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Evaluation of anti-diarrheal activity of *Diospyros malabarica* bark extract

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Abstract

The present study was undertaken to evaluate the anti-diarrheal activity of the ethanolic extracts of *Diospyros malabarica* Kostel bark at concentration (200, 400 mg/kg) using different experimental model such as castor oil-induced diarrhea, gastrointestinal motility and enteropooling. The *D. malabarica* extract showed significant anti-diarrheal activity in a dose dependent manner. The results collectively demonstrate the *D. malabarica* could act by attributed to an anti-electrolyte permeability action, inhibit the PGE_2 centrally and also anti-muscarinic activity to give anti-diarrheal effects. Result of charcoal meal test also suggested its anti-muscarinic activity. These findings indicate that ethanolic extract of the *D. malabarica* displays good anti-diarrheal activity, corroborating the folk use of *D. malabarica* preparations and contributing for its pharmacological validation.

Introduction

Diarrhea is one of the major health threats to populations in tropical and subtropical countries, responsible for about 5 million deaths annually, of which 2.5 million are children less than 5 years (Adeyemi and Akindele, 2008). Medicinal plants are promising source of anti-diarrheal drugs, the important advantages claimed therapeutic uses of medicinal plants in various ailments are their safety besides being economical, effective and their easy availability (Anonymous, 1979). A range of medicinal plants with anti-diarrheal properties is widely used by traditional healers. For these reason international organizations such as WHO have encouraged studies for treatment and prevention of diarrheal diseases depending on traditional medicinal practices. It is, therefore, important to identify and evaluate available natural drugs as alternatives to currently used anti-diarrheal drugs.

Diospyros malabarica Kostel (Synonym: *D. embryopteris, D. peregrina*; Ebenaceae) is a medium size evergreen plant found in India. It is commonly known as '*Indian persimon*' or *Gubh* in Hindi. In Indian, traditionally the bark of *D. malabarica* is recommended for astringent, acrid, anti-inflammatory and hemorrhages, diarrhea and dysentery (Warrier et al., 1994). The plant also possesses antifertility activity and useful in menstrual problems (Choudhary et al., 1990). Stem bark of the plant is reported to have hepatoprotective and hypoglycemic activity (Mondal et al., 2005; Dhar et al., 1968). Despite of its traditional uses, literature review reveled that, plant is not investigated for anti-diarrheal potential. So, the present study has been directed to investigate the anti-diarrheal activity of *D. malabarica* bark in different in experimental animal models.

Material and methods

Bark of *D. malabarica* kostel was collected in bulk quantities from college campus area, Malegaon, Baramati in winter season of 2008-2009 and identification of the plant was established by Department of Botany, S. P. M.

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M. Baramati (Voucher specimen number DPG: B–O3/08). The barks were shade dried and were crushed to moderately coarse powder for further use.

Extraction and phytochemical analysis of extract

The powder obtained was subjected to successive soxhlet extraction with ethanol, which was used for our studies. The extract were concentrated under reduced pressure and stored in desiccators for use.

The extract showed the presence of different classes of secondary metabolites including alkaloids, flavonoids, phenolics, tannins, saponins, sterols and triterpenoids were confirmed using conventional phytochemical tests. The presence of gallic acid in ethanol extract of *D. malabarica* was confirmed by HPLC using catechin (Sigma-Aldrich Chemie, Germany) as the standard marker. A Shimadzu HPLC system with LC-10AT, UV detector (Spectra System UV1000), and Luna C18 reverse-phase column (250 mm X 4.6 mm, i.d. particle size 5 µm) was used. The mobile phase consisted of 0.1% H_3PO_4: acetonitrile (85:15) mixture with flow rate 0.7 mL/min at 25°C, and the detection wavelength was set at 280 nm. Data was acquired and analyzed using Chromquest version 3.0 software.

