ORIGINAL ARTICLE

Anti-ulcer Potential of Aqueous and Ethanolic Bark Extracts of "*Saraca indica*" Using Different Screening Models

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Abstract

Objective: *Saraca indica* (*Cesalpiniaceae*) is a plant, reported for its variety of ethnic medicinal uses, and widely grown in Asia, Africa, and the Caribbean for its edible bark. The present work has been planned to screen the anti-ulcer activity of bark of the plant with the ethanolic and aqueous extracts. **Materials and Methods:** Bark powder was successively extracted with ethanol (95%) and water using Soxhlet extraction and subjected to phytochemical screening to identify different phytoconstituents. Ld_{50} studies for both (ethanolic and aqueous) extracts were conducted up to the dose level of 2 g/kg by following OECD up-and-down method of guidelines No.425. The anti-arthritic activity was performed using pylorus ligation, aspirin, and stress-induced ulcer models in rats. Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Dunnett's test. $P < 0.05$ was considered statistically significant. **Results:** Preliminary phytochemical studies revealed the presence of saponins, sterols, mucilage, glycosides, and alkaloids, steroidal saponins in both the ethanolic and aqueous extracts of *S. indica*. No mortality was observed with aqueous and ethanolic extracts up to the maximum dose level of 2 g/kg. Both the extracts except with low dose 100 mg/kg other two doses, that is, medium and high have significantly reduced the ulcer number (0.83 ± 0.0.16, 1.5 ± 0.34 and 0.50 ± 0.11, 0.66 ± 0.21), ulcer score (1.5 ± 0.12, 1.66 ± 0.16 and 0.83 ± 0.10, 0.91 ± 0.15), and ulcer index (13.64, 9.80 and 11.20, 8.25), and a significantly ulcer inhibition (41.10%, 27.30% and 68.76%, 54.25%) is noted. **Conclusion:** From the present experimental findings of both pharmacological and biochemical parameters observed from the current investigation, it is concluded that the doses of 200 mg/kg and 400 mg/kg aqueous extract of *S. indica* possess potentially useful anti-ulcer activity since it gives a positive result in ulcer score and ulcer index and a significant ulcer inhibition.

Key words: Anti-ulcer, aspirin, pylorus ligation, *Saraca indica*

INTRODUCTION

Gastric ulcers the most wide state disease and are a very common global problem today. A peptic ulcer is a lesion of the gastric duodenal mucosa[1,2] which occurs at a site where the mucosal epithelium is exposed to acid and pepsin. Peptic ulcers occur due to an imbalance between the offensive and defensive factors. Physical, chemical, and psychological factors may lead to gastric ulceration in humans and experimental animals. Reactive oxygen species (ROS) are reported in the pathophysiology of human diseases,[3,4] such as neurodegenerative inflammation, viral infections autoimmune, GI. inflammation, and gastric ulcer. *Saraca indica* is used as astringent to the bowels,[5] alexiteric, anthelmintic, demulcent, emollient, cures dyspepsia, thirst, burning sensation, diseases of the blood, biliousness, effects of fatigue, tumors, enlargement of the abdomen, colic, piles, ulcers, bloody discharge from the uterus, menorrhagia,[6] and useful in fractures of bones. Indian Ayurvedic system of medicine was reported the anti-ulcer activity of *S. indica* for its anti-ulcer activity and no studies were reported. Therefore, the present investigation was aimed at studying the anti-ulcer

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activity of alcoholic and aqueous extracts of bark of *S. indica* in different models of experimental animals like rats.

MATERIALS AND METHODS

Plant material

Bark of *S. indica* was collected in the month of June from the Alva Pharmacy, Mangalore and was dried in shade at room temperature then subjected to size reduction to a fine powder with the help of a mixer grinder. The image of the *S. indica* bark is shown in Figure 1.

Preparation of ethanolic extract

The bark powder (750 gm) was packed in a Soxhlet apparatus and extracted with 1 L ethanol (95%) for 18 h at > 78°C. The appearance of colorless solvent in the siphon tube was taken as the termination of extraction. The extract was then transferred into a previously weighed empty beaker and evaporated to a thick paste on the water bath, maintained at <50°C. The ethanolic extract of bark of *S. indica* (EEBSI) was appeared dark brown amorphous in nature with a percentage yield of 1%.

