In Vivo Anti-diarrheal Activity of Methanolic Extract of *Streblus asper* Leaves Stimulating the Na^+/K^+ -ATPase in Swiss Albino Rats

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Abstract *Streblus asper* Lour., traditionally used for anti-diarrheal effects as like dysentery and diarrhea. The present study aims to prove the anti-diarrheal activities of methanolic extract of leaves of *S. asper* in animal models. The anti-diarrheal activity was evaluated using castor oil-induced diarrhea and magnesium sulphate-induced diarrhea models whereas anti-motility activities were investigated using gastrointestinal transit test examined in animal models. In castor oil-induced diarrhea model, methanolic extract of *S. asper* (MESA) at the doses of 100, 200, and 400 mg/kg produced statistically significant ($P < 0.001$) decreased the number of diarrheal feces of rats against castor oil-induced diarrhea as well as magnesium sulphate-

induced diarrhea model also showed the same manner. In gastrointestinal transit test, delayed gastric emptying time decreased significantly ($P < 0.001$), the propulsion of charcoal meal in the gastrointestinal tract which also showed a dose-dependent manner in rats. The recent study indicates that MESA possesses anti-diarrheal property. The findings represent a rational explanation for its use in traditional medicine for the management of diarrhea management.

Keywords *Streblus asper* Lour. · Anti-diarrheal · Anti-secretory · Anti-motility · Castor oil

Abbreviations

MESA	Methanolic extract of <i>Streblus asper</i>
ICDDR, B	International Center for Diarrhoeal Disease and Research, Bangladesh
Na^+/K^+ -ATPase	Sodium–potassium adenosine triphosphatase

Introduction

Diarrheal disease is a leading cause of mortality and morbidity. Especially among children in developing countries, resulting in a major impact on health care. The World Health Organization (WHO) induced a diarrheal disease control program to study traditional medicine practices and other related aspects, together with the evaluation of health education and prevention approaches [1] Diarrhea is a disorder characterized by discharge of semisolid or watery fecal matter from the bowel three and more times in a day. Diarrhea occurs when secretory processes exceed the absorptive capacity of

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the gastrointestinal tract through four identifiable mechanisms: (1) increased secretion from mucosal; (2) decreased absorption (ions and/or solutes and water); (3) altered motility; and (4) increased permeability [2]. Plants have a valuable source of natural products for maintaining human health for many years. The use of herbal drugs in the treatment of diarrhea is a common practice medicinal plants would be the best source from which to develop a variety of medications [3].

Streblus asper Lour. is a small tree belongs to family Moraceae found in tropical countries. It is locally known as “Shaora” in different parts of Bangladesh. *S. asper* traditionally used the medicinal plant in Asian countries including Bangladesh, India, Sri Lanka, Malaysia, Philippines, Vietnam, Cambodia and Thailand [4]. According to Indian medicinal plant data *S. asper* known as “Mittli mara”. In the Philippines, it is commonly known as “bog-talay”, and “Kalyos”. In Cambodia, it is known as Snay. Several rural communes in Cambodia were named after the tree such as and Krang Snay (Hill of Snay) of Kampot Province. In Malaysia, it is known as “kesinai” [5]. Several remarkable pharmacological application of this plant have reported. *S. asper* extracts traditionally used in wide range of specific ailments including diarrhea and dysentery. The marma tribes in Bangladesh used the roots juice of *S. asper* to treat irregular menstruation and promote to delay menstruation [6]. Various parts of the *S. asper* plant used for different purposes. The bark extract used to relieve toothache and gingivitis; the branch is used as a toothbrush for strengthening teeth and gums; and the leaves exhibit insecticidal activity toward mosquito larvae, antibacterial action, and inhibitory effect on oral and dental diseases [7]. The plant roots applied to ulcers, sinuses and locally as an antidote to snake bite, and the milky juice has used as an antiseptic and astringent [8].

