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Antibacterial, antidiarrhoeal and ulcer-protective activity of methanolic extract of *Spondias mangifera* bark

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Abstract

PURPOSE: *To evaluate Spondias mangifera bark for indomethacin-induced ulcer protective and castor-oil induced antidiarrhoeal activity.*

METHODS: *The extracts of S. mangifera were tested for castor-oil induced diarrhea, and intestinal fluid accumulation and propulsion in rats using diphenoxylate hydrochloride and atropine as standard drug. The effect of the extracts on indomethacin-induced ulceration in rats was also evaluated. using cimetidine as positive control. In-vitro antibacterial activity of methanolic and aqueous extract was also evaluated against Escherichia coli, Salmonella typhimurium and Vibrio cholerae bacteria.*

RESULTS: *There was a significant ($p < 0.01$) inhibition activity against castor oil induced diarrhoea and fluid accumulations in rats when tested at 100 and 200mg/kg. The extracts also showed significant ($p < 0.01$) reduction in intestinal propulsion in charcoal meal test in rats. The methanol extract inhibited the ulcerogenic effect of indomethacin and good antibacterial activity.*

CONCLUSION: *S. mangifera possesses significant antidiarrhoeal, ulcer protective and antibacterial activities.*

Keywords: *Antidiarrhoeal; Antibacterial; Spondias mangifera bark; Ulcer protective effect.*

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Introduction

Majority of people living in rural areas and those that are homeless or working in automobile garages, refineries and lather industries in India often suffer from diarrhea due to eating of contaminated food and/or drinking of contaminated water. In developing countries, a quarter of infant and childhood mortality is related to the diarrhoea.[1] The highest mortality rates have been reported to be in children less than five years of age. There are several reports on the epidemiological and experimental issues pertaining to world-wide acute-diarrheal disease.[2] Although many currently available synthetic drugs can cure the diarrhea they often produce other gastrointestinal side effects. Due to the growing awareness about limited side effects and complications, the usage of traditional medicines has gained importance both in eastern and western countries. Several studies have shown that prior administration with some plant extracts had a protective effect on the intestinal tract.

*Spondias mangifera (W*illd, Family (Anacardiaceae), is a glabrous tree with characteristic pleasant smell of wood, widely distributed on tropical and subtropical beaches.[3] The trunk barks have been used traditionally as refrigerant, tonic, antiseptic, astringent and for the treatment of dysentery, diarrhea and prevent vomiting.[4] A paste or lotion of the bark extract is rubbed on to the body for treatment of sprain and strain in case of both articular and muscular rheumatism.[5-6] All parts of the plant have a foeted, turpentine like odour when broken or brushed and provide the unique aroma and taste.[7] Previously, ellagitannins, galloylgeranin, lignoseric acid and β-carotein have been isolated from the plant.[8-9]

In the present study, we evaluated methanolic extracts of traditionally used *Spondias mangifera* bark (that have not been studied so far) for its anti-diarrhoeal potential against castor oil-induced diarrhoea, small intestine propulsion in charcoal meal test and indomethacin-induced gastric ulceration in Albino Wistar rats.

Materials and Methods

Chemicals and drugs

Indomethacin and atropine sulphate were purchased from Ranbaxy labs (New Delhi, India) while diphenoxylate hydrochloride was procured from Sigma-Aldrich (MO, USA).

Animals

Wister rats (150–200g) of either sex were used in this investigation. Animals were maintained under standard environmental conditions and had free access to feed and water *ad libitum*. The rats were randomly assigned into groups of 6 animals per group. Approval for the study protocol was granted by the Institutional Ethical Committee of Jamia Hamdard, Hamdard University New Delhi-62, India (Reg.173/01BC/CPCSEA).

Collection and authentication of plant materials

The stem bark of *Spondias mangifera* was collected from Jokoai forest of Dibrugarh, Assam in the month of January 2007. The plant specimen was authenticated by Prof Muhibul Islam Department of Life Sciences, Dibrugarh University, Assam with voucher specimen number, L-32/07. The material was dried under shade, pulverized and pass through 40 mesh sieve before being stored in a closed vessel for further use.

Extraction of plant materials

The *S. mangifera* bark powder was extracted with methanol (90%) by using soxhlet extractor. The solvent was removed under vacuum to give a semisolid residue (13.8% w/w). Phytochemical screening indicated the presence of reducing sugar, flavonoids, peptides, phenolic compounds and tannins.

Safety profile study

For acute toxicity study of the methanolic extract of the plant material, LD_{50} was determined by adopting fixed dose method.[10] The number of animals that died or survived after 24 hr was recorded and LD_{50} was extrapolated graphically.

