INTERNATIONAL RESEARCH JOURNAL OF PHARMACY
www.irjponline.com
ISSN 2230 – 8407

Research Article

EVALUATION OF ANTI-ULCER ACTIVITY OF ETHANOLIC EXTRACT OF *DALBERGIA SISSOO* LEAVES IN EXPERIMENTAL ANIMALS

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Article Received on: 10/10/13 Revised on: 29/11/13 Approved for publication: 16/12/13

DOI: 10.7897/2230-8407.041205

ABSTRACT
Gastric ulcer is one of the major health problems in developing countries. Furthermore, high cost and of adverse effects are seen with the long term use of allopathic drugs in treatment of ulcer which are diminished by the use of herbal drugs. The aim of this study is to evaluate antiulcer activity of crude extract of *Dalbergia sissoo* leaves on experimentally induced gastric ulcer in wistar albino rats. Methodology involves screening for antiulcer activity of the plant using pylorus ligation and Indomethacin induced ulcer models in albino rats using 4 groups as; control (Tween 80 1 % v/v solution, 5 ml/kg), standard (Ranitidine 80 mg/kg), 250 mg/kg leaves extract and 500 mg/kg leaves extract given respective doses orally (p.o.). The parameters *viz.* mean ulcer index, percentage protection, gastric pH, protein, carbohydrate, pepsin, free and total acidity and ratio of total carbohydrates and proteins (TC:TP) were determined. The result showed the significant decrease in mean ulcer index of the leaves extract treated group in both the models compared to control. Furthermore, as obvious from pylorus ligation model the offensive factors like free and total acidity, pepsin content and protein content were decreased to significant levels whereas the defensive factors like total carbohydrate content and TC:TP ratio were increased significantly compared to control in dose dependent manner. The study concluded that leaves extract of *Dalbergia sissoo* had significant antiulcer activity and was not only effective in reducing the development of gastric ulcer but also increasing the healing of the gastric ulcer in dose dependent manner.
Keywords: *Dalbergia sissoo*, pylorus ligation, indomethcin induced ulcer, ulcer index, peptic ulcer.

INTRODUCTION

Peptic ulcer disorder (PUD) is a chronic inflammatory condition involving a group of disorders characterized by ulceration in regions of the upper gastrointestinal (GI) tract[1]. Peptic ulcer disorder embraces both gastric and duodenal ulcer and has been a major threat to the world's population over past two centuries, with a high morbidity and substantial mortality. Peptic ulcer and gastritis have been associated with multi-pathogenic factors such as disturbances in natural balances between aggressive (acid and pepsin) and maintenance of mucosal integrity through the endogenous defence mechanism (e.g. defensive mechanism of mucus, bicarbonates, mucosal blood supply, PGs etc.)[2-3]. With the greater understanding of the causes and pathogenesis of peptic ulcer, various treatment regimens have been established since 25-30 years for the effective management of the disorder. Despite the substantial advances, this disorder remains an important clinical problem, largely because of the increasingly widespread use of NSAIDs and low dose aspirin[4]. Almost 5–15 % of adult population of world is suffering from peptic ulcer disorder and its consequences[5]. Though prevalence of duodenal ulcer has been significantly decreased by 50 % than in past[6], its incidence seems to be in still alarming trend in developing countries. According to the WHO data published in 2011 peptic ulcer disorder deaths in India reached 108,392 or 1.20 % of total deaths being ranked 5th position in the world[7]. The availability of the proton pump inhibitors, histamine receptor blockers, drugs affecting the mucosal barrier and prostaglandin analogues have given relief to the disorder[8]. However, the clinical evaluation of these drugs showed development of tolerance and incidence of relapses and side effects that make their efficacy arguable[9]. Herbal drugs are more widely used than allopathic drugs as antiulcer because of them being inexpensive, better cultural acceptability, better compatibility with the human body and minimal side effects. *Dalbergia sissoo* (Family: *Fabaceae*[10]) is a deciduous tree with a light crown and an often crooked trunk found in the foothills of the Himalaya of Pakistan, Nepal, India and Bangladesh[11]. It's common name are Shinshapa, Indian Rosewood, Sisau, Sisham, Beeti (Sanskrit, English, Neapli, Hindi and Kannada)[12] which is extensively used in Ayurveda and other system of medicine for its clinical effects like aphrodisiac, abortifacient, expectorant, anithelminthic, antipyretic, and in the treatment of various digestive and skin disorders[13-14]. The sufficient scientific evidence to prove the antiulcer activity of leaves extract of the plant has not been established yet. Hence, the present study was undertaken to evaluate the effect of crude extract of *Dalbergia sissoo* leaves on experimentally induced gastric ulcer.