Preparation of aqueous extract

About 100 g of bark powder[7] were taken in a round bottom flask (2000 ml) and macerated with 500 ml of distilled water for 24 h with occasional shaking in a closed vessel. Ten milliliters of chloroform were added as a preservative. Then, the marc was removed by filtering the extract and then concentrated on a water bath maintained at 50°C. The extract was finally air dried thoroughly to remove all traces of the solvent. The aqueous extract of bark of *S. indica* (EEBSI) was appeared dark brown sticky in nature with a percentage yield of 1%. The two extracts were examined for their color and consistency. Their percentage yield was calculated with reference to air dried sample used for extraction then stored in an air tight containers in a refrigerator below −4°C.

Figure 1: Bark of *Saraca indica*

Experimental animals

Albino rats (Wistar strain) of both sex weighing between 150 and 200 gm and albino mice of either sex weighing between 16 and 25 g were procured from National Centre for Laboratory Animal Sciences, C/O Sri Venkateswara Enterprises, Bengaluru, for experimental purpose. After procuring, all the animals were acclimatized for 7 days under standard husbandry condition as, 26 ± 2°C room temperature, with relative humidity 45–55% and kept light/dark cycle for 12:12 h. The animals were fed with synthetic standard diet Amrut Laboratories (Pranava Agro Industries Ltd. Sangli.). Water was allowed *ad libitum* and strict hygienic conditions were maintained. After obtaining prior permission from the Institutional Animal Ethical Committee (IAEC) of V. L. College of Pharmacy Raichur (Karnataka), all animal studies were performed as per rules and regulations in accordance with the guidelines of CPCSEA (Registration Number 557/02/c/CPCSEA).

Chemicals and drugs

The chemicals used for the anti-arthritic study were – distilled water (Mysore petro chemicals, Raichur, India), aspirin (Dr. Reddy's laboratories, Hyderabad), and anesthetic ether (Sigma Solvents and Pharmaceuticals – Mumbai). All the drugs and chemicals used were of pharmaceutical grade.

Determination of acute oral toxicity (LD_{50})

The acute oral toxicity study[8] of bark extracts of *S. indica* was determined in female albino mice (16–25 g) maintained under standard husbandry conditions. The animals were fasted 4 h before the experiment and up-and-down procedure (OECD Guidelines No. 425) method of CPCSEA was adopted for acute toxicity studies. Animals were administered with single doses of each extract and observed for their mortality during 48 h study period (short-term toxicity). Based on the short-term profile of extracts, the doses for the next animals were determined. All the animals were observed for long-term toxicity (7 days). The LD_{50} studies of the test extracts were conducted up to the maximum dose level of 2000 mg/kg body wt. $1/20^{th}$, $1/10^{th}$, and $1/5^{th}$ doses of the LD_{50} dose of the individual extracts that were selected for the study as low, medium, and high doses. The protocol was approved by the Institutional Animal Ethical Committee of V. L. College of Pharmacy, Raichur, Karnataka, with with CPCSEA Number -557/02/e/CPCSEA.

Pylorus ligation-induced ulcer model

In this method, albino rats were fasted in individual cages for 24 h care that was being taken to avoid coprophagy. In

the control group (vehicle), EEBSI, AQEBSI, and reference drug (ranitidine 30 mg/kg) were administered by the oral route. The pylorus ligation[9] was carried out 30 min after the drug administration in each group of animals. Under light ether anesthesia, the abdomen was opened and the pylorus was ligated. The abdomen was then sutured. After 4 h of pylorus ligation, the animals were sacrificed with an excess of anesthetic ether and the stomach was dissected out. Gastric juice was collected and its volume and free acidity and total acidity were measured. The glandular portion of the stomach was opened along the greater curvature, and the severity of hemorrhagic erosions in the acid secreting mucosa was assessed on a scale of 0–3, that is, ulcer index was determined.

