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Original Research Article

Cytotoxicity, analgesic and anthelmintic studies of the different plant parts of *Saraca indica* Linn. (Family: Caesalpiniaceae)

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ABSTRACT

Background: *Saraca indica* or *Saraca asoca* belonging to the family Caesalpiniaceae. It is popularly known as Ashoka, is the most ancient tree of Bangladesh. This versatile plant has anti-cancer, anti-menorrhagic, anti-oxytocic, anti-microbial activity and has extended uses in ayurveda, unani and homeopathy. The present study attempts to investigate the cytotoxic and analgesic activities of the methanol extract of bark and the anthelmintic activity of the leaf extract of *Saraca indica*.
Methods: The cytotoxic activity of the methanol extract of the bark was performed by brine shrimp lethality bioassay and analgesic potential of the bark extract was evaluated using both acetic acid induced writhing test and tail-flick tests to understand the central analgesic activity using Swiss Albino mice as experimental animal. Anthelmintic activity of the leaf extract was performed by observing the time of paralysis and the time of death of earthworms, *Pheretima posthuma*.
Results: The methanol extract of the bark showed moderate cytotoxic activity when compared with the standard drug vincristine sulphate. The results of analgesic activities suggested that the methanol extract of the bark possess significant analgesic activity in mice, which is comparable to the standard drug, diclofenac-Na. The fresh juice extract of the leaf of *S. indica* showed potent anthelmintic activity when compared with standard drug, albendazole.
Conclusions: Our present study, demonstrates the cytotoxic, analgesic and anthelmintic, properties of *Saraca indica*, which validate its use in traditional medicine.

Keywords: *Saraca indica*, Cytotoxic, Analgesic, Anthelmintic

INTRODUCTION

Patients are treated with herbal medicines for its extraordinary influence since little knowledge about the mode of action is known. Ashoka is one of the most fabulous and revered trees of Caesalpiniaceae family that is scientifically known as *Saraca indica* L. or *Saraca asoca*.[1] It is a medium sized evergreen tree up to 9 m in height with various spreading and drooping glabrous branches. The bark of the plant is dark brown to grey or black; flowers are odorous, numerous, dance and orange or red color; leaves are pinnate, 15-25 cm long having 4-6 pairs of oblong-lanceolate leaflets.[2]Leaves are beneficial in stomachalgia and flowers are used in vitiated condition of pitta, syphilis, hyperdipsia, inflammation, dysentery, haemorrhoids and scabies in children.[2-4]

Bark of the plant is bitter and conventionally used as astringent, anthelmintic, demulcent, emollient, stomachic and in blood disease, biliousness, colic, piles, ulcers, fractures, menorrhagia, metropathy, dyspepsia, visceromegaly. Stem bark is astringent, antileucorrhoeic, antibilious and uterine sedative; flowers are used as uterine tonic, antidiabetic and antisyphilitic traditionally. Plant is also vital for CNS depressant activity as aerial part is important for its CNS active, hypothermic, CNS depressant and diuretic activity.[5,6] The plant is also used

in diabetes and for stopping bleeding. The plant possesses hypertensive and inotropic effects due to the presence of the catecholamines.[7] Taking into account of the above findings; it was decided to investigate cytotoxicity, analgesic and anthelmintic activities of different plant parts of *Saraca indica*.

METHODS

Chemicals and Drugs

Drugs and chemicals used in the study include: methanol (Merck, Germany), vincristine sulphate (Gedeon Richter), DMSO (Merck, Germany), diclofenac-Na, and acetic acid (Merck, Germany), albendazole (Square Pharmaceuticals Ltd., Bangladesh), DMF (Merck, Germany).

Plant materials and extraction

Saraca indica was collected from Ramna Park, Dhaka, Bangladesh. The time of collection was February, 2013. Later the plant was identified by the respective scientist of Bangladesh National Herbarium Institute, Mirpur, Dhaka. An accession number was given from there and a voucher specimen (DACB: 38223) has been deposited in the herbarium for future reference. *S. indica* leaf and bark were first separated from undesirable materials.

The barks were dried for one and half week in a shaded place. After drying, the plant part was grinded by blender machine (NOWAKE, JAPAN). Coarse powder was obtained after grinding. Following the method of cold extraction, 100g of powdered bark of *S. indica* was separately soaked in 500 ml of methanol for 20 days and then all the extracts were filtered through a cotton plug followed by Whatman filter paper number 1 and then concentrated by using a rotary evaporator at low temperature (40-50) °C and reduced pressure which provide greenish color extract (1.40 g).

