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Research Article

ANTIULCEROGENIC EFFECT OF RESIN FROM *SHOREA ROBUSTA* GAERTN. F. ON EXPERIMENTALLY INDUCED ULCER MODELS

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ABSTRACT

Objective: The natural resin obtained from *Shorea robusta* Gaertn. F. is a reputed folklore remedy in its natural form for the treatment of ulcer, inflammation and wounds. The objective of the study was to evaluate the gastroprotective potential of the resin obtained from the bark of *Shorea robusta* Gaertn. f. on gastric ulcer models.

Methods: Gastroprotective potential of *S. robusta* resin (dissolved in water) at two different doses (150 and 300 mg/kg bw p.o.) was studied on ethanol and pyloric ligation (PL) induced gastric ulcer models in rats.

Results: Pretreatment with the resin (SRR) produced 62.69% inhibition of gastric mucosal damage in ethanol induced model and 64.55% inhibition in PL-induced model which was comparable to the reference drug omeprazole. The protective effect was associated with normalization of antioxidant markers (superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione-S-transferase (GST) and lipid peroxidation (LPO)) in ethanol induced model. In PL rats, SRR showed significant ($P<0.001$) decrease in gastric juice volume (65.44%), free acidity (33.06%), total acidity (26.98%) pepsin (44.39%) and protein (23.82%) with subsequent increase in carbohydrate (22.67%) and mucin (41.46%) in gastric juice. Further, the pH of the gastric juice increased from 1.23 to 4.54.

Conclusion: The results of the present study clearly suggested that *S. robusta* resin possess significant gastroprotective activity, supporting the folk use of resin preparations and contributing for its pharmacological validation.

Keywords: *Shorea robusta*; Resin; Antiulcer; Pyloric ligation; Antioxidant

INTRODUCTION

Gastric hyperacidity and gastro duodenal ulcer is a very common global problem today. The gastric lesions develop, when the delicate balance between some gastroprotective and aggressive factors is lost. Among various causes of gastric ulceration, lesions caused by stress, alcohol consumption, *H. pylori* infection and use of Non Steroidal Anti Inflammatory Drugs (NSAIDs) have been shown to be mediated largely through the generation of reactive oxygen species (ROS), especially hydroxyl radical (OH•) [1]. Hypersecretion of gastric acid is a pathological condition, which occurs due to uncontrolled secretion of hydrochloric acid from the parietal cells of the gastric mucosa through the proton pumping H+K+ATPase [2]. Most of the antisecretory drugs such as proton pump inhibitors (omeprazole, lansoprazole, etc.) and histamine H2-receptor blocker (ranitidine, famotidine, etc.) are extensively used to control increased acid secretion and acid related disorders, but there are reports of adverse effects and relapse in the long run [3,4]. On the other hand, most of the herbal drugs reduces the offensive factors and are proved to be safe, clinically effective, better patient tolerance and relatively less expensive [5]. In traditional medicine, several plants and herbs have been used to treat gastrointestinal disorders, including gastric ulcers [6].

Shorea robusta Gaertn. f. (Dipterocarpaceae) is a moderate deciduous tree commonly known as Sal. It is widely distributed in tropical regions of India, Indonesia, Malaysia and Philippines. It has been traditionally used for various ailments. The leaves and bark are used to treat wounds, ulcers, leprosy, cough, gonorrhea, earache and headache [7]. The bark is also used to treat diarrhea, dysentery and vaginal discharges [8]. The fruits are useful in tubercular ulcers, seminal weakness, burning sensation and dermopathy [7]. The oleoresin exuded from the plant has astringent, carminative and stomachic properties. It is useful in vitiated conditions of pitta, wounds, ulcers, neuralgia, burns, fractures, fever, diarrhea, dysentery, splenomegaly, obesity and burning of the eyes [7]. In Unani medicine, the resin is used for treating menorrhagia, enlargement of spleen and for relieving eye irritation. In Ayurveda, it is used with honey or sugar in treatment of dysentery and bleeding piles. It is also given in gonorrhea and for weak digestion [8]. It is suggested for ulcers, wounds and menopausal disorders by Siddha practitioners [9]. Earlier pharmacological studies confirmed its antibacterial [10], anti-aging [11], analgesic, anti-inflammatory [12] and wound healing [8] effects. Despite it being used as potential antiulcer agent in traditional system, the scientific evidence for supporting the antiulcer activity of *S. robusta* resin is lacking. Hence it was decided to illustrate the ethnobotanical use of the plant and the study was aimed to evaluate the gastroprotective potential of the *S. robusta* resin on experimentally induced gastric ulcer models.

