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ANTIDIARRHEAL ACTIVITY OF ETHANOLIC EXTRACT OF STREBLUS ASPER LEAVES

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ABSTRACT
Diarrhea is one of the most common causes for thousands of deaths in every year in developing countries and mainly affects children and infants. It has been reported that the leaf of *Streblus asper* is used as an antidiarrheal agent and traditionally used for anti-diarrheal effects like dysentery and diarrhea. The present study aims to prove the anti-diarrheal activities of ethanolic extract of leaves of Streblus asper in animal models. The ethanolic leaf extract of *Streblus asper* was evaluated for its activity against castor oil-induced diarrhea, enteropooling, and gastrointestinal motility in swiss albino rats. In castor oil-induced diarrhea model, ethanolic extract of *Streblus asper* at the doses of 200 and 400 mg/kg produced statistically significant. The recent study indicates that EESA possesses anti-diarrheal property. The findings represent a rational explanation for its use in traditional medicine for the management of diarrhea.

KEYWORD: *Streblus asper*, castor oil, anti-diarrheal activity.

INTRODUCTION
Streblus asper (family-Moraceae) is a small tree which is indigenous to tropical countries such as India, Srilanka, Philippines and Thailand. In India it is distributed in the Himalayas from Himachal Pradesh to West Bengal and in hills and plains of Assam and Tripura. It is also found in the drier parts of India. Various parts of this plant are used in Ayurveda and other folk medicine for the treatment of different ailments such as Filariasis, Leprosy, Tooth ache, Diarrhea, Dysentery, and Cancer. Root is used as an application to the unhealthy ulcers and sinuses, and as antidote to snakebite, in epilepsy and obesity. Stem is used in toothache, Stem bark is given in fever, dysentery, diarrhea, stomach ache, urinary complaints, piles, edema and wounds. Decoction is effective against lymphodema, chylurea, and other effects of Filariasis. Leaves are used in eye complaints; milky juice is used as antiseptic, astringent, applied to chapped hand and sore feet, in pneumonia and swells of cheeks. The branch of *Streblus asper* has been used as tooth brush for strengthening teeth and gums. *Streblus asper* extracts thus has the potential for being used as a natural product for controlling dental carries. *Streblus asper* is a rigid shrub or gnarled evergreen tree; bark light grey and a rich source of cardiac glycosides. More than 20 cardiac glycosides from the root bark of *Streblus asper* have been reported and were able to structurally characterize about 15 such compounds, kamloside, asperosides, trebloside, glucokamloside, glucostrebloside, Strebloside, and β –sitosterol were reported. The literature mentions that S. asper is a rich of amyrin acetate, lupeol actate**,** a-amyrin lupeol, diol, strebloside and mansonin already isolated. A pregnane glycoside has also been isolated,

MATERIALS AND METHODS
Collection of Plant Material
The leaves of Streblus asper were collected in locally from local area of Azamgarh, Uttar Pradesh, India in the month of September and were authenticated by Professor Dr. N. K. Dubey Taxonomist, centre of Advanced study in botany, institute of science, Banaras Hindu University, Varanasi (India) as *Streblus asper* (Moraceae) leaves. A voucher specimen has been kept in the herbarium (voucher specimen no. mora. 2019/1). Department of botany. The leaves were dried under shade and coarsely powdered.

Drugs and chemicals
Caster oil (Dabur India Ltd), 0.9% sodium chloride solution (normal saline), charcoal meal (10% activated charcoal in 5% gum acacia), and loperamide (Bafna Parmaceuticals, Ltd, India), ethanol (Lab Chemicals, India) were used for antidiarrheal activity test.

Preparation and Extraction of plant material
The leaves of *streblus asper* were cleaned with distilled water and air-dried under shade at room temperature. The dried leaves were coarsely powdered by pulverization in electric grinder. Then, 500gm of

powdered leaves was weighted using electronic balance and extracted by Soxhlet apparatus using ethanol for three consecutive days at room temperature and then filtered through a cotton plug followed by Whiteman Filter Paper. The solvent was evaporated under vacuum at room temperature to yield semisolid extract. Then the ethanolic extract of *streblus asper* leaves was collected in Eppendorf tube and preserved in a refrigerator at 4^0C for further use.

Experimental Animals

Swiss albino rats (95-100g) were used. The rats were bred at the animal house of the department of pharmacology under standard conditions. They were housed in plastic cages with softwood shavings and chips as bedding, in a room with 12: 12 hrs. dark-to-light cycle, at room temperature and 55% humidity, and clean water and pelletized food *ad libitum.* All rats were acclimatized to the working lab. Environment one week prior to the experiment.

Preliminary Phytochemical Screening

Qualitative phytochemical screening tests were carried out to determine the presence of alkaloids, tannins, glycosides, flavonoids, saponins, phenols, steroids and anthraquinones in the ethanolic extract of *streblus asper*.

