ANTI-ULCER ACTIVITY OF HYDRO-ALCOHOLIC EXTRACT OF *SARACA ASOCA* STEM BARK IN RATS

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ABSTRACT

In the present study effort has been made to establish the antiulcer activity of *Saraca asoca* (Roxb.), De Wild (family-Caesalpinaceae) stem bark hydroalcoholic-extract using ethanol induced ulceration model in albino rats, because no systematic study has been reported for its antiulcer activity. The effect of hydro-alcoholic extract (60:40) of *S. asoca* bark was investigated by carrying out an acute toxicity study in albino rats to evaluate the antiulcerogenic activity by using ethanol induced gastric ulcer model compared with omeprazole as standard drug. The preliminary photochemical screening of this medicinal plant identified the presence of important secondary metabolites like flavonoids and tannins. Acute toxicity study did not manifest any toxicological signs in rats. Oral administration of absolute ethanol (99.5 %, 5 mL kg^{-1}, p.o.) produced significant ulcers index (41.00 ± 1.75) in the control group. Standard drug omeprazole (40 mL kg^{-1}, p.o.) produced ulcer index (12.17 ± 0.74). Ulcer index (24.17 ± 1.40) is significantly ($p<0.001$) protected by hydro-alcoholic extract of *S. asoca* (stem bark) at a dose of 200 mL kg^{-1}, p.o. The present finding suggests that hydro-alcoholic extract of *S. asoca* (stem bark) promotes ulcer protection activity.

Keywords: *Saraca asoca*, omeprazole, ethanol induced ulcer model, gastric ulcer

INTRODUCTION

An inflamed break in the continuity of the covering epithelium, either skin or mucous membrane of the alimentary canal is known as ulcer. Ulceration occurs due to disturbance of the normal equilibrium caused by either enhanced aggression or diminished mucosal resistance. About 19 out of 20 peptic ulcers are duodenal. Gastric ulcers, found in the stomach wall, are less common[1]. Peptic ulcer arises when the normal mucosal defensive factors (mucus, mucosal blood flow, formation of HCO_3 and PGE_2) are impaired or over powered by aggressive factors (acid, pepsin, $NSAID_S$ and *Helicobacter pylori*)[2]. Gastric ulcer is a serious injury caused by spicy food, stress, alcohol, gastric surgery and *H. pylori*[3]. Mucosal stasis leads to hemorrhage, necrosis and lipid peroxidation caused by ethanol as they trigger radical reactions[4,5]. The enzyme H^+/K^+-ATPase pumps protons in exchange for potassium ions across the apical membrane to secrete gastric acid by parietal cells[6,7].

Saraca asoca is a medicinally important and globally vulnerable plant species found in the evergreen forests of India[8]. India has often been referred to as the medicinal garden of the world and the medicinal plant *S. asoca* has been regarded as one of the foremost plants utilized from antiquity till date. *S. asoca* (Roxb.) de Wilde, is a small evergreen tree, belongs to the family Caesalpiniaceae and is commonly known as Asoka, Sita Asoka and Haempushpam[9,10]. All the plant parts i.e. leaves, bark, stem, flowers etc. contain medicinal properties. Barks and flowers contain glycosides, steroids, saponins, carbohydrates and tannins[11,12]. Bark of *S. asoca* is the most important part due to its medicinal value due to the presence of flavonoids, tannins, steroids, volatile oil, glycosides and steroidal glycosides such as sitosterol glucoside. Lignin glycosides such as lyoniside, nudiposide, 5-methoxy-9-xylopyranosyl, isolariciresinol, schizandriside and flavonoids such as catechin, epicatechin, epiafzelechin-(48)-epicatechin, procyanidin B2, deoxyprocyanidin B, leukocyanidins, leucopelargonidin and leucopelargonidin glucoside have been reported from *S. asoca* bark. Antioxidants such as

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Table I: Determination of acute toxicity testing of hydro-alcoholic extract of *S. asoca* Roxb. (stem bark)

Treatment	Dose	No. of animals used	Parameter	Observations		
Hydro-alcoholic (60 % ethanol) extract of *S. asoca* Roxb. (stem bark)	2000 mg kg^{-1}	6		24 h	72 h	14 days
			Weight loss	Nil	Nil	Nil
			Death rate	Nil	Nil	Nil
			CNS Toxicity	Nil	Nil	Nil
			Neurological disorder	Nil	Nil	Nil

Table II: Effect of hydro-alcoholic extract of *S. asoca* Roxb. de Wilde (stem bark) on ethanol induced gastric ulcer in albino rats

Group	Treatment	Total area of lesion (mm^2)/ ulcer index (mean± SEM)	Percentage protection
I (Control)	Ethanol (99.5 %) (5mL kg^{-1})+Vehicle	41.00 ± 1.75	-
II (Standard)	Ethanol (99.5 %) (5mL kg^{-1})+ Omeprazole (40 mg kg^{-1})	12.17 ± 0.74***	70.31 %
III(HA/Soxhlet)	Ethanol (99.5 %) (5mL kg^{-1})+Extract I (200 mg kg^{-1})	24.17 ± 1.40***	41.04 %

*Values are mean ± SEM, (n=6); p<0.05 considered as significant, *** p<0.001, One- way ANOVA followed by Dunnett's test as compared to control*

polyphenolics, gallic acid and ellagic acid have also been described from *S. asoca* bark[13-21].