MATERIALS AND METHODS

Sample preparation

Resin of *Shorea robusta* was collected from Siruvani hills, Western Ghats, Coimbatore, TN, India, authenticated and deposited in the Herbarium, Manian Institute of Science and Technology with Acc. No. MISAT-H-52. The freshly collected resin was processed according to Siddha tradition [9]. 100 g of powdered yellow resin was mixed with 200 mL of tender coconut water and boiled until the resin started to appear on the surface of the boiling liquid in a molten state. The resin was separated by filtration and cooled. The procedure of melting and recovery was repeated for an additional five times. After completely air dried in room temperature, the resultant fine white resin powder was freshly dissolved in water and used for further antiulcer studies.

Chemicals

Omeprozole was purchased from Sigma- Aldrich Chemicals, Mumbai. All other chemicals used were of analytical grade obtained from Merck, HiMedia and SD Fine Chemicals, Mumbai. All drugs and reagents were prepared immediately before use.

Experimental animals

Male Wistar albino rats (120–150 g), purchased from the Small Animals Breeding Station, Mannuthy, Kerala, India were used for the study. All the animals were housed in polyacrylic cages and maintained under standard environmental conditions (14h dark /10h light cycles; 25 ± 2°C temperature; 35-60% humidity; air ventilation) and were fed with a balanced commercial diet (M/s. Hindustan Lever Ltd., Mumbai, India) and fresh water *ad libitum*.

The animals were acclimatized to laboratory conditions for 15 days before the commencement of experiment. All animal experiments were conducted with the permission from Institutional Ethical Committee (1454/po/c/11/CPCSEA).

Experimental design

Ethanol- induced ulcers

The experiment was performed according to the method of Morimoto et al., [13]. Five groups of male wistar rats, each group consisting of six animals were fasted overnight prior to the start of the experiment. The first and the second groups received distilled water (10 mL/kg/day, p.o.), the third group was treated with the standard drug omeprazole (10 mg/kg/day, p.o.), while the fourth and fifth groups were administered with the *S. robusta* resin (SRR) (150 and 300 mg/kg/day, p.o. respectively). All the pretreatments were administered orally one time only. On the same day, 1 h after treatment, all rats received 1ml of absolute ethanol as a single oral dose to induce gastric ulcer [13]. After 1 h, the animals were sacrificed with overdose of diethyl ether and each stomach was examined for ulcer index.

Pyloric ligation (PL) induced ulcers

Male Wistar rats were divided into four groups of six animals each. Animals were fasted overnight prior to the start of the experiment and fresh water *ad libitum*. Control group of animals received distilled water (10 mL/kg/day, p.o.). Experimental groups received SRR, suspended in distilled water at a dose of 150 and 300 mg/kg/day p.o. and another group of animals received the reference antiulcer drug, omeprazole (10 mg/kg/day) orally for 3 days before subjecting them to ulcerogen. PL was performed by ligating the pyloric end of the stomach of all the rats on 3rd day under mild diethyl ether anesthesia [14]. Animals were allowed to recover and stabilize in individual cages and were deprived of water during postoperative period. After 4 h of surgery, rats were euthanized with chloroform and gastric juice was collected for performing gastric secretion study. The stomach was examined for ulcer index.