The literature mentions that *S. asper* is a rich of amyirin acetate, lupeol acetate [6], β -sitosterol, a-amyirin lupeol, diol, strebluside and mansonin already isolated. A pregnane glycoside has also been isolated [9], Tetraiacontan-3-one, n-Triacontane [10], β -sitosterol, stigmasterol, botulin and oleanolic acid [11], threo-streblusignan [12], magnolol [13], glycosmistic acid [14], 9-b-xylopyranosyl-isolariciresinol; 5-methoxy-9-b-xylopyranosyl-isolariciresinol; 9-b-xylopyranosyl-lyoniresinol; erythro-4-hydroxyphenylpropane-7,8-diol; threo-4-hydroxyphenylpropane-7,8-diol; 1,2-di-O- b-D-glucopyranosyl-4-allylbenzene [15], quercetin; quercetin-3-Ob-D-glucopyranoside; quercetin-3-O-rhamnoside; 4'-methoxyisoflavone-7-O- a-L-rhamnopyranosyl(1,6)- b-D-glucopyranoside; kaempferol-3,7-O- a-L-rhamnopyranoside; isorhamnetin-3-O-glucoside [16] identified from the aerial parts. One research group reported that in vitro study of some isolates of *S. asper* actively inhibiting the secretion of HBsAg and HBeAg 66 without causing any adverse effects of liver [17]. Several bioactive constituents

have also been indicated from *S. asper* with antimicrobial [18], antioxidant [19], antihyperglycemic [20], anticancer [21], anti-inflammatory [22], and neuropharmacological activities [23]. Based on the above-said information of traditional practice about *S. asper* extract, the present study investigated the anti-diarrheal activity of the methanolic extract of *S. asper* on various rats' models.

Materials and Methods

Collection of Plant Material

The plant *S. asper* collected from the Burichong, Comilla district, Bangladesh during September 2013 in the daytime. The plant identified by the experts Bushra Khan, Principle Scientific officer of Bangladesh National Herbarium at Mirpur in Dhaka (Accession No. DACB 38720) and a voucher specimen deposited at the Pharmacy Department, East West University. The date of the investigation done by Bangladesh National Herbarium was 12th September 2013.

Preparation of Extract

The dried leaves ground separately by commercial grinder (Hammer mill) into a fine powder and about 100 g of each powered materials macerated with methanol and occasional shaking in 900 mL methanol in a beaker at $25 \pm 2^\circ\text{C}$ for 72 h and stirred every 18 h using a sterile glass rod. The whole mixtures then underwent a coarse filtration by a piece of clean and white sterilized cotton material. These filtered through Whatman 102 filter paper. The filtrates evaporated under (BC-R 201 Shanghai Biochemical Equipment Co. Ltd.) rotary evaporator. After drying, 15.7 g of dried extract (yield 15.70%) obtained from 100 g of powder. It rendered two gummy concentrates of greenish black color. This extract used for investigation. The methanolic extract of *S. asper* subjected to the investigations of anti-diarrheal activity studies.

Experimental Animals

Adult Swiss albino rats, weighing 120–150 g of both sexes collected from the Animal Research Branch of the International Center for Diarrhoeal Disease and Research, Bangladesh (ICDDR, B). The standard environmental conditions maintained for mice (temperature: $25 \pm 2^\circ\text{C}$, humidity: 55–65% and 12 h light/dark cycle). Pelleted foods of mice collected from ICDDR, B given to the rats with water. Rats acclimatized to the laboratory environment for 7 days before performing the experiments. Animals kept overnight without food before the experiments. All the experimental mice treatment followed the Ethical

Principles and Guidelines for Scientific Experiments on Animals (1995) formulated by The Swiss Academy of Medical Sciences and the Swiss Academy of Sciences. The experimental process approved by the Institutional Ethics Committee (EWU/IAEC/13.07).

Treatments

The methanolic extract of *S. asper* suspended in 0.9% saline water. Castor oil (WELL's Health Care, Spain), Magnesium sulphate, 0.9% normal saline (Opsonin Pharma Ltd., Bangladesh), charcoal meal (10% activated charcoal in 5% gum acacia), and loperamide (Square Pharmaceuticals Ltd., Bangladesh) used for anti-diarrheal activity test. Test groups of rats treated orally (p. o.) with methanolic extract of *S. asper* at the doses of 100, 200, and 400 mg/kg whereas the control group received 0.9% saline water (2 mL/kg) by the same routes. The positive control group received loperamide (5 mg/kg) was also administered the orally. All the groups received drug and samples via gavages. All other chemicals and reagents were of analytical grade and high purity.