Different parts of the plant exhibit a number pharmacological effects like antihyperglycemic, antipyretic, antibacterial and anthelmintic activities[22, 23]. The aqueous suspension extract of *S. indica* flowers, dried flower buds, bark and seeds were shown to curtail ulcers in albino rats. The anti-ulcer effect of the aqueous extract of *S. indica* flowers was demonstrated in albino rats by employing two models, namely pyloric ligation (which increases the acid content in the stomach) and aspirin-induced gastric ulceration[24-28].

However, no scientific study has been reported for antiulcer activity of *S. asoca* stem bark extract. So, in the present study, an effort has been made to establish the scientific validity to the antiulcer activity of *S. asoca* stem bark hydroalcoholic-extract using ethanol induced ulceration model in albino rats, so that we will be able to come up with new potent extract with less side effects as compared to synthetic drugs.

MATERIALS AND METHODS

Collection and authentication of plant material

The plant material (stem bark of *S. asoca* Roxb. de Wilde) was collected from local vendor (Batliwala and Sons) of Udaipur (Raj.) in the month of September to October. The collected material was dried at room temperature under a well ventilated shade by distributing them homogenously. *S. asoca* Roxb. de Wilde was authentified from Botanical Survey of India (BSI), Dehradun (UK), letter no. BSI/NRC/ Tech (Indent)/2011/166 and Acc no. is 113548.

Preparation of extract

The stem barks of *S. asoca* Roxb. de Wilde was shade dried for about 48 h and was finely powdered. The powdered drug material was extracted by hot percolation process in a Soxhlet apparatus (Borosil, Mumbai, India) using ethanol 60 % (V/V) as solvent. The extract was then filtered and dried in vacuum evaporator at 38 °C and percentage yield was calculated.

Group I (Treated with alcohol + vehicle)

Group II (Treated with alcohol + standard drug omeprazole)

Group III (Treated with alcohol + hydroalcoholic extract of *S. asoca* prepared by Soxhlet extraction method)

Fig. 1: Ulcer lesions in excised stomach

Experimental animals

Wistar albino rats of either sex weighing between 175-200 g were used in the present study. The animals were used for studying the acute toxicity and antiulcer activity. The animals were stabilized for 1 week; they were maintained in standard condition at room temperature, 60 ± 5 % relative humidity and 12 h light dark cycle. They had been given standard pellet diet and food was withdrawn 18-24 h before experiment, water *ad libitum* throughout the course of the study. The study protocol was approved by the Institutional Animal Ethical Committee, B. N. College of Pharmacy, Udaipur, Rajasthan, India (Registration no. 870/ac/08/CPCSEA).

Determination and selection of doses

The acute toxicity study was carried out in adult female *albino* rats by "fixed dose" method of OECD (Organization for Economic Co-operation and Development) guideline no. 420. Test procedure with a starting dose of 2000 mL kg^{-1}, b.w. was adopted. The hydro-alcoholic extract was then administered (p.o.) and the animals were observed for general behavioral, neurological, autonomic profiles, any toxicity and mortality up to 48 h[29].

Selection of doses

For the assessment of *in vivo* activity, two dose levels were chosen in such a way that one dose was

approximately 1/5th and 1/10th of the maximum dose during acute toxicity studies (200 mL kg^{-1} and 400 mL kg^{-1}).

EVALUATION OF ANTI-ULCER ACTIVITY

Chemicals and Reagents

Omeprazole (40 mL kg^{-1}, standard reference antiulcer drug), absolute alcohol 99.5 % (necrotizing agent), normal saline solution (0.9 % NaCl) and vehicle (2 % V/V Tween 80 in distilled water) were used.

Preparation of dosing

The dose 200 mL kg^{-1} of extracts were made by suspending appropriate quantity of extracts in water (suspended in 5 % V/V tween-80).

Ethanol Induced gastric ulceration

Adult rats of either sex weighing 175-200 g were fasted 24 h, allowing water *ad libitum,* control (vehicle)/ standard/ extract was administered per oral and after 30 min., ulceration was induced by oral administration of 99.5 % absolute alcohol (5 mL kg^{-1}, p.o.). The animals were sacrificed 1 h after the administration of ethanol and the stomach was excised. Each stomach was incised along the greater curvature and examined for linear hemorrhagic lesions in the glandular region. The lesion area, length × width (mm^2) of each lesion was determined at 10x magnification and each lesion area (mm^2) was summed per stomach. The sum of lesion areas (mm^2) for each stomach was used as the ulcer index (UI). The percentage inhibition was calculated by the following formula[30].

$$\text{Percentage protection} = 1 - \frac{Ut}{Uc} \times 100$$

where Ut = Ulcer index of treated group

Uc = Ulcer index of control group

Experimental protocol

Group I: Kept as control i.e. treated with absolute alcohol 99.5 % (5 mL kg^{-1}, p.o.).