Ulcer index

Ulcer index was determined by following the scoring method of Suzuki et al., [15]. The sum of the length (mm) of all lesions for each stomach was used as the ulcer index (UI), and the protection percentage was calculated from the following formula: [(UI control - UI treated)/UI control] X100.

Biochemical parameters of stomach tissue in ethanol- induced ulcers

The stomach was washed in saline, blotted to dryness and homogenized in 0.15 M Tris HCl buffer (pH – 7.4) to give a 10% homogenate which was used for assaying the activities of enzymatic antioxidants like superoxide dismutase (SOD) [16], Catalase (CAT) [17], glutathione peroxidase (GPx) [18] and glutathione sulfonyl transferase (GST) [19] and also the level of lipid peroxidation (LPO) in terms of malondialdehyde (MDA) [20].

Collection of gastric juice

The gastric juice was collected 4 h after PL and centrifuged for 10 min at 3000 rpm. The volume of the supernatant was expressed as the amount of gastric juice secreted (mL/kg body weight). The pH of the gastric juice was measured using the pH meter (Cyber scan, India). Then gastric juice was subjected to biochemical estimation as follows.

Biochemical parameters of gastric juice in PL induced ulcerogenic rats

The gastric juice was subjected to the estimation of free and total acidity [21], total carbohydrate [22], pepsin [23] and protein [24] contents. The ratio of total carbohydrate to protein was taken as the index of mucin activity [25].

Statistical analysis

Data were analyzed for statistical significance using one way analysis of variance (ANOVA) followed by Dunnet's multiple comparison test and results were expressed as mean ± standard deviation using SPSS version 17.

RESULTS

Ethanol-induced ulcer

The antiulcerogenic effect of SRR on ethanol induced ulcer model in rats is presented in Table 1. Oral administration of ethanol produced severe ulcer index in the untreated animals (43.75 ± 3.59). However, pretreatment with SRR decreased the formation of ethanol induced ulceration in a dose dependent manner. At a dose of 300 mg/kg, SRR (ulcer index 16.32 ± 1.24; 62.69% protection) significantly ($P<0.001$) reduced the intensity of gastric mucosal damages which is comparable to that of the standard drug omeprazole (ulcer index 11.13 ± 3.22; 74.56% protection). The effect of SRR on enzymatic antioxidants and lipid peroxidation of stomach tissue is given in Table 2. The gastric damage induced by ethanol resulted in significant decrease in the activities of antioxidant enzymes like SOD, CAT, GPx and GST and subsequent increase in LPO. Pretreatment with SRR significantly ($P<0.001$) reversed the levels of enzymatic antioxidants and lipid peroxidation in a dose dependant manner. SRR at a dose of 300 mg/kg b.w. exhibits an equivalent activity to the standard drug omeprazole.

Pyloric ligation (PL) induced ulcers

The efficacy of treatment with SRR was evaluated on PL-induced ulcerogenic rats and the results are presented in Table 1 & 3. Oral pretreatment with SRR at a dose level of 300 mg/kg for 3 days remarkably prevented the adverse changes when compared with the induced group (28.38 ± 2.81), registering 64.55% protection and reduced ulceration index to 10.06 ± 1.32 which is comparable to that of the standard drug omeprazole (ulcer index 8.75 ± 1.27; 69.16% protection).

Compared with induced group, the rats treated with SRR 300mg/kg showed significant ($P<0.001$) reduction in gastric juice volume (65.44%), free acidity (33.06%), total acidity (26.98%) pepsin (44.39%) and protein (23.82%) with subsequent increase in carbohydrate (22.67%) and mucin (41.46%) in gastric juice. Further, the pH of the gastric juice increased from 1.23 to 4.54 (Table 3). These changes, however, were in line with the changes in the gastric juice of the animals treated with omeprazole, the reference antiulcer drug.