Test animal

For the experiment swiss albino mice of either 3-4 weeks of age, weighing between 20 to 25g were collected from the Animal Resources Branch of the International Center for Diarrheal Disease and Research, Bangladesh (ICDDR, B). Soft wood shavings were used as bedding of cages. Animals were maintained under standard environmental conditions: temperature (24.0±1.0°C), relative humidity (55-65% and 12 hrs. light /12 hrs. dark cycle). Husk and excreta were removed from the cages every day. Adult earthworm *Pheretima posthuma* were collected (due to its anatomical and physiological resemblance with the intestinal roundworm parasites of human being) from moist soil. For anthelmintic study, all the worms were washed with normal saline to remove fecal matters. In brine shrimp lethality bioassay, brine shrimp nauplii are used as a favorable monitor for screening and fractionation in the discovery of new bioactive natural products.[8]

Brine shrimp lethality bioassay

Brine shrimp lethality bioassay technique was applied for the determination of general toxic property of the plant extractive. Brine shrimp eggs collected from pet shops were used as the test organism. Seawater was taken in the small tank. Shrimp eggs were added to one side of the tank, and then this side was covered. Two days were allowed to hatch the shrimp and to be matured as nauplii. Constant oxygen supply was provided throughout the hatching time. The hatched shrimps were attracted to the lamp through the perforated dam and with the help of a pasteur pipette 10 living shrimps were added to each of the vials containing 5 ml of simulated seawater.

Preparation of positive control group

Vincristine sulphate was used as the positive control. 0.4 mg of vincristine sulphate was dissolved in 100 µl of DMSO to get an initial concentration of 40 µg/ml from which serial dilutions are made using DMSO to get 20, 10, 5, 2.5, 1.25, 0.625, 0.3125, 0.15625 and 0.078 µg/ml, respectively. Then the positive control solutions are added to the remarked vials containing ten living brine shrimp nauplii in 5 ml simulated sea water.

Preparation of negative control group

30 µl of DMSO was added to each of three pre-marked glass vials containing 5 ml of simulated sea water and 10 shrimp nauplii. If the brine shrimps in these vials show a rapid mortality, then the test is considered as invalid as the nauplii died due to some reasons other than the cytotoxicity of the compounds.

Preparation of test groups

4 mg of sample was dissolved in 100 µl of DMSO to get the stock solutions. Then 50 µl of solution was taken in each test tube containing 5 ml of simulated seawater and 10 shrimp nauplii. Thus final concentration of the prepared solution in the test tube was 400 µg/ml. Then a series of solutions of varying concentrations 200, 100, 50, 25, 12.5, 6.25, 3.125, 1.563, and 0.781 µg/ml, respectively were prepared by serial dilution.

Counting of nauplii

After 24 hours, the vials were inspected using a magnifying glass and the number of survived nauplii in each vial was counted. From this data, the percent (%) of lethality of the brine shrimp nauplii was calculated for each concentration. The median lethal concentration (LC_{50}) of the test samples was obtained by a plot of percentage of the shrimps killed against the logarithm of the sample concentration.[9]

Analgesic activity

The study of analgesic activity of the *S. indica* was performed in animal models for both central and peripheral mechanism of pain. For the screening of analgesic activity against peripheral mechanism of pain, acetic acid-induced writhing test was considered. On the other hand, to evaluate the analgesic activity against centrally mediated pain tail immersion test was used.

Acetic acid-induced writhing test

The acetic acid-induced writhing method is an analgesic behavioral observation assessment method that demonstrates a noxious stimulation in mice. Test sample and vehicle were administered orally 30 min before intraperitoneal administration of 0.7% acetic acid but diclofenac-Na was administered 15 min before injection of acetic acid. After an interval of 5 min, the mice were observed for specific contraction of body referred to as "writhing" for the next 10 min.[10]