Group II: Treated with standard drug omeprazole (40 mL kg^{-1}, p.o.).

Group III: Treated with absolute alcohol 99.5 % (5 mL kg^{-1}, p.o.) + 200 mg kg^{-1} hydro-alcoholic extract of *S. asoca* Roxb. (stem bark) prepared by Soxhlet extraction method.

Statistical analysis

Data were analyzed statistically by one-way ANOVA followed by Dunnet's *t*-test using computerized Graph Pad Prism version 5.4 (Graph Pad Software, U.S.A.). The data are expressed as mean ± S.E.M. P-values less than 0.05 imply significance.

RESULTS AND DISCUSSIONS

Acute toxicity studies

No test substance related mortality was observed at 2000 mL kg^{-1}. So, testing at higher dose may not be necessary and the extract was said to be practically non-toxic (Table I).

Antiulcer activity

In control animals, oral administration of absolute alcohol (99.5 %) produced characteristic lesions in the glandular portion of rat stomach, which appeared as elongated bands of thick, black and dark red lesions. Oral administration of absolute ethanol (99.5 %) produced significant ulcers index (41.00 ± 1.75) in the control group. Standard drug omeprazole (40 mL kg^{-1}) produced percentage protection of 70.31 % with ulcer index (12.17 ± 0.74). Ulcer index (24.17 ± 1.40) is significantly ($p<0.001$) protected by hydro-alcoholic extract of *S. asoca* (stem bark) and percentage protection is 41.04 % at 200 mL kg^{-1}, showed ulcer protection activity in alcohol induced gastric ulcer (Table II, Fig.1).

DISCUSSION

There are various factors (stress, chronic use of anti-inflammatory drugs and intake of alcohol for a longer period of time) responsible for ulceration. The protective effect of antiulcer drug is shown either by reducing the aggressive factors on gastro duodenal mucosa or by increasing mucosal resistance against them[31]. Ulcer results due to an imbalance between aggressive factors and the maintenance of the mucosal integrity through the endogenous defence mechanism. To regain the balance, different therapeutic agents including plant extracts are used to inhibit the gastric acid secretion or to encourage the mucosal defence mechanisms by increasing mucus production, stabilizing the surface epithelial cells, or interfering with the prostaglandin synthesis[32].

The penetration of alcohol in the gastric mucosa is rapid, causing cell and plasma membrane damage, leading to increased intracellular membrane permeability to sodium and water. Intracellular accumulation of calcium is the major step in the pathogenesis of gastric mucosal

injury, leads to cell death and exfoliation in the surface epithelium[33]. Oral administration of absolute alcohol (99.5 %) at (5 mL kg^{-1}) to the control group clearly produced a mucosal damage characterized by multiple hemorrhagic tracks of different sizes along the long axis of the glandular stomach, producing significant ulcer index (41.00 ± 1.75). Pretreatment with standard drug omeprazole (40 mL kg^{-1}) produced significant ($p < 0.001$) ulcer index (12.17 ± 0.74) decrease in the intensity of gastric mucosal damages induced by necrotizing agent (ethanol) compared with the control group.

Preliminary phytochemical investigation of the extracts of *S. asoca* Roxb., revealed the presence of glycosides, reducing sugars, flavonoids, phenolic compounds, tannins and steroids[34,35]. A number of flavonoids present in different plants are known to reduce gastric ulcer formation[36]. Hence, it is assumed that flavonoids may be responsible for the antiulcer effect of extract.

Ethanol is metabolized in the body and releases superoxide anion and hydroperoxy free radicals. Oxygen derived free radicals are implicated in the mechanism of acute and chronic ulceration in the gastric mucosa and scavenging these free radicals can play an appreciable role in healing ulcers[37]. Mohan et al*.,* 2016 proved the antioxidant activity (reduction in the generation of free radicals) of stem bark extracts of *S. asoca* Roxb. and also the previous results (free radical scavenging activity) are confirmed by the present investigation[38- 40].

In a previous scientific study, a correlation between the antioxidant and antiulcer activities of the flavonoids has already described[41]. These results suggest that the stem bark extracts of *S. asoca* Roxb. (200 mL kg^{-1}), have an ability to protect ulceration from ethanol induced damage, which may be due to its direct antioxidative effect.

CONCLUSION

The present study was attempted to scientifically validate the antiulcer potential of *S. asoca*. The present study may support through at least one or more possible mechanisms i.e. inhibition of basal gastric secretion, stimulation of mucus secretion and endogenous gastric mucosal prostaglandin synthesis. In view of the medicinal properties of the selected plant, more detailed research on fractionization and isolation of active constituents can be done to explore new, less toxic and potent phytoconstituents for present activity that will play a great role in healthcare.

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