Animals

Albino Wistar rats of both sex weighing between 150-250 g and nd female albino mice weighing 20-25 g were used. Animals were housed under standard conditions of temperature (27 ± 2°C,) and relative humidity (30-70%) with a 12:12 light: dark cycle. The animals were given standard diet and water *ad libitum*.

Acute toxicity study

The acute toxicity of ethanolic extract of *D. malabarica* was determined in either sex albino mice. The animals were fasted overnight prior to the experiment. Fixed dose OECD guideline No. 420; (Annexure-2d) method of CPCSEA was adopted for toxicity studies (Anonymous, 2008). Group of three mice were used for each test dose and LD_{50} value was used for selection of screening dose.

Castor oil-induced diarrhea (Kalaskar et al., 2010)

Rats of either sex (150-250 g) were divided into four groups (n=6) and fasted for 18 hours. The first group received 1% tragacanth suspension as control. The second group received loperamide suspension (2 mg/kg) orally as standard drug. The ethanolic extract was administered orally at 200 mg/kg dose to third group and 400 mg/kg dose to fourth group as suspension. After 60 min of treatment, all the animals of were challenged with 1 mL of castor oil orally and the watery fecal material and number of defecation was noted up to 4 hours in the transparent metabolic cages with filter paper at the base. Weight of paper before and after defecation was noted.

Gastrointestinal motility test (Jayakumari et al., 2011)

Rats of either sex (150-235 g) were fasted for 18 hours and divided into four groups (n = 6). The first group which served as control was administered with aqueous 1% tragacanth suspension. The second group receives standard drug atropine (0.1 mg/kg) subcutaneously. The ethanolic extract was administered orally at 200 mg/kg to third group and 400 mg/kg to fourth group as suspension. The animals were given 1 mL of 10% activated charcoal suspended in 10%aqueous tragacanth powder p.o., 30 min after treatment. Animals were euthanized 30 min after charcoal meal administration by ether anesthesia and sacrificed. The intestinal distance moved by the charcoal meal from pylorus to cecum was measured and expressed as a percentage of distance traveled from pylorus to cecum. The mean percentage movement of charcoal meal in ratio to the intestinal length, and percentage of inhibition was calculated.

PGE_2-induced enteropooling (Biswas et al., 2002)

Rats of either sex were divided into four groups of six in each group. The first group, which served as negative control was administered with vehicle (1% tragacanth suspension) and 1 mL of 5% v/v ethanol and normal saline by oral route. The second group, which served as positive control, was administered with PGE_2 in 5% v/v ethanol (100 µg/kg p.o.), only. The extract was administered orally at 200 and 400 mg/kg to third and fourth group, respectively. Immediately after extract administration, PGE_2 was administered. After 30 min following administration of PGE_2 each rat was sacrificed and the whole length of the intestine from pylorus to cecum was dissected out, its content collected in measuring cylinder and volume measured.

Results

Preliminary phytochemical investigation on ethanolic extract of *D. malabarica* reveled the alkaloids, flavonoids, phenolics, tannins, saponins, sterols and triterpenoids. Further HPLC analysis of the ethanol extract confirmed the presence of an important polyphenolic constituent, gallic acid (Rt = 3.36 min) (Figure 1). All the extracts of bark appeared to be non-toxic as no lethality was observed at the highest dose, i.e. 2,000 mg/kg p.o. in mice.

In present study, anti-diarrheal activity against castor-oil challenged diarrhea in rats the ethanolic extract of *D. malabarica* exhibited significant protection in dose dependant manner. The extract remarkable reduces the frequency of defecation, fecal droppings and mean weight of feces when compared to control group. The extract at a dose of 400 mg/kg had shown more significant effect when compared to the standard drug loperamide (Table I).