Preliminary Phytochemical Screening

Preliminary phytochemical screening of methanolic extract of *S. asper* qualitatively tested was carried out by employing standard procedure and the presence of glycosides, flavonoids, saponins, and sterols [24].

Determination of Flavonoid Content

1 ml of extract solution (100 mg/mL) mixed with 3 ml methanol, 200 mL (10%) aluminum chloride solutions, and 200 mL (1 M) potassium acetate solutions. Then 5.6 mL of distilled water added to the mixture and incubated for 30 min at room temperature. The absorbance of the solution measured at 415 nm against blank. The total flavonoid content calculated in quercetin equivalents [25].

Acute Toxicity Study

Adult Wister albino rats arranged into four test groups and a control group (n = 10) within the weight of 150–200 g. 0.9% Saline water used for the control group (2 mL/kg). The test groups received in 500, 700, 1000, 1500, and 2000 mg/kg doses of the methanolic extract of *S. asper* respectively through the oral route. The LD₅₀ of MESA is 2000 mg/kg. After gavages, the animals kept in separate cages and allowed for food and water ad libitum. The animal's observations continued for the next 72 h for any abnormal behaviors, allergic symptoms, and mortality [26].

Castor Oil-Induced Diarrhea

The method of Awouters followed for carrying out the study [27]. Rats of both sexes fasted (without food, but water) for 18 h. The selected rats for castor oil-induced diarrheal test divided into five groups (n = 10). Animals of the positive control group received loperamide (5 mg/kg) body weight while the control group received 0.9% normal saline (2 mL/kg) orally. On the other hand, test groups received MESA at the doses of 100, 200, and 400 mg/kg body weight. After 1 h, all groups received castor oil 1 mL each orally. Then they placed in cages lined with adsorbent papers and observed for 4 h for the presence of characteristic diarrheal droppings. 100% considered as the total number of feces of the control group the activity expressed as % inhibition of diarrhea. The percent (%) inhibition of defecation measured.

Magnesium Sulphate-Induced Diarrhea

A similar procedure as for castor oil induced diarrhea maintained for magnesium sulphate-induced diarrheal model. Every experimental group administered magnesium sulphate orally (2 g/kg). After 1 h, the positive control received loperamide (5 mg/kg) as standard. The control group received 0.9% normal saline (2 mL/kg) while the tested group received 100, 200, and 400 mg/kg doses of MESA. Then placed in cages lined with adsorbent papers and observed for 4 h for the presence of characteristic diarrheal droppings. 100% considered as the total number of feces of the control group the activity expressed as % inhibition of diarrhea. The percent (%) inhibition of defecation measured [28].

Gastrointestinal Transit Test

The method adopted for the determination of the effect of MESA on gastrointestinal transit in the rats [29]. The experimental animals fasted (without food, but water) for 18 h. The selected rats for castor oil-induced diarrheal test divided into five groups (n = 10). After 30 min, all the animals again administered orally with 1 mL of the charcoal meal (10% charcoal suspension in 5% gum acacia). After 30 min post administration of the charcoal meal, all animals sacrificed and the distance covered by the charcoal meal in the intestine, from the pylorus to the caecum, measured and expressed as the percentage of distance moved.

Table 1 The antidiarrheal effect of MESA on castor oil-induced diarrhea models in rats

Treatment	Dose (mg/kg)	Total number of feces	% of inhibition	Total number of diarrheal feces	% of inhibition
Control	2 mL/kg	19.80 ± 1.62	–	12.60 ± 1.16	–
Loperamide	5	8.80 ± 0.86***	55.55	5.20 ± 0.73***	58.73
MESA	100	15.20 ± 0.58**	23.23	9.20 ± 0.80*	26.98
MESA	200	12.60 ± 0.67***	37.37	6.80 ± 0.86***	40.03
MESA	400	10.20 ± 0.58***	48.48	5.80 ± 0.58***	53.96

Each value is presented as the mean ± SEM (n = 10)

MESA = methanolic extract of *Streblus asper* leaves

****P* < 0.001 compared with the control group (Dunnett's Test)

***P* < 0.01 compared with the control group (Dunnett's Test)

**P* < 0.05 compared with the control group (Dunnett's Test)

Table 2 The antidiarrheal effect of MESA on magnesium sulphate-induced diarrhea models in rats

