Evaluation of Analgesic Activity of *Sterculia villosa* Roxb. Bark in Swiss-Albino Mice

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ABSTRACT: The methanolic crude extract and different fractions of *Sterculia villosa* bark were investigated for their possible analgesic activity in experimental animal models. Analgesic activity was evaluated using acetic acid induced writhing inhibition and radiant heat tail-flick methods in swiss albino mice. In peripheral method of anti-nociception, the methanolic crude extract (400 mg/kg) and petroleum ether fraction (400 mg/kg) showed significant analgesic activity having 50.76% and 51.72% ($P < 0.001$) of writhing inhibition, respectively compared to standard aspirin (71.03% inhibition). In the radiant heat tail-flick method of central anti-nociception, the methanolic crude extract (400 mg/kg) and petroleum ether fraction (400 mg/kg) of *S. villosa* showed significant analgesic activity having 71.25% ($P < 0.001$) and 66.77% ($P < 0.001$) elongation of reaction time, respectively at 30 minutes after administration of sample compared to the standard morphine (144.4% elongation). The findings of the studies demonstrated analgesic activity of the bark of *S. villosa* which could be the therapeutic option against pain.

Key words: *Sterculia villosa*, analgesic activity, writhing inhibition, % time elongation.

INTRODUCTION

Sterculia villosa Roxb. (Bengali name: Udal, Family: Sterculiaceae) is a small to large, often spreading deciduous tree having large long-stalked deeply lobed leaves and yellow flowers. It is found all over the tropics and subtropics including Bangladesh.[1] Traditionally the plant is used as an agent in diuretic, cooling and aphrodisiac properties[2] and also used by Indians for traditional remedy of inflamation.[3] Sherbet, prepared from the petiole of the plant along with water and sugar is given in urinary problems and rheumatism. The bark and the petiole are used as a remedy in seminal weakness. White exudates of the tree are used for throat infection. Root infusion is taken as food adjunct while the whole plant extract is useful for skin diseases.[4] The plant also has anthelmintic,[5] anti-inflammatory, antidiabetic,[6] antimicrobial, membrane stabilization and antithrombotic activity.[7] Some chemical constituents like flavonoids, chrysoeriol, diosmetin-7-O-β-D-glucoside and hrysoeriol-7-O-β-D-glucoside were isolated from *S. villosa*.[8] As the plant contains flavonoids which are responsible for analgesic effect and no scientific data has been reported on its analgesic activity, therefore, our present work was undertaken to prove the analgesic potential of the methanolic crude extract and its different partitioning fractions of *S. villosa* bark using acetic acid induced writhing inhibition and radiant heat tail flick methods respectively in experimental albino mice for the first time.

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MATERIALS AND METHODS

Collection and preparation of plant material. Plant sample of *Sterculia villosa* was collected from Rangamati in September, 2011. The bark of *S. villosa* were identified and authenticated by taxonomic experts in the Department of Botany, University of Dhaka and a voucher specimen (accession No: DUSH-6905**,** Call No. 01) was deposited there for future reference. Bark of *S. villosa* was washed properly, cut into small pieces and then air dried for several days. The pieces were then oven dried for 24 hours at considerably low temperature and ground into coarse powder.

Extraction of the plant material. About 600 g of the powdered material was taken in a clean, round bottomed flask (5 liters) and soaked in 2.5 liter of methanol. The container with its content was sealed by foil and kept for a period of 7 days accompanying occasional shaking and stirring. The whole mixtures were then filtered through a fresh cotton plug and finally with a Whatman No.1 filter paper. The volume of the filtrate was then reduced using a Büchi rotary evaporator at low temperature and reduced pressure. The weight of the crude extract was 19 g.

Solvent-solvent partitioning. Solvent-solvent partitioning was done using the protocol designed by Kupchan and modified by Van Wagenen.[9] The crude extract (5 g) was dissolved in 10% aqueous methanol. It was then extracted with petroleum ether (PE), carbon tetrachloride (CTC), finally with ethyl acetate (EA). All three fractions were evaporated to dryness and were used for the study.