Acute Oral Toxicity test

Acute oral toxicity test was conducted according to the OECD guideline on five swiss albino rat at a single oral limit dose of 2000 mg/kg body weight. Five Swiss albino rat were randomly selected for the test. One animal was first dosed at the limit test dose and survived in the 24-hour follow-up. Then the other four additional animals were sequentially dosed at 2000mg/kg so that a total of five animals were tested. The animals were observed individually for signs of toxicity at least once in the first 30 minutes after dosing. periodically during the first 24 hours, and daily for an additional 13 days, for a total of 14 days.

Caster oil- Induced Diarrhea in rats

Rats of both sexes (95-100g) were fasted for 18 hours and divided into five groups with six animals in each group. The Group I received normal saline orally as control group and Group II received loperamide (5mg/kg) as standard group. Groups III and IV received EESA (200 and 400 mg/kg wt. i. p resp.). After one hour, all the animals received castor oil 1ml. each orally. The animals were kept in separate metabolic cages. The severity of diarrhea was assessed for 4 hours for the presence of characteristic diarrheal dry and wet droppings 100% was considered as the total number of feces of control group. The activity was expressed as % inhibition of diarrhea. The percent (%) inhibition of defecation was measured using the following formula.

Percent (%) inhibition of defecation = [(A-B/A)]X100

Where A is mean number of defecation time caused by caster oil and B is mean number of defecation time caused by drug or extract.

Caster oil- Induced Enteropooling

Castor oil -induced enteropooling test helps to determine the prevention of fluid accumulation ability of extract. Rats of both sexes (95-100) were fasted for 18 hours. The selected rats for this test were divided into four groups (n= 5), Group I (controlled group) was given normal saline orally while group II (standard group) received loperamide (5ml/kg). The rest of the groups III and group IV received EESA (200 and 400 mg/kg b. wt., i.p resp.). After 1 h, all groups received castor oil, 1ml orally per animal. Two hours later, all rats were sacrificed and the small intestine form the pylorus to the caecum was isolated. The intestinal contents were collected into a graduated tube and their volume was measured.

Gastrointestinal Motility Test

This test was done according to the method of mascolo et al. and Rahman et al. for this test, selected rats were divided into four groups of five rats in each. At first, 1 ml. caster oil was given orally in every rat of each group to produce diarrhea. After 1 h, Group I (control group) received saline (2 ml/kg) orally. Group II received standard drug (loperamide 5 mg/kg b, wt., i.p) and groups III-IV received EESA (200 and 400 mg/kg b. wt. i.p resp). After 1h, all animals received 1 ml of charcoal meal (10% charcoal suspension in 5% gum acacia) orally. One hour after the charcoal meal administration, all animals were sacrificed and the distance covered by the charcoal meal in the intestine, form the pylorus to the caecum, was measured and expressed as percentage of distance moved.

Table 1: Effect of EESA leaves on castor oil-induced diarrhea in rats.

S.NO	Group	Treatment	Dose	Total no. of feces	% inhibition of defecation	Total no. of diarrheal feces	% inhibition of diarrhea
I	Control	Vehicle	------	17.56±1.12**	----------	12.00±1.18**	--------
II	Standard	Loperamide	5mg/kg	7.23±0.51**	58.82	5.45±0.39**	54.58
III	Test-I	EESAL	200mg/kg	9.85±0.86**	43.90	6.15±0.91**	48.75
IV	Test-II	EESAL	400mg/kg	7.85±1.31**	55.29	5.59±0.50**	53.41

Values were expressed as mean ± SEM. (n=5). $*p < 0.05$, $**p < 0.01$ when compared with control group (ANOVA followed by Dunnett's t-test).

Table 2: Effect of EESA leaves on castor oil induced enteropooling in rats.

S.NO	Group	Treatment	Dose	Wt. of intestinal content (g)	Vol. of intestinal content (ml)	% inhibition of intestinal content
I	Group	Castor oil + vehicle	----------	3.45±0.05**	2.79±0.28**	---------
II	Standard	Caster oil + Loperamide	5mg/kg (i.p)	1.80±0.43**	1.49±0.47**	46.59
III	Test-I	Castor oil + EESAL	200mg/kg (i.p)	2.25 ±0.09*	2.19±0.02*	21.50
IV	Test-II	Caster 0il + EESAL	400mg/kg (i.p)	1.90 ±0.03**	1.67±0.10**	40.14

Values were expressed as mean ± SEM. (n=5). $*p < 0.05$, $**p < 0.01$ when compared with control group (ANOVA followed by Dunnett's t-test).

Table 3: Effect of EESA leaves on small intestinal transition in rats.

