind of *P. granatum* at the doses of 200 and 400 mg reduced defecation by 31.25 and 53.25%, respectively, while the standard drug Loperamide exhibited 71.25% inhibition of defecation. It is clear that the inhibition of defecation is dose dependent (Table 1).

Effect on magnesium sulfate-induced diarrhoea

In magnesium sulfate-induced diarrhoeal model, both doses of extract reduced the severity of diarrhoea in test animals. The extract at 200 mg/kg dose exhibited 45.71% inhibition of defecation and

Table 1: Antidiarrhoeal activity of PGR in castor oil-induced diarrhoea.

Treatment	Dose (p.o.)	No. of faecal droppings in 4 h	% Inhibition of defaecation
Vehicle	0.2 ml/mouse	16.00±0.45	-
Loperamide	3 mg/kg	4.60±0.75*	71.25
PGR	200 mg/kg	11.00±0.63*	31.25
PGR	400 mg/kg	7.40±0.51*	53.75

PGR refers to the methanol extract of rind of *Punica granatum*
Each value is presented as the mean ± SEM (n = 5)
*$p < 0.001$ compared with the control group (Dunnett's test)

Table 2: Antidiarrhoeal activity of PGR in $MgSO_4$ induced diarrhoea.

Treatment	Dose (p.o.)	No. of faecal droppings in 4 h	% Inhibition of defaecation
Vehicle	0.2 ml/mouse	14.00±0.71	-
Loperamide	3 mg/kg	5.00±0.84*	64.29
PGR	200 mg/kg	7.60±0.51*	45.71
PGR	400 mg/kg	6.00±0.71*	57.14

PGR refers to the methanol extract of rind of *Punica granatum*
Each value is presented as the mean ± SEM (n = 5)
*$p < 0.001$ compared with the control group (Dunnett's test)

400 mg/kg dose exhibited 57.14% inhibition. The standard drug Loperamide gave 64.29% inhibition of defecation (Table 2). The effect was also dose dependent in this test.

DISCUSSION

The castor oil-induced diarrhoea demonstrates secretory diarrhoea, since recinolic acid, the active ingredient of castor oil, induces diarrhoea by a hypersecretory response. Castor oil is reported to produce changes in intestinal mucosal membrane permeability to electrolytes and water and thus produces diarrhea (Bruton, 1996; Galves *et al.*, 1993). Since the methanol extract of *P. granatum* significantly inhibited the castor oil-induced diarrhoea, it can be assumed that the antidiarrhoeal action was exerted by anti-secretory mechanism. This was also evident from the reduction of total number of wet faces in the test groups in the experiment. The extract may also reduce prostaglandins secretion from intestinal mucosa. Liberation of ricinoleic acid by castor oil results in irritation and inflammation of intestinal mucosa, which lead to the release of prostaglandins and stimulation of intestinal secretion (Pierce *et al*., 1971; Ramakrishna *et al*., 1994).

On the other hand, magnesium sulfate has been reported to induce diarrhoea by increasing the volume of intestinal content through prevention of reabsorption of water. It has been demonstrated that it promotes the liberation of cholecystokinin from the duodenal mucosa, which increase the secretion and motility of small intestine and thereby prevents the reabsorption of sodium chloride and water. The extract offered an increased absorption of water from the gastrointestinal tract. Since the extract delayed the gastrointestinal transit in mice as compared to the control, it might have anti-motility property. The delay of gastrointestinal transit prompted by the extract might have contributed, at least to some extent, to their antidiarrhoeal activity by allowing a greater time for absorption (Gaginella *et al.*, 1975).

CONCLUSION

Previous phytochemical screening revealed the presence of various phytoconstituents like flavonoids, tannis, alkaloids, glycosides in *p. granatum*. Our present study also confirms the presence of flavonoids, alkaloids, tannins, glycosides in *P. granatum*. Flavonoids and alkaloids are known for inhibiting release of autacoids and prostaglandins; thereby inhibit secretion induced by castor oil (Vimala *et al.*, 1997; Veiga *et al.*, 2001). The given antidiarrhoeal activity of the rind extract of *P. granatum* may be due to the presence of previously mentioned phytoconstituents present. It may be concluded that the present study supports the traditional use of the rind of *P. granatum* by traditional medical practitioners in the treatment of diarrhoea and associated disorders.