Drugs and reagents. Methanol, carbon tetra-chloride, petroleum ether, ethyl acetate, acetic acid, tween 80 (Sigma chemicals, USA), aspirin, morphine (Gonoshasthaya Pharmaceuticals Ltd.), normal saline (Opsonin Pharma) were collected from the mentioned sources. All the chemicals and solvents were of analytical grade.

Experimental animals. Swiss albino mice (25-30 g) of either sex, aged 4-5 weeks were obtained from the animal house of Jahangirnagar University. They were housed in standard polypropylene cages and kept under controlled room temperature (24 ± 2ºC; relative humidity 60-70%) in a 12 hour light-dark cycle in the animal house of Institution of Nutrition and Food Science, University of Dhaka and fed ICDDR,B formulated rodent food and water *ad libitum*. As these animals are very sensitive to environmental changes, they were kept before the test for 3-4 days in the environment where the experiment would take place.

Analgesic activity study.

Peripheral analgesic activity study. The peripheral analgesic activity of the crude extracts and its different fractions of *S. villosa* were determined by the acetic acid-induced writhing inhibition method according to procedure described by Koster and Turner.[10,11] Thirty six swiss albino mice were divided into six groups consisting of six animals each. Each group received particular treatment as shown in Table 1. After experiment, the responses of the extract and aspirin treated groups were compared with those of the animals in the control group. Percentage inhibition of writhing in comparison to control group was taken as an index of analgesia and was calculated using the following formula:

$$\text{Inhibition (\%)} = [(Wc-Wt) \times 100]/Wc$$

Where Wc is the average number of writhing reflex in the control group and Wt is the average number of writhing reflex in the test group.

Central analgesic activity study. Central anti-nociceptive activity for both crude extracts of *S. villosa* and its different fractions was determined by radiant heat tail-flick method.[12] In this experiment, test samples and normal saline were orally fed to the test mice and control group, respectively at zero hour whereas the positive control received morphine subcutaneously. Thirty minutes interval was given to ensure proper absorption of the administered substances. Then the mice were kept into cages leaving the proximal third of their tail exposed over a holder having a thin wire. Tail flicking time was measured by analgesiometer (Medicraft, India). In order to make the wire hot, current was allowed to pass through the wire at a low intensity (3 amperes). The animals flick the tail aside or try to escape. The time required to withdraw the tail was recorded.

Percent time elongation due to the effect of various fractions and standards were calculated using the following formula:

Percent elongate of reaction time = (Average reaction time of test group - Average reaction time of control group)/ Average reaction time of control group.

Statistical analysis. The data were statistically analyzed using one-way ANOVA for individual comparison of group with control. All values are expressed as mean ± SEM. $p < 0.05$ was considered as significant.

RESULTS AND DISCUSSION

In peripheral analgesic activity study methanolic crude extract and its different fractions of bark of *S. villosa* decreased the number of acetic acid induced abnormal constrictions (writhings) in mice, when compared to control ($P < 0.001$) as mentioned in Table 1. Statistical evaluation of the data confirmed that the crude methanolic extract, petroleum ether and ethyl acetate soluble fraction at a dose of 400 mg/kg had shown significant analgesic activity. They produced an inhibition of 50.76, 51.72 and 49.66%, respectively. Carbon tetrachloride fraction also had promising analgesic activity.

Table 1. Peripheral anti-nociceptive activity of methanolic crude extract and its different fractions of *S. villosa* bark.

Animal group	Writhing Count						Number of writhing (Mean ± SEM)	Writhing (%)	% of Inhibition of writhing
	M_1	M_2	M_3	M_4	M_5	M_6			
NC	25	21	24	27	25	23	24.17 ± 0.83	100	-
PC	8	6	7	8	7	6	7.00 ± 0.36	28.97	71.03
MCE_{400}	14	13	12	11	11	12	11.83 ± 0.75	49.24	50.76***
PEF_{400}	13	11	11	13	12	10	11.66 ± 0.49	48.28	51.72***
CTF_{400}	13	14	16	13	16	15	14.50 ± 0.56	60.00	40.00***
EAF_{400}	13	12	11	12	13	12	12.17 ± 0.30	50.34	49.66***

Values expressed as Mean± SEM (n = 6). ***P <. 001, **P <.01, *P <.05 significant compared to negative control. NC = negative control, PC=positive control, MCE = Methanolic crude extract, PEF = Petroleum ether fraction, CTF = Carbon tetrachloride fraction, EAF = Ethyl acetate fraction.