Tail immersion test

The procedure is based on the observation that morphine-like drugs selectively prolong the reaction time of the typical tail withdrawal reflex in mice. The animals of the control, positive control and test groups were treated with diclofenac-Na (5 mg/kg body weight), water (10 mL/kg body weight) and test samples at the doses of 200 and 400 mg/kg body weight, respectively. 1 to 2 cm of the tail of mice was immersed in warm water kept constant at 55 °C. The reaction time was the time taken by the mice to deflect their tails. The first reading was discarded and the reaction time was recorded as a mean of the next three readings. A latency period of 20 sec was defined as complete analgesia and the measurement was stopped when the latency period exceeded to avoid injury to mice. The latent period of the tail-flick response was taken as the index of anti-nociception and was determined at 0, 30, 60 and 90 min after the administration of the test drugs and standard.[11]

Anthelmintic activity

Helminthiasis is a macro parasitic disease of humans and animals in which a part of the body is infested with parasitic worms such as pinworm, roundworm, or tapeworm. Anthelmintics or antihelminthics are the drugs or the agents that destroy or cause the expulsion of such parasitic intestinal worms and helps to treat helminthiasis, one of the most common infections in humans and cattle. Resistant worms accumulate and finally treatment failure occurs. To overcome the resistance, plant derived drugs can serve as prototype to develop more effective and less toxic medicines.[12] Fresh juice extract of the leaves of *S. indica* were dissolved in minimum amount of DMF and the volume was adjusted to 10ml with saline water. All drug and extract solutions were freshly prepared before starting the experiment.

In each case, 6 earthworms released into 10 ml of desired formulations as follows: vehicle (5% DMF in normal saline), albendazole (25, 50 and 100 mg/ml) and fresh juice extract of the leaf (5% DMF in normal saline). Observation was made for the time taken to paralysis and death of individual worm. Paralysis was said to occur when the worms were not able to move even in saline solution.[13]

Statistical analysis

Statistical analysis for animal experiment was carried out using one-way ANOVA followed by Dunnett's multiple comparisons. The results obtained were compared with the vehicle control group; $p < 0.05, 0.001$ were considered to be statistically significant.

RESULTS

In brine shrimp lethality bioassay, the lethality of the methanolic bark extract of *S. indica* to brine shrimp was determined. The plant extract of *S. indica* showed significant cytotoxic activity against brine shrimp nauplli with LC_{50} value 2.38 µg/ml when compared with the standard drug vincristine sulphate (LC_{50} value: 0.812µg/ml) as shown in Table 1 and the regression analysis data for vincristine sulphate and methanolic extract of the bark of *S. indica* are given in the Table 2. DMSO has no effects on Brine shrimp nauplii.

The analgesic effect of the methanolic bark extract of *S. indica* on acetic acid-induced writhing and tail immersion test/ tail-flic method in mice was determined. The extract significantly inhibited writhing response induced by acetic acid in a dose-dependent manner. In this test, the methanol extract of bark showed inhibition of writhing of 56.47% and 69.44% at the dose of 200 and 400 mg/kg body weight, respectively as in Table 3 that was comparable to diclofenac-Na (73.09% of inhibition).

In case of tail-flick method, the methanol extract of the bark of *S. indica* exhibited significant analgesic activity in tail immersion test. It seems possibly that the higher doses of the extract have more potent analgesic effect as shown in Table 4.

Anthelmintic activity was performed by observing the time of paralysis and the time of death of earthworms *Pheretima posthuma* compared with the standard drug, albendazole. The range of minimum to maximum time of paralysis and death of albendazole at the conc. 100 mg/ml was 2 min 10 sec to 9 min 30 sec whereas; the range of minimum to maximum time of paralysis and death of the fresh leaf juice extract was 3 min 10 sec to 7 min 15 sec respectively. The results confirmed the presence of potent anthelmintic activity of the leaf extract of *S. indica*. Vehicle (5% DMF in normal saline) has no effect on both paralysis and death of individual worm. The results of anthelmintic activity of *S. indica* are given in Table 5.

Table 1: Effects of methanol soluble extract of bark of *S. indica* on brine shrimp nauplii.

Methanol extract of the bark				Vincristine Sulfate			
Conc. (C) (µg/ml)	Log C	% Mortality	LC_{50} (µg/ml)	Conc. (C) (µg/ml)	Log C	% Mortality	LC_{50} (µg/ml)
400	2.602	100	2.38	40	1.602	100	0.812
200	2.301	100		20	1.301	100	
100	2	100		10	1.000	90	
50	1.699	90		5	0.698	80	
25	1.397	80		2.5	0.397	70	
12.5	1.097	70		1.25	0.096	50	
6.25	0.796	60		0.625	-0.204	40	
3.125	0.495	50		0.312	-0.505	30	
1.563	0.194	40		0.156	-0.806	30	
0.781	-0.107	40		0.078	-1.107	20	

Table 2: Regression analysis data for vincristine sulphate and methanolic extract of the bark of *S. indica*.