The mean ulcer score for each animal is expressed as ulcer index. The percentage protection was calculated using the formula,

$$\text{Percentage ulcer protection} = U_t / U_c \times 100$$

Where,
U_t = Ulcer index of treated group and
U_c = Ulcer index of the control group

Aspirin-induced gastric ulcers

Albino rats of either sex weighing between (140 and 200 g) will be divided into eight groups of six rats in each. Group A served as normal control given with vehicle only. Group B with standard drug and Groups C, D, E, F, G, and H will be treated with low, medium, and high doses of ALEBSI and AQEBSI. After 30 min, aspirin[10] is administered at a dose of 250 mg/kg p.o, and after 6 h, rats will be sacrificed using anesthetic ether and their stomachs dissected out for determination of gastric lesions, washed in warm water, and examined for ulcers microscopically with the help of hand lens (×10). The mean ulcer score for each animal in each group is expressed as the ulcer index. Gastric juice collected into centrifuge tubes and centrifuged at 1000 rpm for 10 min and volume will be noted. The pH of the gastric juice was recorded by pH meter. The gastric content is subjected to analysis of free and total acidity.

Stress-induced ulcers (cold water immersion method)

Albino rats of either sex weighing between (140 and 200 g) will be divided into eight groups of six rats in each. Group A served as normal control, given with vehicle only. Group B with standard drug and Groups C, D, E, F, G, and H treated with low, medium, and high doses ALEBSI and AQEBSI. After 30 min of oral administration of the vehicle/standard/extracts, rats are placed in cold water vertically in individual restraint cages maintained at 22°C for 1 h. Then, they are taken out, dried, and injected with 30 mg/kg Evans blue i.v through the tail vein. Ten minutes later, sacrificed with ether and stomachs are removed. Formal saline (2%v/v) is then injected into the totally ligated stomachs for overnight storage.[11] The next day, the stomachs opened along the greater curvature, washed in warm water, and examined microscopically for ulcers with the help of a hand lens (×10). The mean ulcer score for each animal is expressed as the ulcer index. Gastric juice collected into centrifuge tubes and centrifuged at 1000 rpm for 10 min and volume will be noted. The pH of the gastric juice was recorded by pH meter. The gastric content is subjected to analysis of free and total acidity.

Data analysis

The obtained values in all three models were expressed as mean ± SD from six animals, subjected to statistical analysis using one-way ANOVA followed by Dunnett's "*t*" test to verify significant difference if any among the groups. $P <$ 0.05*, 0.01**, and 0.001*** were considered significant.

RESULTS AND DISCUSSION

Ethanolic and aqueous extracts of bark of *S. indica* were subjected to phytochemical screening for all types of phytoconstituents, for example, alkaloid, glycoside, terpenoids, tannins, saponins, and flavonoids. The ethanolic extract was found positive for lead acetate test and ferric chloride test, which confirms the presence of flavonoids. Positive Salkowski test confirms the presence of triterpenes. Liebermann–Burchard test was found positive which confirms the presence of sterols. Foam and froth test confirms the presence of saponins. The phytochemical study of aqueous extract confirms the presence of triterpenes and flavonoids. Ethanolic and aqueous extracts of *S. indica* bark were administered orally to different groups of mice at different dose levels. It was found that even up to the dose level of 2000 mg/kg, body weight either of the extracts did not produce any behavioral symptoms or mortality.

Pylorus ligation-induced ulcer model in rats

In pylorus ligation-induced ulcer model in rats, a significant increase in ulcer number (5.33 ± 0.66), ulcer score (2.58 ± 0.30), and ulcer index (15.93) is noted. In the same model, a significant increase in gastric volume (6.03 ± 0.37 ml), free acid (6.53 ± 0.14 mEq/L), and total acid (11.03 ± 0.30 mEq/L) is noted. Standard drug ranitidine 30 mg/kg treatment has significantly reduced ulcer number (0.16 ± 0.16), ulcer score (0.58 ± 0.08), ulcer index (4.32), gastric volume (4.90 ± 0.41 ml), free acidity (2.60 ± 0.20 mEq/L), and total acidity (7.05 ± 0.24 mEq/L). In pylorus ligation-induced ulcer model, both ALEBSI and AQEBSI except with low dose 100 mg/kg other two doses, that is, medium and high have significantly reduced the ulcer number (0.83 ± 0.0.16, 1.5 ± 0.34 and 0.50 ± 0.11, 0.66 ± 0.21), ulcer score (1.5 ± 0.12, 1.66 ± 0.16 and