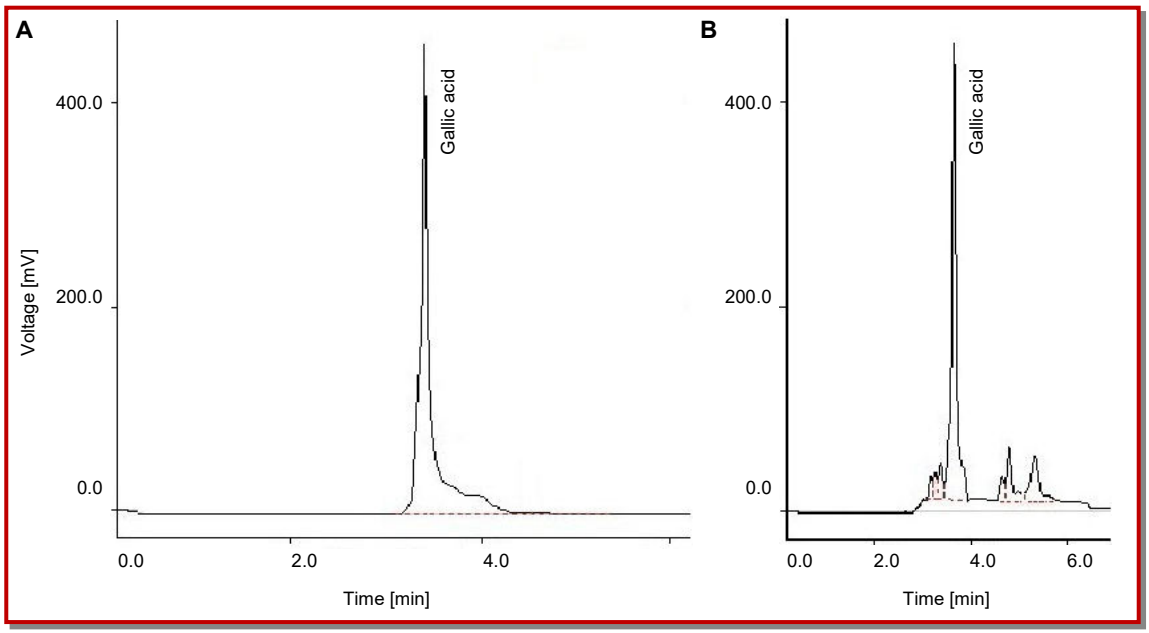

Figure 1: HPLC chromatogram of an authenticate standard gallic acid (A), and gallic acid identified in ethanol extract of *D. malabarica* (B)

The ethanolic extracts decreased propulsion of the charcoal meal through the gastrointestinal tract as compared with control group (1% gum tragacanth). These results are almost equivalent to standard anticholinergic drug, atropine. The extract inhibited significantly the intestinal propulsion in dose-dependent manner (Table II).

The ethanolic extract (200 and 400 mg/kg) dose dependently reduced the intestinal fluid accumulation with respect to PGE_2 control group. The effect of extract at dose of 400 mg/kg was found to be more potent when compared with standard and control. There was significant increase in intestinal fluid accumulation was seen in PGE_2 control group when compared to negative control received only ethanol in normal saline (Table III).

Discussion

In the present investigation, the anti-oxidant properties and anti-diarrheal activity of ethanol extracts of *D. malabarica* were established in animals by different

Table I

Evaluation of anti-diarrheal activity of ethanolic extract of *D. malabarica* by castor oil-induced diarrhea

Treatment	Total number of feces	Total number of diarrheal feces	Delay in defecation time
Control	21.5 ± 2.5	15.2 ± 1.8	18.6 ± 2.7
Loperamide (2 mg/kg)	5.7 ± 0.8[b]	4.9 ± 0.8[b]	86.5 ± 9.0[b]
Ethanolic extract (200 mg/kg)	14.8 ± 1.2[a]	8.7 ± 1.9[b]	40.9 ± 5.6[b]
Ethanolic extract (400 mg/kg)	6.5 ± 1.2[b]	5.6 ± 0.9[b]	68.9 ± 7.7[b]

Values are expressed as mean ± SD (n = 6), [a]p<0.05, [b]p<0.01

Table II

Evaluation of anti-diarrheal activity of ethanolic extract of *D. malabarica* by charcoal-induced gut transit time