Castor-oil induced diarrhea

Four groups of animals (n = 6 per group) were fasted for 18 hr and water was provided *ad libitum*. Group 1 rats received vehicle (0.5% v/v aqueous Tween 80, 10 ml/kg, p.o.) which served as control. Group 2 rats received diphenoxylate hydrochloride (5mg/kg) and served as standard. The methanolic extract of *S. mangifera* bark (MESM, 100 and 200 mg/kg, p.o.) were administered orally to the third and fourth groups of rats. After 1 hr of treatment, all the animals were challenged with 1 ml of castor oil orally and observed for consistency of faecal material.[11] The number of wet faecal droppings were measured for 3 hr with changing of filter papers beneath the individual rat cages.[12]

Castor-oil induced fluid accumulation

Four groups of animals (n = 6 per group) were fasted for 18 hr and water was provided *ad libitum*. Group 1 was administered vehicle (0.5% v/v aqueous Tween 80, 10 ml/kg, p.o.) and served as control. Group 2 rats received diphenoxylate hydrochloride (5mg/kg) and served as standard. The MESM (100 and 200 mg/kg, p.o.) were administered orally to the third and fourth groups of rats. Immediately after the drug dosing, 1 ml of castor oil was administered orally to each rat. After 30 min, each rat was sacrificed and the whole length of the intestine, from the pylorus to the caecum, was dissected out and its content collected in a measuring cylinder and the volume measured.[13]

Small intestinal propulsion

Rats were divided into four groups (n = 6) and fasted for 18 hr before the experiment. Each animal was orally administered 1 ml of charcoal meal (5% deactivated charcoal in 10% aqueous tragacanth) followed by oral administration of MESM to the third and fourth groups of animals in the dose of 100 mg/kg and 200 mg/kg. The first group was treated with 0.5% v/v aqueous Tween 80, 10 ml/kg, p.o.) and served as a negative control. The second group received atropine (0.1 mg/kg, i.p.), and served as the positive control. Thirty minutes later, each animal was killed by cervical dislocation and the small intestinal was rapidly dissected out and placed on a clean surface. The distance moved by the charcoal meal from the pylorus to caecum was measured the intestinal propulsion was calculated as the difference between the whole length of small intestine and the distance traveled by the suspended charcoal head divided by the whole length of small intestine. The value was expressed as a percentage of distance moved.[14]

Indomethacin-induced gastric ulceration

Gastric ulceration was achieved by administering different doses of indomethacin (30, 60 and 100 mg/kg) to rats orally and 100 mg/kg was found to be the most effective for producing gastric ulceration in the rats. The rats were then divided into four groups (n=6) and food and water were withdrawn 24 hr and 2 hr, respectively, before drug administration.[15] Rats in group 1 received 100 mg/kg indomethacin while those in group 2 were pretreated with 100 mg/kg cimetidine. The rats in groups 3 and 4 were pretreated with 100-200 mg/kg of bark extract 1 hr prior to administration of 100 mg/kg indomethacin. The drugs were administered intragastrically via the aid of an orogastric cannula. After 4 hr, the animals were killed by cervical dislocation and their stomachs were removed and opened along the greater curvature. Tissues were washed and treated with 10% formaldehyde in saline. Number of lesions were determined by

examination using hand lens.[16] Ulcer index (UI) and preventive ratio of group of each of the animals treated with extract was calculated by following formulae:[17]

Degree of ulceration = Total ulcer score / number of animals ulcerated

UI = Degree of ulceration x percentage of group ulcerated / 100

Preventive ratio = UI (ulcerated group – pretreated group) / UI (ulcerated group) x 100

Antibacterial Activity

In-vitro antibacterial activity of the methanolic and aqueous extract was evaluated by cup plate diffusion method at the concentration of 50 mg, 100 mg and 150 mg. The activity was tested against *Escherichia coli, Salmonella typhimurium* and *Vibrio cholerae*. Activity of the extract was compared with penicillin and streptomycin used as standard drug.[18]

Statistical methods

The data were analysed statistically using one-way analysis of variance followed by Dunnett's `*t*' test. The data were expressed as mean ± S.E.M. At 99% confidence interval p-values less than 0.01 were considered to be significance.

Results

Castor-oil induced diarrhea

A significant decrease in the number of wetness and frequency of defecation was observed in castor-oil induced diarrhea by *S. mangifera* bark extract (100-200 mg/kg, p.o). This effect was enhanced in the presence of diphenoxylate hydrocholide (5mg/kg, p.o), an anti-cholinergic drug (Table 1).