S. N0	Group	Treatment	Dose	Total length of intestine (cm)	Distance traveled by marker (cm)	% Inhibition
I	Control	Castor oil + saline	--------	108.10 ± 1.70	105.45 ±1.60	--------
II	Standard	Caster oil + Loperamide	5mg/kg (i.p)	106.79 ± 1.25	50.02 ±1.15***	52.56
III	Test-I	Caster oil +EESAL	200mg/kg (i.p)	107.90 ± 1.05***	60.79 ±1.80***	42.32
IV	Test- II	Caster oil + EESAL	400mg/kg (i.p)	107.01 ± 1.67***	53.15 ±0.66***	49.59

Values were expressed as mean ± SEM. (n=5). $*p < 0.05$, $**p < 0.01$ when compared with control group (ANOVA followed by Dunnett's t-test).

RESULT

Castor Oil-Induced Diarrhea. In case of castor oil induced diarrheal test, the ethanolic extract of *Streblus asper* showed a marked antidiarrheal effect in the rats (Table 1). In both doses, 200 mg/kg and 400 mg/kg, extract produced significant ($p < 0.01$) defecation. The leaves extract doses of 200 mg/kg and 400 mg/kg decrease the total amount of wet feces produced upon administration of castor oil (6.15 ± 0.91 and 5.59 ± 0.50 g) at doses 200 mg/kg and 400 mg/kg compared to the control group (5.45 ± 0.39 g) at the dose of 5 mg/kg.

Castor Oil-Induced Enteropooling. In this test, the ethanolic extract of EESA leave at both of the 200 and 400 mg/kg doses produced significant and dose dependent reduction in intestinal weight and volume (Table 2). The leaves extract decreased intestinal volume by 21.50% and 40.14% at doses 200 and 400 mg/kg, respectively. The standard drug loperamide (5 mg/kg) also significantly inhibited ($p < 0.01$) the intestinal fluid accumulation (46.59%).

Gastrointestinal Motility Test. The ethanolic extract of Streblus asper. Lessened gastrointestinal distance (105.45 ± 1.60 cm to 53.15 ± 0.66 cm) traveled by the charcoal meal in the rats compared with the control group. Loperamide (5 mg/kg) produced a marked (52.56%) decrease in the propulsion of charcoal meal through gastrointestinal tract (Table 3).

DISCUSSION

Traditionally, people use plant(s) or plant-derived preparations considering them to be efficacious against diarrheal disorders without any scientific basis. These experimental models were therefore employed to antidiarrheal efficacy of ethanolic extract of *Streblus asper* leaves in the current study. Diarrhea can be described as the abnormally frequent defecation of feces of low consistency due to a disturbance in the transport of water and electrolytes in the intestines. The study of causation of diarrhea due to (i) increased electrolytes secretion, (ii) increased luminal osmolarity, (iii) deranged intestinal motility causing a decreased transit time, and (iv) decreased electrolytes absorption may be

responsible for pathophysiology. There are several mechanisms proposed to explain the diarrheal effect of castor oil including inhibition of intestinal Na+ K+ ATPase activity, consequently reducing normal fluid absorption, activation of adenylate cyclase or mucosal cAMP-mediated active secretion, and stimulation of prostaglandin formation and platelet activating factor. Usually castor oil is metabolized into ricinoleic acid in the gut, which causes irritation and inflammation in the intestinal mucosa, resulting in the release of inflammatory mediators (e.g., prostaglandins and histamine). The released prostaglandins initiate vasodilatation, smooth muscle contraction, and mucus secretion in the small intestines. In experimental animals as well as in human beings, prostaglandins of the E series are considered to be good diarrheagenic agents. Our study showed that the overall antidiarrheal study reveals the dose dependent activity. Moreover, our results directly demonstrate an inhibition of castor oil induced enteropooling with reduction of the weight and volume of intraluminal contents respectively. These results suggest that leaves of *Streblus asper* contain antidiarrheal components. Also, from these results, it can be predicted that reduction of water and electrolytes secretion into the small intestine may enhance electrolyte absorption from the intestinal lumen consistent with inhibition of hypersecretion. In gastrointestinal motility, EESA suppressed the propulsive movement or transit of charcoal meal through the gastrointestinal tract which demonstrates that the leaves extract may be able to reduce the frequency of stool in diarrheal conditions. It was reported that flavonoids and polyphenols were responsible for the antidiarrheal activity properties. However, previous studies also have shown that flavonoids have ability to inhibit intestinal motility and water and electrolytes secretion. Thereby, flavonoids as the inhibitors of prostaglandins biosynthesis are considered to delay castor oil-induced diarrhea. So, the antidiarrheal activity of ethanolic extract of the leaves of *Streblus asper* could therefore be due to the presence of flavonoids and phenols. 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 antidiarrheal action.

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