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DOES *DALBERGIA SISSOO* LEAVES POSSESS ANTI-ULCER ACTIVITY: AN EXPERIMENTAL STUDY IN ALCOHOL AND ASPIRIN INDUCED ULCERATIVE RAT MODELS

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Abstract

Ulcer is a common problem associated with NSAID's use. The use of plants to treat and cure diseases is common practice since ancient times because of people blind faith on safety of plant's use. The present study aimed to evaluate anti-ulcer activity of *Dalbergia sissoo* in alcohol and aspirin induced ulcerative rat models. Animals were divided into four groups. Group I was disease control, group II received standard drug and III-IV groups were administered leaves ethanol extract (orally) at 100 mg/kg and 200 mg/kg dose levels. Cumulative ulcer index score was calculated and anti-ulcer activity was determined on the basis of this calculated score. Results revealed significant anti-ulcer activity of leaves ethanol extract at 200 mg/kg dose in both ulcer models. This study provides scientific evidence of folklore use of leaves of *Dalbergia sissoo* in ulcer.

Keywords: Cumulative ulcer index score, anti-ulcer activity, *Dalbergia sissoo*

Introduction

Dalbergia sissoo, commonly called "shisham" and also known as Indian rosewood belongs to Family: *Fabaceae*. It is a perennial tree, available in India, Pakistan, Bangladesh, Afghanistan and sub-Himalayan region of Nepal. Besides its use as a timber or firewood, different ethnic groups use it to treat various ailments [1-3]. Traditionally leaves are used in breast swelling, sore throat, heart problem, dysentery, syphilis, gonorrhea, curing of boils and pimples, alleviation of perfuse menstruation and painful maturation. The mucilage of leaf is mixed with sweet oil for application on excoriations [4] Roots have astringent effect which is helpful in inflammation and infections; smoke obtained by burning the roots is helpful in bronchitis and headaches [5, 6]. The bark is useful as antidiarrhetics [5]. Pharmacological investigations revealed antioxidant, anti-inflammatory, antimicrobial and anidiarrhoeal activities of this plant [7-10]. Peptic ulcer is a gastrointestinal disorder that develops due to excessive secretion of gastric acid, pepsin or presence of *Helicobacter pylori*. The patients who are using NSAID's are prone to have gastric / peptic ulceration [11]. The use of medicinal plants to combat diseases is common from ancient times. Still the use of natural drugs is popular worldwide to cure the diseases [12]. Antioxidants help to fight against diseases [13]. *Dalbergia sissoo* possessed good antioxidant property so it is hypothesized that it may be helpful in the treatment of ulcer. This study was aimed to evaluate anti-ulcer activity of the leaves of *Dalbergia sissoo* in rats.

Materials and methods

Plant collection and authentication

Plant was collected from suburbs of Faisalabad. Identification and authentication was done by a Taxonomist of Botany Department, University of Agriculture, Faisalabad-Pakistan. Plant was shade dried for seven days and ground to fine powder.

Preparation of extract

Extract was prepared by cold maceration method using ethanol as solvent. Pulverized leaves were soaked in ethanol in 1:4 ratio for five days with occasional shaking at ambient temperature. The excess solvent was removed with rotary evaporator at 40 ^{o}C.

Approval by animal ethical committee

The study was conducted after getting approval from Animal Ethical Committee of Government College University, Faisalabad-Pakistan.

Animal husbandry

Wistar albino rats (150-200grams) of either sex were purchased from National Institute of Health (NIH), Islamabad. Animals were kept in animal house of the Faculty of Pharmaceutical sciences, Govt. College University, Faisalabad-Pakistan one week prior to start of study for the purpose of acclimatization. Animal house was operated at room temperature 22 ± 2ºC , humidity of 44 to 56%, and 12 hours night and day cycle. Rats were provided chow and water *ad libitum.*

Induction of ulcer

Ulcer was induced by two methods,

1. by administering 1 mL of alcohol (80 %) orally to all the rats
2. by giving aspirin (200 mg/kg) orally in the form of aqueous suspension to all experimental animals.

Study design for alcohol induced ulcer model

Rats were divided into four groups (n=6). Group I, served as control, received normal saline orally. Group II was administered ranitidine (20 mg/kg), orally; this is called standard group. Group III and IV were administered extract orally at 100 mg/kg and 200 mg/kg dose levels respectively. After one hour of dosing, all the rats were given 1 mL of 80 % alcohol to induce ulcer. All the rats were sacrificed after one hour of alcohol administration. Stomachs were excised, opened along the greater curvature and pinned on the soft board. Lesion were seen with magnifying glass and scored according to selected scale. Ulcer index and antiulcer activity were calculated.