Sample	LC_{50} (µg/ml)	Regression equation	R^2
Vincristine sulphate (Positive control)	0.812	Y=33.219x + 52.781	0.9717
Methanol extract of *S. indica*	2.38	Y=26.37x + 40.09	0.961

Table 3: Effect of the *S. indica* on acetic acid-induced writhing in mice.

Treatment	Dose (mg/kg)	Number of writhing	% of inhibition
Control (DMSO+water)	0.1 mL/mice, oral	33.43 ±1.47	0.00
Diclofenac-Na	1.0, intraperitoneal	9.00 ±2.08	73.09
Group-I	200, oral	14.56 ±1.30	56.47
Group-II	400, oral	10.23 ±1.45	69.44

Values are expressed as mean ± SEM, where n = 5, p <0.05 compared with the control group (Dunnett's test), Control: DMSO+Water (0.1 mL/Mouse), Positive control: Diclofenac-Na (1.0 mg/kg), Group I = *S. indica* (200 mg/kg), Group II = *S. indica* (400 mg/kg).

Table 4: Effect of *S. indica* on tail withdrawal reflex in mice.

Test group	Dose (mg/kg b.w.) and route of administration	Number of movements			
		0 min	30 min	60 min	90 min
Control (DMSO + water)	0.1 mL/mice, oral	1.42 ±0.07	1.96 ±0.08	2.08 ±0.20	1.94 ±0.25
Diclofenac-Na	1.0, intraperitoneal	2.84 ±0.43	5.32 ±0.32	7.45 ±0.46	7.1 ±0.33
Group-I	200, oral	3.55 ±0.75	10.40 ±2.5	12.15 ±3.12	9.35 ±5.0
Group-II	400, oral	5.32 ±2.35	13.22 ±4.17	11.20 ±3.53	8.56 ±2.27

Values are expressed as mean ± SEM, where n = 5, p <0.05 compared with the control group (Dunnett's test), Control: DMSO+Water (0.1 mL/Mouse), Positive control: Diclofenac-Na (1.0 mg/kg), Group I = *S. indica* (200 mg/kg), Group II = *S. indica* (400 mg/kg).

Table 5: *In vitro* anthelmintic activity of *S. indica.*

Test Samples	Conc. (mg/ml)	Time taken for paralysis (min)	Time taken for death (min)
Fresh juice extract of leaves	25	6 min 30 sec	11 min 5 sec
	50	5 min 40 sec	10 min 20 sec
	100	3 min 10 sec	7 min 15 sec
Albendazole	25	5 min 50 sec	12 min 20 sec
	50	4 min 10 sec	10 min 5 sec
	100	2 min 10 sec	9 min 30 sec

DISCUSSION

In brine shrimp lethality bioassay, the methanolic extract of the bark of *S. indica* showed significant cytotoxic activity when compared with the standard vincristine sulphate. Comparison with positive control vincristine sulphate the plant extract signifies moderate cytotoxicity. The lethal concentration LC_{50} of the test samples after 24 hr. was obtained by a plot of percentage of the shrimps killed against the logarithm of the sample concentration (toxicant concentration) and the best-fit line was obtained from data by means of regression analysis. This cytotoxic activity of *S. indica* is principally contributed by the presence of alkaloids, glycoside, flavonoids, tannins and saponins.[14] The analgesic effect of the methanolic bark extract of *S. indica* on mice was determined by both acetic acid-induced writhing test and tail immersion test. In case of acetic acid-induced writhing test, the extract significantly inhibited writhing response induced by acetic acid in a dose dependent manner. The result was statistically significant and was comparable to the reference drug, diclofenac-Na. The *S. indica* caused dose-dependent anti-nociception against chemical-induced pain in mice. The methanolic extract of *S. indica* was found to exhibit significant writhing response inhibitory effect. The analgesic activity tests of tail-flick method were carried out in the laboratory on five groups of mice by tail-flick method. Time interval for the test was 30 minutes. The tail withdrawal reflex time after administration of the *S. indica* was found to increase with increasing dose of the extract. The tail immersion test is widely used for assessing analgesic activities. In our experiments, *S. indica* exhibited significant analgesic activity in tail immersion test. It seems possibly that the higher doses of the extract have more potent analgesic effect. The analgesic activity might have been attributed to the presence of alkaloids and steroids in this plant. [15]It is evident from experimental data that the fresh juice extract of the leaf of *S. indica* showed potent anthelmintic activity when results are compared with standard drug, albendazole. Sarojini et al was performed the phytochemical screening that showed the presence of several important constituents like glycosides, tannin, flavanoids and terpenoids seems to be the accountable phytochemical constituents for signify anthelmintic activities of ethanolic and methanolic extracts of leaf.[16]