The central anti-nociceptive effects of methanolic crude extract and its different fractions of the bark of *S.villosa* in radiant heat tail flick method are presented in Table 2. In this test, the extract and fractions effectively elongate the reaction time. The methanolic crude extract (400 mg/kg), petroleum ether fraction (400 mg/kg) and ethyl acetate fraction (400 mg/kg) showed significant analgesic activity having 71.25% ($P < 0.001$), 66.77% ($P < 0.001$)and 63.57% ($P < 0.001$) elongation of reaction time, respectively at 30 minutes after administration of sample compared to that exhibited by standard morphine (144.4% elongation), whereas carbon tetrachloride fraction showed the least elongation of reaction time (9.58%). At 60 minutes, both the methanolic crude extract and petroleum ether fraction (400 mg/kg) increased the tail flick time by 34.69% ($P < 0.001$) and 32.65% ($P < 0.001$), respectively. The central anti-nociceptive property decreases with the passage of time.

Table 3. Central analgesic activity of methanolic crude extract and its different fractions of *S. villosa* bark.

Group	Dose (mg/kg)	Reaction time (sec)	
		(% elongation) (30 min)	(% elongation) 60 min
Control	-	6.26 ± 0.61	5.88 ± 0.46
Morphine	2	(144.4) 15.3 ± 0.50***	(119.04) 12.68 ± 1.05***
MCE	400	(71.25) 10.72 ± 0.29***	(34.69) 7.92 ± 0.59**
CTF	400	(9.58) 6.86 ± 0.18	(6.9) 6.26 ± 0.45**
PEF	400	(66.77) 10.44 ± 0.55***	(32.65) 7.8 ± 0.58*
EAF	400	(63.57) 10.24 ± 0.49***	(11.22) 6.54 ± 0.43

Each value represents the mean ± SEM (n = 5). ***P < 0.001, **P < 0.01, *P < 0.05 compared with control. (One-Way ANOVA followed by Dunnett's test). Standard = Morphine, MCE = Methanolic crude extract (400 mg/kg), CTF = Carbon tetrachloride fraction (400 mg/kg), PEF = Petroleum ether fraction (400 mg/kg), EAF = Ethyl acetate fraction (400 mg/kg).

Both in the acetic acid-induced writhing and tail flick methods, methanolic crude extract and its fractions (400 mg/kg) and standard drug showed

significant results as compared to control group. As the extracts appeared to be active in both animal models of nociception, it may possess peripherally and centrally acting compounds for its anti-nociceptive action.

The anti-inflammatory, analgesic and antipyretic activities of many plants have been attributed to their saponin,[13] terpenoids, flavonoids and steroids contents.[14] Moreover, the flavoloids, triterpenoids are known to inhibit prostaglandin systhesis.[15] The phytochemical investigation revealed that *Sterculia villosa* possessed the maximum phytochemicals like alkaloids, glycosides, steroids, flavonoids, tannin and phenolic compounds which possibly are responsible for the observed analgesic effect.

CONCLUSION

In conclusion, for the first time we have reported the anti-nociceptive properties of *Sterculia villosa* bark by two distinct methods *i.e.* peripheral anti-nociception by acetic acid induced writhing and central anti-nociception by radiant heat tail flick method. Based on the results of the present studies, it can be concluded that the methanolic crude extract and other fractions of the barks of *S. villosa* have promising peripheral analgesic activity. Again, the extracts also possess promising central anti-nociceptive potential as the reaction time increased significantly for the test samples and standard drug in comparison with control. Therefore, the above-mentioned plant can be a better alternative against pain to synthetic toxic drugs. However, further detail studies are essential to find out the underlying mechanisms and to isolate the active compounds responsible for these pharmacological properties.

Declaration of Interest. The authors declare no conflict of interest.

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