Treatment	Dose (mg/kg)	Total number of feces	% of inhibition	Total number of diarrheal feces	% of inhibition
Control	2 mL/kg	17.20 ± 1.35	–	15.60 ± 1.16	–
Loperamide	5	7.80 ± 0.37***	54.65	7.20 ± 0.86***	53.84
MESA	100	13.60 ± 0.92*	20.93	12.60 ± 0.24*	19.20
MESA	200	11.40 ± 0.74***	33.72	10.80 ± 0.58***	30.76
MESA	400	8.60 ± 0.81***	50	8.40 ± 0.51***	46.15

Each value is presented as the mean ± SEM (n = 10)

MESA = methanolic extract of *Streblus asper* leaves

****P* < 0.001 compared with the control group (Dunnett's Test)

***P* < 0.01 compared with the control group (Dunnett's Test)

**P* < 0.05 compared with the control group (Dunnett's Test)

Table 3 The anti-motility effect of MESA on gastrointestinal transit test in rats

Treatment	Dose (mg/kg)	Total length of intestine (cm)	Distance travel by charcoal (cm)	% of inhibition
Control	2 mL/kg	106.80 ± 1.24	104.20 ± 1.74	–
Loperamide	5	104.40 ± 1.12	49.80 ± 1.24***	52.21
MESA	100	105.80 ± 1.59	86.80 ± 1.82***	16.70
MESA	200	108.40 ± 1.07	62.80 ± 1.15***	39.73
MESA	400	105.00 ± 1.67	55.20 ± 0.66***	47.02

Each value is presented as the mean ± SEM (n = 10)

MESA = methanolic extract of *Streblus asper* leaves

****P* < 0.001 compared with the control group (Dunnett's Test)

***P* < 0.01 compared with the control group (Dunnett's Test)

**P* < 0.05 compared with the control group (Dunnett's Test)

Effect of MESA and Loperamide in Castor oil-induced test

Effect of MESA and Loperamide in Castor oil-induced test

Fig. 1 The antidiarrheal effect of MESA on castor oil-induced diarrhea (total number of feces and total number of diarrheal feces) of models in rats

Results and Discussion

The preliminary phytochemical screening of MESA revealed the presence of glycosides, flavonoids, saponins, and sterols. Total flavonoids calculated as 73.607 ± 1.61 mg_{QE}/g_{extract}, respectively. In case of castor-oil induced diarrheal model, MESA, at the doses of 100, 200, and 400 mg/kg exerted statistically significantly ($P < 0.001$) decreased and dose-dependent inhibition of the total number of diarrheal feces compared with the control

groups (Table 1). In magnesium sulphate-induced diarrheal model, MESA, at the doses of 100, 200, and 400 mg/kg showed statistically significant ($P < 0.001$) and dose-dependent prevention the total number of diarrheal feces compared with the control groups (Table 2). The distances traveled by charcoal meal through the small intestine results, statistically significant ($P < 0.001$) and the effects of MESA at the doses of 100, 200, and 400 mg/kg in a dose-dependent manner while compared with control group and the ($P < 0.001$) (Table 3).

Many plants available in Bangladesh have found to be effective against diarrhea and dysentery and are used by local people and in traditional medicine. It has shown that preceding treatment with plant extracts had a protective effect on the intestinal tract. In our present study, the methanolic extract of *S. asper* leaves evaluated for its anti-diarrheal potential against castor oil induced diarrhea and Magnesium sulphate-induced diarrhea model in rats. In respect to demonstrating the probable mechanisms, the anti-motility effects was also tested using gastrointestinal transit test in rats.

Preliminary phytochemical screening of the plant extract in the present study revealed the presence of glycosides, flavonoids, saponins, and sterols which have all been reported to possess activity and therefore explain its anti-diarrheal action [30]. The anti-diarrheal activity of flavonoids has ascribed to their ability to inhibit intestinal motility and hydro-electrolytic secretion. According to the in vitro and in vivo experiments, it elucidated that flavonoids able to inhibit the secretory response in the intestine. It is thus apparent that the anti-diarrheal effect of aqueous extract of leaves of *S. asper* may be due to the presence of flavonoids. Hence, the result of this investigation revealed that the aqueous extract of *S. asper* leaves contains pharmacologically active substances with anti-diarrheal properties.