Table 1: Anti-ulcer effect of ALEBSI and AQEBSI in different models of ulcers in rats (Mean±SEM)

Group	Treatment (p.o)	Pylorus ligation-induced ulcers				Stress-induced ulcers				Aspirin-induced ulcers			
		Ulcer number	Ulcer score	Ulcer index	(%) Inhibition of ulcers	Ulcer number	Ulcer score	Ulcer index	(%) Inhibition of ulcers	Ulcer number	Ulcer score	Ulcer index	(%) Inhibition of ulcers
Control	Vehicle 10 ml/kg	5.33±066	2.58±0.30	15.93	-	3.83±0.60	2.41±0.23	16.86	-	5.0±0.44	2.66±0.16	17.96	-
Standard	Ranitidine 30 mg/kg	0.16±0.16**	0.58±0.08**	4.32	86.67	0.66±0.21**	0.75±0.11**	4.70	58.18	0.50±0.22**	0.75±0.17**	5.37	78.28
ALEBSI	100 mg/kg	3.33±0.66 ns	2.25±0.17 ns	15.78	12.76	1.83±0.40*	1.75±0.11 ns	14.16	11.45	3.16±0.54ns	2.16±0.16ns	16.07	14.90
ALEBSI	200 mg/kg	0.83±0.16*	1.5±0.12*	13.64	41.10	1.16±0.16**	1.41±0.08*	13.52	24.78	1.16±0.40*	1.58±0.08*	11.82	39.23
ALEBSI	400 mg/kg	0.50±0.11**	0.83±0.10**	11.20	68.76	0.83±0.16**	0.83±0.10**	8.42	49.25	0.66±0.21**	0.83±0.10**	7.41	67.10
AQEBSI	100 mg/kg	3.5±0.3ns	2.41±0.16ns	13.71	11.80	2.50±0.42ns	1.91±0.23ns	13.28	10.26	3.5±0.34ns	2.33±0.21ns	18.51	11.62
AQEBSI	200 mg/kg	1.5±0.34*	1.66±0.16*	9.80	27.30	1.33±0.21**	1.6±0.08*	11.36	23.61	2.33±0.33*	1.91±0.15*	14.61	30.52
AQEBSI	400 mg/kg	0.66±0.21**	0.91±0.15**	8.25	54.25	1±0.0**	0.91±0.15**	9.56	39.68	0.83±0.16**	0.66±0.10**	6.25	58.21

n=6, Significant at *P*<0.05*, 0.01** and 0.001***, ns=not significant, ALEBSI-Alcoholic extract of bark of *Saraca indica*, AQEBSI-Aqueous extract of bark of *Saraca indica*.

0.83 ± 0.10, 0.91 ± 0.15), and ulcer index (13.64, 9.80 and 11.20, 8.25) and a significantly ulcer inhibition (41.10%, 27.30% and 68.76%, 54.25%) is noted. Similar to the above, a significant reduction in gastric volume (5.18 ± 0.16, 6.0 ± 0.24 ml and 5.03 ± 0.07, 5.51 ± 0.12 ml), free acidity (3.19 ± 0.16, 5.21 ± 0.19 and 3.0 ± 0.12, 3.58 ± 0.21 mEq/L), and total acidity (8.43 ± 0.22, 8.58 ± 0.20 and 7.48 ± 0.16, 7.65 ± 0.17 mEq/L) is noted with medium and high doses but not with the low doses of ALEBSI and AQEBSI, respectively [Table 1].