Study design for aspirin induced ulcer model

Rats were divided into four groups (n=6). Group I, served as control and received normal saline orally. Group II was administered ranitidine (20 mg/kg), orally; this is called standard group. Group III and IV were administered extract orally at 100 mg/kg and 200 mg/kg dose levels respectively for one week. After one hour of dosing, all the rats were given aqueous suspension of aspirin (200 mg/kg) orally to induce ulcer. All the rats were sacrificed four hours after aspirin administration. Stomachs were excised, opened along the greater curvature and pinned on the soft board. Lesion were seen with magnifying glass and scored according to selected scale [Table 1]. Ulcer index and anti-ulcer activity were calculated.

Ulcer index was calculated by applying formula:

$$Ulcer\ index = \frac{(Un + Us + Up)}{10}$$

Where, Un is the average of number of ulcers per animal, U_S is the mean severity of ulcer score and U_p is the percentage of animals with ulcer incidence [14].

Anti-ulcer activity was calculated by applying formula:

$$\text{Anti} - \text{ulcer activity (\%age)} = \frac{\text{Ulcer index (control)} - \text{Ulcer index (treated)}}{\text{Ulcer index (control)}} * 100$$

Statistical analysis

Results were presented as mean ± S.D. One way ANOVA with tukey posthoc test was applied using SPSS verion 16 software. $P < 0.05$ was set as statistically significant value.

Results

Ulcer index score and anti-ulcer activity were determined in two ulcerative models of rats, a) alcohol induced model and b) aspirin induced model. In alcohol induced ulcerative model, cumulative ulcer index score of ranitidine (positive control), negative control and leaves ethanol extract (100 mg/kg and 200 mg/kg) were 0.80±0.037, 3.70±0.093, 1.87±0.22 and 1.40±0.22 respectively. There was significant decrease ($p < 0.05$) in cumulative ulcer index score of leaves ethanol extract treated group at 200 mg/kg dose level [Figure 1]. Leaves ethanol extract at 200 mg/kg concentration showed greater (61.67 %) anti-ulcer activity as compared to that of 100 mg/kg concentration whereas ranitidine had 78.23 % anti-ulcer activity [Figure 3]. In aspirin induced ulcerative model, cumulative ulcer index score for ranitidine (positive control),negative control and leaves extract (100mg/kg and 200mg/kg)were 0.80±0.037, 3.70±0, 1.92±0.217, 0.80±0.09 3 respectively.

Figure 2 showed that the treatment with *D. sissoo* extract at dose of 100 mg/kg and 200 mg/kg (Group 4) decreased the cumulative score of ulcer index to 1.92±0.217 and 0.80±0.093 respectively. Anti-ulcer activity of standard and leaves ethanol extracts (100 mg/kg and 200 mg/kg) were 78.23 %, 47.69 %, 78.28% respectively [Figure 4]. The maximum anti-ulcer activity was exhibited by leaves ethanol extract at 200mg/kg and this result was comparable to that of standard (ranitidine).

Discussion

Only cause of peptic ulcer was thought to be the acid secretion and sole approach towards therapy was reduction in acid secretion. However, the recent advances have changed this concept. The disturbance of natural balance between the aggressive factors and the defensive mechanisms is also major cause in peptic ulcer. First step towards ulcer formation is the alteration in the defensive mechanism of gastric mucosa although acid and pepsin are also involved as causative agents [15,16].

Various factors are involved in induction of ulcer and stomach mucosal injury in ulcer induced models of animals. The causative factors include weakening of stomach wall, mucosal injury as integrated by NSAID's and due to production of free radicals. NSAID's including aspirin cause injury to the stomach mucosa by causing reduction in prostaglandins level by causing inhibition of the synthesis of prostaglandins [17]. Ethanol induced stomach ulcer is assumed to be due to the appearance of stasis in the blood flow of stomach which is a causative factor in the development of the gastric hemorrhages and also the tissue injury due to necrosis. Alcohol is having an ability of rapid penetration into the mucus lining of the stomach thus enhancing the permeation of water and sodium into the intracellular membrane and resulting in cell and plasma membrane damage [18]. Damage caused to the stomach due to alcohol induced ulcer is also accompanied with significant production of oxygen free radicals and ultimate effect is generation of lipid peroxidation [19].The necrosis of the stomach mucosa by the ethanol takes place in multifactorial manner. Ethanol causes cell rupturing in the wall of blood vessels and by upsetting the mucus bicarbonate barrier; it can easily approach the mucosal lining of the stomach. Biological factors are the cause of such changes, such as lipid peroxidation, free radical generation, stress due to intracellular oxidation, variations in permeability and depolarization of the mitochondrial membrane preceding to cell loss [20, 21].