Castor-oil induced fluid accumulation

MESM (100-200mg/kg, p.o) dose dependently reduced the intestinal fluid accumulation by 32.3 – 42.3% relative to the control. Diphenoxylate hydrochloride (5 mg/kg, p.o) reduced the intestinal fluid accumulation by 53.4% (Table 1).

Small intestinal propulsion

All the tested doses of the extract significantly inhibited the intestinal propulsion from 40.0 to 54.9% in a dose dependent manner (Table 2). Atropine (an anti-cholinergic drug), produced 66.4% inhibition of the intestinal propulsion.

Indomethacin-induced gastric ulceration

The orally given extract produced a dose dependent inhibition of the ulcerogenic effect of indomethacin, reducing the ulcer index from 17.7 (control) to 8.7 and 6.7 for the 100 mg/kg and 200 mg/kg, respectively, resulting in preventive ratio of 50.4 and 62.0, respectively. Cimetidine (H_2-receptor blocker), reduced ulcer index to 4.6 (equivalent to preventive ratio of 74.01) (Table 3).

Antibacterial activity

Data from the antibacterial study suggest that the methanolic extract exhibited good antibacterial activity while the aqueous extract showed mild antibacterial activity against *Escherichia coli, Salmonella typhimurium* and *Vibrio cholerae*.

Discussion

Since *Spondias mangifera* is known to have many constituents, it is not possible to specifically identify the constituents producing the pharmacological effects investigated in this study. However, flavonoids present in the plant have been known to inhibit intestinal motility and hydro-electrolytic secretion, which are known to be altered in diarrhoeal conditions.[23] Therefore it is possible that the antiidiarrhoeal effect demonstrated is due to the presence of the flavonoids.

Table 1: Effect of *Spondias mangifera* bark extract on castor oil-induced diarrhea in rats

Treatment Groups	Mean defecation	Mean number of wet faeces	Mean volume of intestinal fluid	Percentage inhibition
Control vehicle (p.o)	14.0±0.543	8.85±0.580	3.78±0.06	-
Diphenoxylate.HCL 5mg/kg (p.o)	0.84±0.014**	0.538±0-012**	1.76±0.12**	53.43
MESM 100mg/kg (p.o)	5.54±0.032**	3.635±0.031**	2.56±0.04**	32.27
MESM 200mg/kg (p.o)	4.84±0.039**	0.818±0.023**	2.18±0.13**	42.32

*Values are expressed as mean ± S.E.M. **p< 0.01 compared to control; n=6 animals in each groups; MESM= Methanolic extract of Spondias mangifera*

Table 2: Effect of *Spondias mangifera* bark extract on intestinal propulsion in rats

Treatment Groups	Mean intestinal Length	Mean distance travelled by the charcoal meal	% Inhibition
Control vehicle (p.o)	58.59±1.067	52.298±1.365	0.11
MESM 100mg/kg (p.o)	59.70±2.191	35.810±0.332**	40.01
MESM 200mg/kg (p.o)	57.87±1.636	26.086±0.224**	54.92
Atropine sulphate 5mg/kg (i.p)	58.40±1.978	19.61±0.437**	66.42

*Values are expressed as mean ± S.E.M. **p< 0.01 compared to control; n=6 animals in each groups; MESM = Methanolic extract of Spondias mangifera*

Table 3: Effect of *Spondias mangifera* bark extract on indomethacin-induced ulceration in rats

Treatment Group	Ulcer index	Prevention ratio
Control vehicle (p.o)	17.66±0.450	-
Cimetidine, 100mg/kg (p.o)	4.59±0.30**	74.01
MESM, 100mg/kg (p.o)	8.76±0.52**	50.40
MESM, 200mg/kg (p.o)	6.71±0.28**	62.00

*Values are expressed as mean ± S.E.M. **P< 0.01 compared to control; n=6 animals in each groups; MESM= Methanolic extract of Spondias mangifera*

Escherichia coli, Salmonella typhimurium and *Vibrio cholerae* are three pathogens known to cause a variety of diseases including diarrhea and gastroenteritis in humans.[24] Since diarrhea can be caused by these organisms and other agents, the anti-bacterial effect against the three pathogens indicate that the extract could be quite useful in the management of bacterial-induced diarrhea.

In this preliminary work, no attempt was made to demonstrate the mechanism of action of the plant extract. Therefore, the mechanism of action of the extract in producing the different effects demonstrated is unknown. Further studies will be need to

explain demonstrate the mechanisms of action.

Conclusion

Spondias mangifera possesses significant ulcer-protective, antibacterial and anti-diarrhoeal activities. This justified the use of the plant bark as a non-specific anti-diarrhoeal agent in folk medicine.

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