Table 1: Effects of *Shorea robusta* resin (SRR) extract on ethanol and pyloric ligation induced ulcers

Treatment group	Ethanol - induced ulcers		Pyloric ligation - induced ulcers	
	Ulcer index	Protection (%)	Ulcer index	Protection (%)
Control	-	-	-	-
Induced	43.75 ± 3.59$^{\#\#\#}$	-	28.38 ± 2.81	-
Omeprazole (10mg/kg)	11.13 ± 3.22***	74.56	8.75 ± 1.27***	69.16
SRR (200mg/kg)	30.15 ± 1.15***	31.08	18.19 ± 2.10***	35.90
SRR (400mg/kg)	16.32 ± 1.24***	62.69	10.06 ± 1.32***	64.55

Values are expressed as mean ± SD (n=6).

$^{\#}$Change in activities at $P<0.05$ when induced group compared to control group, $^{\#\#}$ $P<0.01$, $^{\#\#\#}$ $P<0.001$; *Change in activities at $P<0.05$ when test groups compared to induced group, ** $P<0.01$, *** $P<0.001$.

Table 2: Effect of *Shorea robusta* resin (SRR) extract on antioxidant markers against ethanol-induced ulcers in rat model

Parameter	Control (10mL/kg)	Induced (10mL/kg)	Omeprazole (10mg/kg)	SRR (200mg/kg)	SRR (400mg/kg)
SOD (Units/min/mg protein)	0.48 ± 0.01	0.20 ± 0.01###	0.40 ± 0.02***	0.31 ± 0.02***	0.35 ± 0.01***
CAT (µ moles of H_2O_2 consumed/min/mg protein)	21.58 ± 1.02	9.91 ± 1.01###	19.33± 0.02***	12.62± 0.12***	18.10± 0.61***
GPx (µ moles of GSH oxidized/min/mg protein)	13.87 ± 0.25	7.68 ± 0.15###	13.14 ± 0.27***	9.54 ± 0.28***	11.94 ± 0.34***
GST (µ moles of CDNB conjugation formed/min/mg protein)	46.69 ± 0.59	19.99 ± 0.76###	44.26 ± 0.27***	30.12 ± 0.65***	40.26 ± 1.02***
LPO (µ moles/mg protein)	0.82 ± 0.07	4.06 ± 0.22###	1.12 ± 0.06***	2.48 ± 0.08***	1.62 ± 0.08***

Values are expressed as mean ± SD (n=6).

#Change in activities at P<0.05 when induced group compared to control group, ## P<0.01, ### P<0.001; *Change in activities at P<0.05 when test groups compared to induced group, ** P<0.01, *** P<0.001.

Table 3: Effect of *Shorea robusta* resin (SRR) extract on certain biochemical parameters of gastric juice in pyloric ligation (PL)-induced ulcers in rat model

Parameter	Induced	Omeprazole (10mg/kg)	SRR (200mg/kg)	SRR (400mg/kg)
Gastric juice volume (mL/100g b.w.)	12.50 ± 0.51	3.12 ± 0.21***	8.45 ± 0.17***	4.32 ± 0.35***
pH	1.23 ± 0.14	4.68 ± 0.32***	3.06 ± 0.22***	4.54 ± 0.30***
Free acidity (meq/l/100g b.w.)	88.62 ± 5.61	55.27 ± 2.83***	70.45 ± 3.15***	59.32 ± 2.54***
Total acidity (meq/l/100g b.w.)	171.62 ± 7.68	115.32 ± 8.51***	145.39 ± 5.72***	125.32 ± 5.94***
Total carbohydrate (µg/mL)	419.00 ± 4.38	561.42 ± 7.15***	478.11 ± 6.04***	541.82 ± 7.91***
Protein (µg/mL)	578.10 ± 10.07	425.00 ± 15.48***	492.32 ± 8.10***	440.32 ± 10.15***
Pepsin (µg/mL)	17.18 ± 0.73	8.49 ± 0.52***	11.34 ± 1.06***	9.42 ± 0.30***
Mucin	0.72 ± 0.005	1.32 ± 0.033***	0.97 ± 0.003***	1.23 ± 0.009***

Values are expressed as mean ± SD (n=6).