Treatment	Total length of intestine	Distance traveled by charcoal meal
Control	95.5 ± 0.8	89.5 ± 5.4
Atropine (0.1 mg/kg)	102.7 ± 0.9[b]	39.8 ± 2.7[b]
Ethanolic extract (200 mg/kg)	97.5 ± 0.8[a]	75.9 ± 4.8[a]
Ethanolic extract (400 mg/kg)	89.5 ± 0.7[b]	53.5 ± 3.4[b]

Values are expressed as mean ± SD (n = 6), [a]p<0.05, [b]p<0.01

Table III

Anti-diarrheal activity of ethanolic extract of *D. malabarica* by PEG_2 induced enteorpooling

Treatment	Volume of intestinal fluid (mL)
Control	1.9 ± 0.1
PGE_2 control (2 mg/kg)	2.7 ± 0.1[b]
Ethanolic extract (200 mg/kg)	1.3 ± 0.2[b]
Ethanolic extract (400 mg/kg)	1.1 ± 0.1[b]

Values are expressed as mean ± SD (n = 6), [a]p<0.05, [b]p<0.01

animal model, the results obtained could suggest mechanism of action in inhibiting diarrhea.

Castor oil chiefly contains triglyceride of ricinoleic acid which hydrolyzeses by lipases in the small intestine to glycerol and ricinoleic acid. Ricinoleic acid supported by the release of prostaglandins, which results from the inflammation and irritation effect results to increased peristalsis. Furthermore, it stimulates secretion of fluid and electrolytes of the small intestine which speed up the intestinal transit. (Helmut et al., 1974). The anti-diarrheal activity of the extract against experimentally induced diarrhea by castor oil may be attributed to an anti-electrolyte permeability action.

Aqueous extracts of *D. malabarica* (200 and 400 mg/kg) and the anti-muscarinic drug, atropine (0.1 mg/kg) decreased the propulsive movement in the charcoal meal study; extract of 400 mg/kg appears to be equipotent as atropine. Thus one the possible mechanism appears to be spasmolytic and an anti-enteropooling property by which the extract produced relief in diarrhea.

The intraluminally administered PGE_2 is known to induce duodenal and jejunal secretion of water and of electrolytes such as Cl^- and Na^+, fluid content is the principal determinant of stool volume and consistency. Net stool fluid content reflects a balance between luminal input (ingestion and secretion of water and electrolytes) and output (absorption) along gastrointestinal tract (Dajani et al., 1975). The extract showed protection against PGE_2 induced enteropooling, which might be due to the inhibition of synthesis of prostaglandins. Anti-enteropooling effect of the extract is more relevant because the prevention of enteropooling helps in the inhibition of diarrhea, especially by PGE_2 induced diarrhea as it is involved in the onset of diarrhea in intestinal mucosal cells.

The preliminary phytochemical screening reveled tannins as one the phytoconstituent. Earlier literature supports that tannins are potential anti-diarrheal property. Tannins present in plant, denature proteins forming protein tannate complex. The complex formed coat over the intestinal mucosa and makes the intestinal mucosa more resistant and reduces secretion (Kalaskar et al., 2010), i.e. anti-electrolyte permeability action, further more due to this complex coat over the mucosa it may prevent the PGE_2 induced enteropooling. On the basis of results, it was concluded that tannin i.e. gallic acid and like phytoconstituents present in the *D. malabarica* extracts may be responsible for the anti-diarrheal activity.

Conclusion

The prolong onset of diarrhea, reduction of gastro-intestinal motility and inhibition of the synthesis of prostaglandin observed in this study support to traditional uses of *D. malabarica* bark. The above effects of it may also be due to the presence of gallic acid and like tannins and polyphenols in the extract.

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Ethical Issue

The experimental protocol was approved from Institutional Animal Ethics Committee.

Conflict of Interest

Authors declare no conflict of interest

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