The ethanolic extract of the *Dalbergia sissoo* has significantly provided the strengthening of stomach mucosa against ethanol induced ulcer in rats as it is shown by decreased values of ulcer index in comparison with the control group. It suggests its potent cytoprotective effect. Similarly, in case of "aspirin induced ulcer model" of albino rats, ethanolic extract of *Dalbergia sissoo* elucidated in the results, reduced ulcer index and increased

antiulcer activity. Histopathological assessment of the tissues of gastric mucosa of rats has also established the fact that the ethanolic extract of *D. sissoo* has shown significant anti-ulcer effect in dose dependent manner [Figure 5]. A drug used in peptic ulcer ameliorates the disease either by reducing the synthesis of aggressive factors that induced ulceration or by enhancing the mucosal integrity against damage by these factors [22].

Conclusion

It was concluded from this study that ethanolic extract of *Dalbergia sissoo* leaves has anti-ulcer activity against alcohol and aspirin induced ulcers.

Conflict of interest

No conflict of interest among authors.

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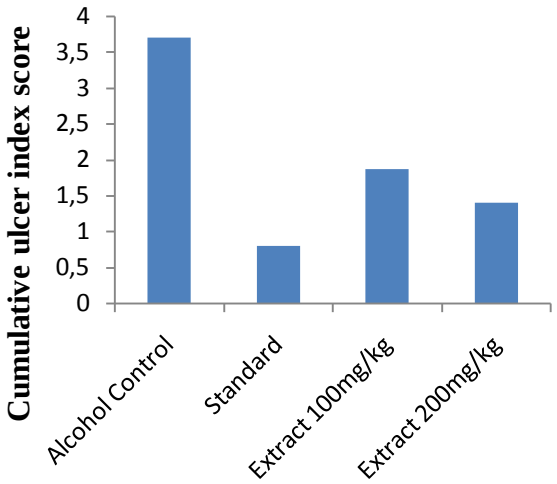

Figure 1. Cumulative ulcer index score for alcohol induced ulcer model

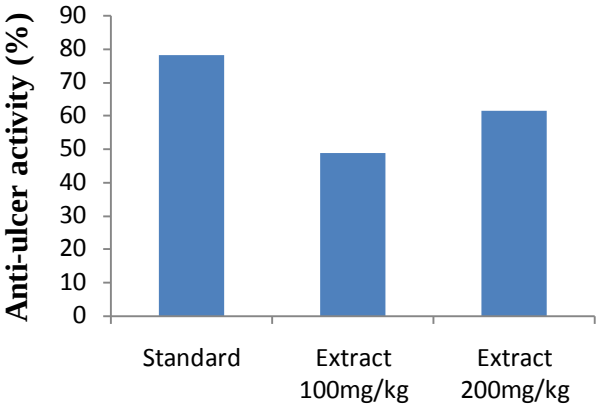

Figure 3. Anti-ulcer activity of leaves ethanol extract in alcohol induced ulcer model

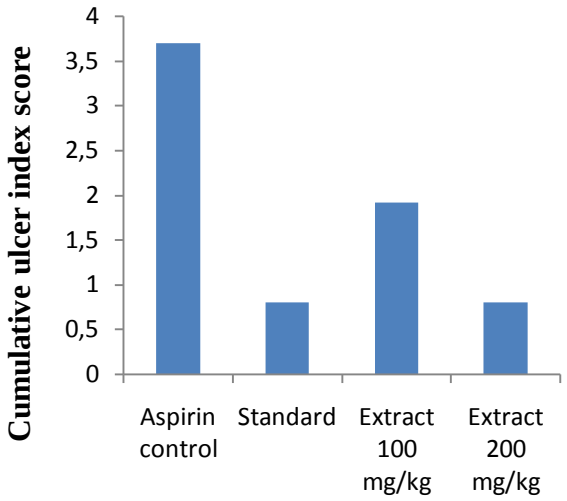

Figure 2. Cumulative ulcer index score for aspirin induced ulcer model

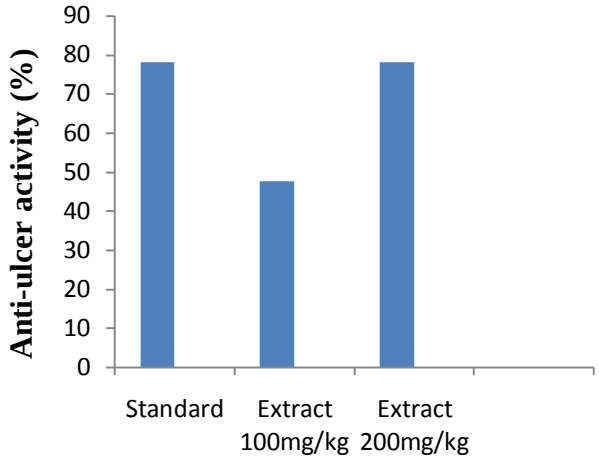

Figure 4. Anti-ulcer activity of leaves ethanol extract in aspirin induced ulcer model

Table 1. Selected scale to measure the ulcer index score

Score	Remarks
0	Normal coloration
0.5	Red coloration
1	Spot ulcer
1.5	Hemorrhagic stress
2	Deep ulcer
3	Perforations