Castor oil derived from the seeds of *Ricinus communis*, a flowering plant belonging to the family Euphorbiaceae. It is well known that it contains ricinoleic acid, an active compound of castor oil formed in the upper small intestine. Due to its polar effects, it poorly absorbed and induced in the mucosal permeability, electrolyte transport, intestinal peristalsis, leading to hypersecretory response and diarrhea [31]. The ricinoleic acid readily forms ricinoleate salts with sodium and potassium in the lumen of the intestine. Ricinoleate has several actions that could account for its anti-absorptive effect on the mucosa. It inhibits the enzyme Na^+/K^+ -ATPase and increases the permeability of the intestinal epithelium, producing a cytotoxic effect on isolated enterocytes. Ricinoleate acts as a calcium ionophore, increasing the influx of extracellular calcium which activates the calmodulin-dependent secretory mechanism. Secretory diarrhea induced by castor oil is associated with

an activation of Cl^- channels, causing Cl^- efflux from the cell, the efflux of Cl^- results in massive secretion of water into the intestinal lumen and profuse watery diarrhea. In our present study, castor oil administration resulted in copious watery diarrhea and intestinal fluid accumulation in rats.

Loperamide, the standard drug, generally produces rapid and sustained inhibition of peristaltic reflex through depression of longitudinal and circular muscle activity. It is well known to reduce the daily fecal volume and decreases

intestinal fluid and electrolyte loss. The anti-diarrheal activity of the plant extract was comparable to the standard drug, loperamide, which at present is one of the most efficacious and widely employed anti-diarrheal drugs. In our investigation, loperamide proved the claimed that causing by effectively antagonizes diarrheal activity which induced by castor oil and magnesium sulphate.

In the castor-oil induced diarrhea model in rats, the MESA showed dose-dependent manner and significant ($P < 0.001$) reduced in the number of feces and the number

Fig. 2 The antidiarrheal effect of MESA on magnesium sulphate-induced diarrhoea (total number of feces and total number of diarrheal feces) of models in rats

Effect of MESA and Loperamide in Magnesium sulphate-induce test

Effect of MESA and Loperamide in Magnesium sulphate-induced test

Effect of MESA and Loperamide in Gastrointestinal transit test

Fig. 3 The anti-motility effect of MESA on gastrointestinal transit test of distance travel by charcoal (cm) in rats

of diarrheal feces with the percentage of inhibition (Fig. 1). Both the effects being less potent as compared to the standard drug loperamide. Magnesium sulphate produces diarrhea by the osmotic properties, preventing reabsorption of water ions, leading to increment of the volume of the intestinal content. This salt also promotes the liberation of cholecystokinin from the duodenal mucosa, which increases the secretions. Moreover, it has a motor effect on the small intestine as well as also prevents reabsorption of sodium chloride and water [32]. *S. asper* exhibited significant anti-diarrheal activity against magnesium sulphate-induced diarrhea (Fig. 2). It significantly ($P < 0.001$) increased the percentage of inhibition and also notably reduced the number of diarrheal feces at all the doses in the study compared to the control in a dose-dependent manner. In respect of gastrointestinal transit test, the extract MESA increases the reabsorption of water by significantly ($P < 0.001$) decrease intestinal transit of charcoal meal thus possesses anti-motility effect in a dose-dependent manner, which in turn allows the absorption of water and electrolytes, thus leading to an anti-diarrheal activity (Fig. 3).

Conclusions

Our present findings validate the traditional use of *S. asper* leaves as an anti-diarrheal agent. The study also supports the traditional claim, further studies needed to identify the chemical constituents that are responsible for the anti-diarrheal effect. The anti-diarrheal activity may confer with the glycosides, flavonoids, and saponins of the plant which

may act by stimulating the activity of Na^+/K^+ -ATPase. The isolation and characterization of the bioactive agent as well as the exact mechanism of actions will be the focus of further studies.

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Compliance with Ethical Standards

Conflict of interest The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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