MATERIALS AND METHODS

Animals and chemicals

Albino wistar rats weighing between 180-220 g of either sex were used in the experiment. Animals were maintained under standard conditions in an animal house approved by Committee for the Purpose of Control, and Supervision of Experiments on animals (CPCSEA). Drugs, Ranitidine and Indomethacin along with the other chemical reagents were used in the experiment and all of them being of analytical grade. The study protocol was approved by Institutional Animal Ethical committee and was carried out in Mallige College of Pharmacy, Bangalore, India.

Plant material and preparation of extract

The leaves of *Dalbergia sissoo* were identified and collected from FRLHT (Foundation for Revitalisation of Local Health Traditions) gardens, Bengaluru, India and authenticated and

verified by senior botanist. A voucher specimen of the plant was preserved in the herbarium (number: MCP/DPCL/2012-13/305/35). The leaves of *Dalbergia sissoo* were chopped into small pieces and dried under shade at room temperature for 20 - 25 days, powdered using properly cleaned mechanical grinder and passed through the sieve (coarse 10). Thus prepared powder was extracted with 90 % ethanol in two lots in Soxhlet's extraction apparatus with each lot being extracted successively for 48 hours maintaining constant temperature (78°C) throughout the process. The extract was concentrated to dryness using rotary evaporator to obtain black to dark brownish mass.

Phytochemical screening

Ethanolic extract of the *Dalbergia sissoo* leaves was subjected to qualitative chemical tests for the identification of their active constituents in order to identify the various phytoconstituents as per the standard procedures[15-17].

Acute toxicity (LD_{50})

The ethanolic extract of *Dalbergia sissoo* having the same chemical constituents was found to be safe up to 5000 mg/kg body weight (P.O.) while tested in mice[18], hence $1/10^{th}$ and $1/20^{th}$ of no lethal dose were taken as effective dose (500 mg/kg and 250 mg/kg, B.W, p.o.) for the ethanolic extract of *Dalbergia sissoo* in evaluation of antiulcer potential in rats.

Pylorus Ligation induced ulcer model in rats (Shay rat 1945)

Healthy wistar albino rats of either sex weighing between 180-220 g were divided into four groups containing six rats each. First group, the control group received vehicle (Tween 80; 1 % v/v solution, 5 ml/kg, p.o.): second group, the standard drug treated group received Ranitidine (80 mg/kg, p.o.): third and fourth group received 250 mg/kg and 500 mg/kg, p.o. leaves extract respectively. All the groups were treated orally with respective agent for 14 days. The animals were fasted for 36 hours with water *ad. libitum* and care taken to avoid coprophagy and cannibalism by placing them individually in the cages. After fasting, different doses of test substances were administered orally with no water given during and after experimentation. Half an hour after drug administration, animals were anesthetized under light ether anaesthesia and the pylorus portion of the stomach was slightly lifted out and ligated, avoiding traction to the pylorus and damage to its blood supply. Stomach was placed back carefully and the abdominal wall was closed with sutures. The animals were deprived of food and water during the postoperative period and they were sacrificed six hours after pylorus ligation by over dose of ether anaesthesia. The stomach were isolated and the content of the stomach collected in labelled test tubes, centrifuged at 3000 rpm, supernatant collected and the parameters like pH, volume of the gastric juice, estimation of free acidity, total acidity, pepsin content, total proteins and total carbohydrates measured according to the standard procedures[22-25]. The stomach were then incised along the greater curvature and then washed with normal saline, pinned on the flat surface to observe for lesions/ulcer in the glandular portion of the stomach. The numbers of ulcer were noted and severity of the ulcer scored microscopically with the help of hand lens (10X) then ulcer index and percentage protection calculated[19-21].

Indomethacin induced gastric ulcer model

Healthy wistar albino rats of either sex weighing 180-220 g were divided into four groups containing six rats each. First group, the control group received vehicle (Tween 80; 1 % solution, 5 ml/kg, p.o.): second group, the standard drug treated group received Ranitidine (80 mg/kg, p.o.): third and fourth group received 250 mg/kg and 500 mg/kg, p.o. leaves extract respectively. All the groups were treated orally with respective agent for 14 days. On the 14^{th} day, gastric ulcer was induced with Indomethacin (40 mg/kg, p.o.) after fasting the entire group for 24 hours. The animals were then given overdose of ether anaesthesia and sacrificed 4 hours after the treatment with ulcerogenic agent, the stomach were removed then incised along the greater curvature and then washed with normal saline, pinned on the flat surface to observe for lesions/ulcer in the glandular portion of the stomach. The numbers of ulcer were noted and area measured severity of the ulcer scored microscopically with the help of hand lens (10X) and ulcer index and percentage protection calculated[19,26-27].