CONCLUSION

Based on the result of the present study, it can be concluded that the methanol extract of the bark posseses significant cytotoxic and analgesic potentials and the leaf extract of *S. indica* possess potent anthelmintic effects. The methanol extract of the bark showed promising analgesic properties compared to respective standard drug. At higher dose, notable analgesic activity was observed from acetic acid-induced writhing and tail-flick test. Significant cytotoxic activity can be suggested from the results of brine shrimp lethality bioassay. Dose dependant activity was observed in all the performed pharmacological investigations. Hence, further investigation is required to explore their activities against various diseases and to isolate bioactive principles.

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REFERENCES

1. Sasmal S, Majumdar S, Gupta M, Mukherjee A, Mukherjee PK. Pharmacognostical, phytochemical and pharmacological evaluation for the antipyretic effect of the seeds of *Saraca asoca* Roxb. Asian Pac J of Trop Biomed. 2012;2(10):782-6.
2. Kirtikar KR, Basu BD, An ICS. Indian Medicinal Plants. Volume 2. Derhadun: International Book Distributors; 2005.
3. Anonymous. Indian Medicinal Plants, A Compendium of 500 Species, Vol 5, Chennai: Orient Longman Pvt Ltd; 2006.
4. Nadkarni AK, KM Nadkarni's. Indian Materia Medica. Volume 1. Mumbai: Bombay Popular Prakashan; 2005.
5. Kashyapa K, Chand R. The Useful Plants of India. National Institute of Science Communication and information Resources, CSIR. New Delhi, 2006.
6. Joy PP, Thomas J, Mathew S, Skaria BP. Medicinal Plants. Kerala Agricultural University, Ernakulam. 1998.
7. Ghani A. Medicinal Plants of Bangladesh. Asiatic Society of Bangladesh, Dhaka. 2003;2:372.
8. Hasan SMR. Phytochemical and Pharmacological Investigations of Commelina benghalensis Linn. M. Pharm. Thesis. Department of Pharmacy, Jahangirnagar University. 2009.
9. Basak A, Hossain ML, Parvin MN. Evaluation of phytochemical and pharmacological activities of *Bacopa monnieri* (L.). Int J Sci Rep. 2016;2(10):242-7.
10. Meera S, Kumar NS, Guptatyam VSS. Screening of Anti-Arthritic, Anti-Inflammatory and Analgesic Activity of a Polyherbal Formulation. Int J pharmacol. 2008;4(5):398–402.
11. Hasan SMR. Phytochemical and pharmacological investigations of *Commelina benghalensis* Linn. M. Pharm. Thesis. Jahangirnagar University. 2009.
12. Singh D, Swarnka RCP. Khan FA. (2002) Anthelmintic resistance in Gastrointestinal nematodes in livestock in India J Vet Parasit. 2002;16:115-30.

13. Patil S, Dave AK, Singh VK, Jagannadham MV. In-vitro Anthelmintic Activity of Indian Medicinal Plant. J Agric Food Chem. 2009;55(14):5809-18.
14. Mishra A, Kumar A, Rajbhar N, Kumar A. Phytochemical and Pharmacological Importance of Saraca indica. JPCBS. 2013;2(2):1009-13.
15. Mohod SP, Jangde CR, Narnawore SD, Rant S. Experimental evaluation of analgesic property of bark of skin of *Saraca indica* (Ashoka) and *Shorea robusta* (Shal). JAPS. 2014;4(2):62-5.
16. Sarojini N, Manjari SA, Kanti CC. Phytochemical screening and anthelmintic activity study of Saraca indica Leaves extracts. In Res J Pharm. 2011;2(5):194-7.

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