*Change in activities at P<0.05 when test groups compared to induced group, ** P<0.01, *** P<0.001.

DISCUSSION

Plant extracts are some of the most attractive sources of news drugs, and have shown promising results for the treatment of gastric ulcer in several experimental models for evaluating antiulcer drugs [26]. In the present study, the possible antiulcerogenic effect of SRR commonly used in traditional medicine was investigated on ethanol and pyloric ligation- induced gastric ulcer models. Ethanol is one of the ulcerogenic agents that induce intense damage in gastric mucosa. Gastric lesion formation may be due to stasis in gastric blood flow which contributes to the development of the hemorrhage and necrotic aspects of tissue injury [27]. The ability of the gastric mucosa to resist injury by ingested irritants (e.g. alcohol) can be attributed to a number of factors that have been generally referred as mucosal defense [28]. The formation of gastric mucosal lesions by necrotizing agents, such as ethanol, has been reported to involve the depression of these gastric defensive mechanisms [29]. On one hand, ethanol administration reduces mucus production, gastric mucosal blood flow, bicarbonate secretion, endogenous glutathione and prostaglandin (PG) levels, and on the other hand it increases the release of histamine, the influx of calcium ions, the generation of free radicals and the production of leukotrienes [30]. The results show that SRR probably exhibit an antiulcerogenic effect related to cytoprotective activity, since it reduced significantly the ethanol-induced ulcer.

Oxidative stress plays an important role in the pathogenesis of various diseases including gastric ulcer, with antioxidants being reported to play a significant role in the protection of gastric mucosa against various necrotic agents. Reactive oxygen species are involved in the pathogenesis of ethanol-induced gastric mucosal injury *in vivo* [31]. In the present study, ethanol induction decreased the activities of SOD, CAT, GPx, GST and with subsequent increase in LPO. Pretreatment with SRR significantly increased the activities of these enzymatic antioxidants with a concomitant decrease in the levels of lipid peroxides, which suggests its efficacy in preventing free radical induced damage.

The effect of aqueous extract of SRR was also evaluated on the gastric secretory activity in rat pyloric ligation model. In pylorus ligated animals, the genesis of ulceration is due to the accumulation of acid and pepsin in the stomach leading to auto digestion of gastric mucosa and ulceration [14,32]. The increased gastric secretory volume and acidity observed in pylorus ligated rats were in accordance with earlier reports [33]. Like omeprazole, SRR decreased gastric acidity, and increased the pH of gastric secretions. Decrease in protein content also signifies decrease in leakage from the mucosal cells, indicating increased mucosal resistance [34]. The current data clearly demonstrated that SRR dose dependently decreased the gastric acid, and pepsin secretion indicating that it has both gastric anti-secretory and gastric cytoprotective effects.

S. robusta resin has been reported to contain several mono-, sesqui- and tri-terpenoids includes ursolic acid, tri and tetrahydroxy ursenoic acid, asiatic acid, α and β-amyrin, α –amyrenone, mangiferonic acid, benthamic acid and uvaol [35]. In general triterpenoids like ursolic acid and amyrin are well known for their antiulcer properties [36,37]. They exert their antiulcer effect by strengthening defensive factors such as stimulation of mucus synthesis or maintenance of prostaglandin contents of gastric mucosa at higher levels [38]. In addition these compounds act as antioxidant which protects gastric mucosa against oxidative damage [39]. It is, therefore, possible that the antiulcerogenic effects observed with the resin of *S. robusta* may be attributable to its phytochemical constituents and potent antioxidant activities.

The present study strongly demonstrated that *S. robusta* resin was able to protect the gastric mucosa from chemically and physically induced ulcers. These findings could justify, at least partially, the ethnomedicinal use of this plant in the management of gastric disorders.

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