Scoring for ulcer and calculation of ulcer index

Based on their intensity of ulceration as observed from the hand lens the scores were given as:

0 = Normal stomach
1 = Superficial ulcer
2 = Deep ulcer
3 = Perforation

The ulcer index was determined using the formula:

$$\text{Ulcer Index} = U_N + U_S + U_P \times 10^{-1}$$

Where, U_N = Average of number of scores per animal
U_S = Average of severity score
And U_P = Percentage of animals with ulcer

The percentage protection was calculated by using the formula:

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

Where, Ut = Ulcer index of treated group and
Uc = Ulcer index of control group.

Statistical analysis

The values were expressed as mean ± SEM. Statistical analysis was performed by one way analysis of variance (ANOVA) followed by Dunnett's comparison tests using Graph Pad Prism version 6.00. P values < 0.05 were considered as significant.

RESULTS

Results for phytochemical screening

Results for the preliminary phytochemical screening of the *Dalbergia sissoo* ethanolic leaves extract revealed the presence of alkaloids, saponins, flavonoids, phenolics and tannins, terpenoids, glycosides, amino acids and proteins.

Results for Indomethacin induced gastric ulcer model

Table 1: Effect of ethanolic extract of leaves of *Dalbergia sissoo* on ulcer index and percentage protection in Indomethacin induced gastric ulcer model in rats

Groups	Ulcer Index	% Protection
Control	21.12 ± 2.01	-
RAN	4.45 ± 0.51***	78.92
250 LE	11.32 ± 0.71*	46.4
500 LE	9.5 ± 0.08**	55.01

N = 6 animals in each group. Values are expressed as Mean ± SEM. *P < 0.05, **P < 0.01 and ***P < 0.001 as compared to control.
Where, RAN: Group treated with Ranitidine. 250 LE: Group treated with 250 mg/kg leaves extract *Dalbergia sissoo.* 500 LE: Group treated with 500 mg/kg leaves extract of *Dalbergia sisssoo.*

Oral administration of ethanolic leaves extract of *Dalbergia sissoo* in two doses, 250 and 500 mg/kg showed significant reduction in the ulcer index values as compared to the control group in a dose dependent manner i.e. 500 mg/kg dose showing more reduction on the ulcer index value than 250 mg/kg dose. Similarly the percentage protection of the leaves extract in doses 250 mg/kg and 500 mg/kg was found to be 46.4 % and 55.01 % where standard drug Ranitidine in dose 80 mg/kg showed the protection of 78.92 % (Table 1).

Results for Pylorus ligation ulcer model

Table 2: Effect of ethanolic extract of leaves of *Dalbergia sissoo* on different parameters in Pylorus ligation induced ulcer model in rats

Parameters	Control	RAN	250 LE	500 LE
Ulcer Index	16.15 ± 1.01	4.8 ± 0.77**	8.51 ± 1.86*	7.2 ± 1.06**
% Protection	-	70.27 %	47.3 %	55.41 %
Volume of Gastric Juice (ml)	9.4 ± 0.87	2.1 ± 0.34**	4.6 ± 0.68**	3.8 ± 0.60**
pH OF Gastric Juice	2.5 ± 0.22	5.4 ± 0.23***	3.7 ± 0.15*	4.2 ± 0.19**
Free Acidity (mEq/L)	34.5 ± 2.08	5.8 ± 0.64***	15.8 ± 0.88**	9.4 ± 0.81***
Total Acidity (mEq/L)	75.8 ± 3.37	19.6 ± 1.89**	38.8 ± 1.76*	32.2 ± 2.52*
Total Pepsin (µg/ml tyrosine)	245.57 ± 3.93	142.63 ± 2.48****	220.38 ± 0.24**	198.76 ± 0.57***
Total Proteins (µg/ml)	405.29 ± 6.31	298.77 ± 5.14***	368.63 ± 4.88*	358.26 ± 3.41**
Total Carbohydrates (µg/ml)	474.81 ± 6.00	665.27 ± 4.73****	507.4 ± 2.57**	552.61 ± 3.21***
TC:TP RATIO	1.17	2.22	1.37	1.54

N = 6 animals in each group. Values are expressed as Mean ± SEM. *P<0.05, **P<0.01, ***P<0.001 and ****P<0.0001 as compared to control

Oral administration of ethanolic leaves extract of *Dalbergia sissoo* in two doses, 250 and 500 mg/kg showed significant reduction in the parameters like ulcer index, volume of gastric juices, free and total acidity, total protein contents, total pepsin content whereas significant increament in the parameters like pH of gastric juice, total carbohydrates content and TC:TP ratio in dose dependent manner. Similarly, the percentage protection against the ulcer induced was also increased in dose dependent manner showing high protection by 500 mg/kg than 250 mg/kg dose of the extrac where the percentage protection of the standard drug Ranitidine in dose 80 mg/kg was found to be 70.25 % which was highest among all the groups (Table 2). The results indicated that the leaves extract of the plant has potent antiulcer activity as revealed from both the experimental model.