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REFERENCES

Akhlaghi, M. and Band, B. (2009). Mechanisms of flavonoid protection against myocardial ischemia-reperfusion injury. Journal of Molecular and Cellular Cardiology, Volume 46, Pages 309-17. [DOI] PMid:19133271

Bruton, L. (1996). Agents affecting gastrointestinal water flux and motility, digestants and bile acids. In: Pharmacological basis of therapeutics, 9th edn., McGraw-Hill, New York, Pages 916.

Caceres, A., Giron, L.M., Alvardo, S.R. and Torres, M.F. (1987). Screening of Antimicrobial activity of plants popu-

larly used in Guatemala for treatment of dermatomucosal diseases. Journal of Ethnopharmacology, Volume 20, Pages 223-237. [DOI]

Chaturvedula, V.S.P. and Prakash I. (2011). Bioactive Chemical Constituents from Pomegranate (*Punica granatum*) Juice, Seed and Peel-A Review. International Journal of Research in Chemistry and Environment, Volume 1, Issue 1, Pages 1-18.

Doherty, S.S. (1981). Inhibition of arachidonic acid release, mechanism by which glucocorticoids inhibit endotxin-induced diarrhea. British Journal of Pharmacology, Volume 73, Pages 549-554. [DOI]

Gaginella, T.S., Stewar, J.J., Olsen, W.A. and Bass, P. (1975). Action of recinolic acid and structurally related fatty acid on the GI tract. II. Effect on water and electrolytes absorption in-vitro. Journal of Pharmacology and Experimental Therapeutic, Volume 195, Pages 355-356. PMid:1185605

Galvez, J., Zavzuelo, A., Crespo, M.E., Lorente, M.D., Ocete M.A. and Jiménez, J. (1993). Antidiarrhoeic activity of *Euphorbia hirta* extract and isolation of an active flavonoid constituent. Planta Medica, Volume 59, Pages 333-36. [DOI] PMid:8372151

Ghani, A. (2003). Medicinal Plants of Bangladesh: Chemical Constituents and Uses. 2nd ed. Dhaka, Bangladesh: The Asiatic Society of Bangladesh.

Jurenka, J. (2008). Therapeutic Application of Pomegranate. Alternative Medicine Review, Volume 13(2), Pages 128-44. PMid:18590349

Kawaii, S. and Lansky, E.P. (2004). Differentiation-promoting activity of pomegranate (*Punica granatum*) fruit extracts in HL-60 human promyelocytic leukemia cells. Journal of Medicinal Food, Volume Spring 7(1), Pages 8-13.

Pierce, N.F., Carpenter, C.C. Jr., Elliott, H.L. and Greenough, W.B. 3rd. (1971). Effects of prostaglandins, theophylline and cholera exotoxin upon transmucosal water and electrolyte movement in the canine jejunum. Gastroenterology, Volume 60, Pages 22-32. PMid:5544090

Rajan, S., Mahalakshmi, S., Deepa, V.M., Sathya, K., Shajitha, S. and Thirunalasundari, T. (2001). Antioxidant potentials of *Punica granatum* fruit rind extracts. International Journal of Pharmacy and Pharmaceutical Sciences, Volume 3, Issue 3, Pages 82-88.

Ramakrishna, B.S., Mathan, M. and Mathan,V.I. (1994). Alteration of colonic absorption by long-chain unsaturated fatty acids, influence of hydroxylation and degree of unsaturation. Scandinavian Journal of Gastroenterology, Volume 29, Pages 54-58. [DOI] PMid:7907428

Said, H.M. (1996). Medicinal Herbal, First ed. Hamdard Foundation Pakistan.

Sangeetha, J. and Vijayalakshmi, K. (2011). Determination of bioactive components of ethyl acetate fraction of *Punica granatum* Rind Extract. International Journal of Pharmaceutical Sciences and Drug Research, Volume 3(2), Pages 116-122.

Shoba, F.G. and Thomas, M. (2001). Study of antidiarrhoeal activity of four medicinal plants in castor oil induced diarrhea. Journal of Ethnopharmacology, Volume 76, Pages 73-76. [DOI]

Veiga, V., Zunino L., Calixto J., Patitucci M. and Pinto, A. (2001). Phytochemical and antiedematogenic studies of commercial copaiba oils available in Brazil. Phytotherapy Research, Volume 15, Pages 476-480. [DOI] PMid:11536374

Vimala, R., Nagarajan S., Alam, M., Susan, T. and Joy, S. (1997). Antiinflammatory and antipyretic activity of *Michelia champaca* Linn (white variety), *Ixora brachiata* Roxb. and *Rhynchosia cana* (Willd.) D.C. flower extract. Journal of Experimental Boilogy,Volume 35, Pages 1310-1314.