Stress-induced ulcer model in rats

In stress-induced ulcer model, a significant increase in ulcer number (3.83 ± 0.60), ulcer score (2.41 ± 0.23), ulcer index (16.86), gastric juice volume (6.86 ± 0.26 ml), free acidity (7.80 ± 0.18 mEq/L), and total acidity (13.06 ± 0.46 mEq/L) is noted. Standard drug ranitidine 30 mg/kg treatment has significantly reduced ulcer number (0.66 ± 0.21), ulcer score (0.75 ± 0.11), ulcer index (4.70), gastric volume (5.31 ± 0.24 ml), free acidity (2.98 ± 0.30 mEq/L), and total acidity (7.83 ± 0.24 mEq/L). Similar to pylorus ligation-induced ulcer models except low dose, other two doses, that is, medium and high of ALEBSI and AQEBSI have significantly reduced the ulcer numbers (1.16 ± 0.16, 1.33 ± 0.21) and (0.83 ± 0.16, 1.0±0.01), ulcer score (1.41 ± 0.18, 1.6 ± 0.08) and (0.83 ± 0.10, 0.91 ± 0.15), and ulcer index (13.52, 11.36 and 8.52, 9.56) and a significant ulcer inhibition (24.74%, 23.61% and 49.25%, 39.68%) also noted. A significant reduction in gastric volume (6.46 ± 0.33, 7.10 ± 0.26 and 5.83 ± 0.13, 6.11 ± 0.18 ml), free acidity (5.11 ± 0.23, 5.50 ± 0.21 and 3.23 ± 0.22, 4.21 ± 0.19 mEq/L), and total acidity (10.91 ± 0.41, 11.76 ± 0.31 and 8.10 ± 0.26, 8.90 ± 0.18 mEq/L) is noted with medium and high doses but not with the low dose of the two extracts [Table 1].

Aspirin-induced ulcer model in rats

In aspirin-induced ulcer model, a significant rise in ulcer number (5.0 ± 0.44), ulcer score (2.66 ± 0.16), ulcer index (17.96), gastric volume (11.73 ± 0.67 ml), free acidity (7.80 ± 0.26 mEq/L), and total acidity (15.51 ± 0.21 mEq/L) is noted. Standard drug ranitidine 30 mg/kg treatment has significantly reduced ulcer number (0.50 ± 0.22), ulcer score (0.75 ± 0.17), ulcer index (5.37), gastric volume (3.90 ± 0.20 ml), free acidity (2.90 ± 0.12 mEq/L), and total acidity (8.15 ± 0.23 mEq/L). Similar to the above two ulcer models, both ALEBSI and AQEBSI except with low dose 100 mg/kg, other two doses, that is, medium and high have significantly reduced the ulcer numbers (1.16 ± 0.40, 2.33 ± 0.33 and 0.66 ± 0.21, 0.83 ± 0.16), ulcer score (1.58 ± 0.08,1.91 ± 0.15) and (0.83 ± 0.10, 0.66 ± 0.10), and ulcer index (11.82, 14.61 and 7.41, 6.25), and significant ulcer inhibition (39.23%, 30.52% and 67.10%, 58.21%) is noted. Hence, a significant reduction in gastric volume (5.53 ± 0.29, 6.21 ± 0.25 and 4.91 ± 0.10, 5.33 ± 0.13 ml), free acidity (5.03 ± 0.19, 5.23 ± 0.19 and 4.10 ± 0.15, 4.68 ± 0.15 mEq/L), and total acidity (10.08 ±

0.20, 11.25 ± 0.39 and 9.13 ± 0.22, 9.70 ± 0.24 mEq/L) is noted with medium and high doses but not with the low doses of the both the extracts [Table 1].

CONCLUSION

Both the extracts were evaluated for their anti-ulcer activity in pylorus ligation, stress, and aspirin-induced ulcer models in rats. Both the extracts produced a significant ($P < 0.01$) anti-ulcer activity, but similar to the above experiment, a relatively better anti-ulcer activity was recorded with the alcoholic extract.

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AUTHORS' CONTRIBUTIONS

The authors Dr. Kola Venu have supervised the research work, whereas the coauthors Dr.Prasenjit Mondal, Rekha Bhai Takur, and Ch. Subba Rao were materially participated in this work. Besides, all authors are contributed in the preparation of the manuscript, experimental, and interpretation of data**.**

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