DISCUSSION

Ulcer index values in both the screening models (Pylorus ligation and Indomethacin induced ulcer model) were reduced significantly as obvious from the results obtained, which provided the preliminary idea that the ethanolic extract of leaves of *Dalbergia sissoo* plant has potent antiulcer activity. Pylorus ligation induced gastric ulcer occurred due to increase in acid-pepsin accumulation due to pylorus obstruction and subsequent mucosal digestion. Results of the leaves extract of the plant *Dalbergia sissoo* revealed an increase in total carbohydrates and TC:TP ratio which suggested significant increase in the glycoprotein content from mucosal cells of the gastric mucosa. The decrease in protein content of gastric juice suggests the decrease in leakage of plasma proteins into gastric juice[28]. The mechanism of action of *Dalbergia sissoo* leaves extract may be due to the coating property leading to protective activity on gastric mucosa as obvious from the results where there is decrease in protein content of the extract treated groups. Acid is considered as an important factor in the development of acute and chronic gastric mucosal lesions. Suppression of gastric acid secretion by surgical and variety of pharmacological means provides effective and rapid healing of ulcer. In the present study, increase in pH, decrease in free and total acidity, decrease in volume of gastric juice and pepsin concentration were evidenced in ulcerated animals treated with leaves extract of the plant *Dalbergia sissoo*, which is highly desirable for gastro protection and antiulcer effect. Ulcerated rats showed an alteration in the peptic activity which is in accordance with the previous report[29]. The modification in pepsin concentration on leaves extract of the plant treatment depicts the efficacy of the extract on gastric secretions and it can be assumed due to the direct action on the acid producing cells. Increase in mucosal resistance and decrease in aggressive factors mainly acid and pepsin are associated with gastric protection offered by prostaglandins. PGE_2 and PGI_2 are the predominant PGs synthesized by the gastric mucosa and are known to inhibit the secretion of gastric acid and stimulate the secretion of mucus bicarbonate and hydrophobic surfactants like phospholipids secretion in gastric epithelial cells[30]. The possible involvement of extract of the plant *Dalbergia sissoo* on enhancing mucosal resistance could have offered gastro protection. NSAIDs such as Indomethacin have been reported to induce gastric ulceration due to inhibition of the

biosynthesis of cytoprotective prostaglandins, PGE_2 and PGI_2 (by inhibition of the cyclooxygenase pathway of arachidonic acid metabolism), resulting in overproduction of leukotrienes and other products of the 5-lipoxygenase pathway[31]. Indomethacin causes a direct irritation effect by increasing H^+ ion transport and free radical formation. It also decreases mucus content, surface-active phospholipid bicarbonate secretion, and mucosal proliferation[32]. The possible protective effect of the leaves extract of *Dalbergia sissoo* in Indomethacin-induced ulcer may be due to increased mucus secretion. Furthermore, phytochemical results and other reported works revealed the presence of flavonoids, saponins, phenolic compounds, alkaloids and tannins in the leaves extract of *Dalbergia sissoo*[33-34] and these substances have proved to be effective against healing the gastric ulcer by different mechanisms[35]. In our study, the efficacy of plant extract in experimental animal ulcer models is attributed to its cytoprotective effect. This cytoprotective effect may be due to the free radical scavenging activity or increase in mucus secretion.

CONCLUSION

In conclusion 90 % ethanolic extract of leaves extract of *Dalbergia sissoo* is effective in reducing the development of gastric ulcer and also increasing the healing of the gastric ulcer in dose dependent manner in both the experimental ulcer induced models. The antiulcer effect of the extract may be due to any of the probable mechanisms *viz.* reduction in gastric acid secretion, antioxidant action, muco protection or gastric cytoprotection attributed to the presence of flavonoids, phenolics, tannins, saponins or alkaloids as discussed earlier.

ACKNOWLEDGEMENT

We would like to provide humble gratitude to all the faculty members of Mallige College of Pharmacy for their inspiration and support. We express our sincere thanks to all the PG students of the college for their support to complete the work in timely manner.

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Cite this article as:

Baral Sanjay Raj, Swamy Shivakumar, Bhattarai Bimbishar, Dahal Prasanna. Evaluation of anti-ulcer activity of ethanolic extract of Dalbergia sissoo leaves in experimental animals. Int. Res. J. Pharm. 2013; 4(12):20-23 http://dx.doi.org/10.7897/2230-8407.041205