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ORIGINAL ARTICLE

Gastroprotective Potential of *Dalbergia sissoo* Roxb. Stem Bark against Diclofenac-Induced Gastric Damage in Rats

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Abstract
Objectives: Dalbergia sissoo Roxb. stem bark possesses anti-inflammatory, antipyretic, and antioxidant properties. This plant is used traditionally in the Indian system of medicine to treat emesis, ulcers, leucoderma, dysentery, stomach complaints, and skin disorders. This study was conducted to evaluate the antiulcer effects of *D. sissoo* stem bark methanol extract (DSME) against the diclofenac sodium-induced ulceration in rat.
Methods: The DSME (200 mg/kg and 400 mg/kg body weight) was orally administered to rats once a day for 10 days in diclofenac-treated rats. The gastroprotective effects of DSME were determined by assessing gastric-secretory parameters such as volume of gastric juice, pH, free acidity, and total acidity. Biochemical studies of gastric mucosa were conducted to estimate the levels of nonprotein sulfhydryls (NP-SHs), lipid peroxidation [thiobarbituric acid reactive substances (TBARSs)], reduced glutathione (GSH), hydrogen peroxide (H_2O_2), levels of scavenging antioxidants, catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), glutathione-S-transferase (GST), and myeloperoxidase (MPO). Moreover, adherent mucus content and histological studies were performed on stomach tissues.
Results: Administration of DSME significantly decreased the ulcer index, TBARSs, H_2O_2, and MPO activity in gastric mucosa of the ulcerated rats. Activities of enzymic antioxidants, CAT, SOD, GSH-Px, GST and GSH, and NP-SH contents were significantly increased with DSME administration in the gastric mucosa of diclofenac-treated rats. Volume of gastric juice, total and free acidity were decreased, whereas pH of the gastric juice was increased with the administration of DSME + diclofenac. Our results show that DSME administration is involved in the prevention of ulcer through scavenging of free radicals. Results of histopathological studies supported the gastroprotective activities of DSME.
Conclusion: The results of this study showed that DSME exhibit potential gastroprotective activity probably due to its antioxidant and cytoprotection ability.

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1. Introduction

Nonsteroidal anti-inflammatory drugs (NSAIDs) such as diclofenac sodium [Sodium {2-[(2,6-dichlorophenyl)amino]phenyl}acetate] is used to induce ulcer in animal models. In humans, chronic administration of diclofenac sodium for the treatment of various diseases such as rheumatoid and osteoarthritis induces gastric ulcer in 35−60% of patients [1]. In general, diclofenac sodium is prescribed for its analgesic, antipyretic, and anti-inflammatory properties; its action is mediated by inhibition of the biosynthesis of prostaglandins, cyclooxygenase, and leukotriene [2].

Diclofenac sodium induces gastric mucosal lesions because of its acidic properties. A highly acidic gastric environment favors the migration of nonionized lipophilic diclofenac sodium into the epithelial cells, and at the surface these are dissociated into ions, trapping hydrogen ions and inducing mucosal injury. This action is further enhanced by the decrease of the following: mucosal blood flow, secretion of mucous and bicarbonates, and the defensive factors of the gastric layer [3]. Diclofenac sodium is also suggested to be involved in oxidative stress in mucosal cells, another etiopathogenic factor inducing gastric ulcer. All these factors cause an imbalance between the acid−pepsin secretion and defensive factors including secretion of mucin and shedding of cells [4].

The effect of diclofenac can be minimized by the proper use of antioxidants that ameliorate the free radicals. Plants possess valuable phytochemicals in the form of secondary metabolites of which flavonoid and phenolics are of great concern for antioxidant properties. In recent years, studies on antioxidants have received much attention as these chemicals can help defend the biological systems from diseases and injuries. The traditional drugs used in the treatment of a gastric ulcer are histamine (H_2) receptor antagonists, proton-pump inhibitors, antacids, and anticholinergics. Most of these drugs have severe unwanted side effects and drug interactions [5]. However, alternative and complementary systems in medicament can provide additional therapeutics for gastric damages.

Dalbergia sissoo is native to Pakistan, India, Bangladesh, Nepal, and Afghanistan. Chemical characterization of *D. sissoo* bark revealed the presence of flavonoids, furans, benzophenone, styrenes, and terpenoids [6]. Its bark exhibits anti-inflammatory, antipyretic, and antioxidant properties [7]. This plant is traditionally used to treat emesis, ulcers, leucoderma, dysentery, stomach complaints, and skin disorders [8].

To the best of our knowledge no experimental evidence is available to prove the gastroprotective effect of *D. sissoo* stem bark extract. This study was undertaken to evaluate the antiulcer effects of crude methanol extract of *D. sissoo* (DSME) stem bark on a diclofenac sodium-induced gastric ulcer in rats.

2. Materials and methods

2.1. Plant collection and extract preparation

Shade-dried bark (2 kg) of *D. sissoo* collected in September 2010 from the Sargodha district (Pakistan) was mechanically grinded into a powdered form and extracted twice in 4 L of 95% methanol for 1 week. The filtrates obtained were combined and evaporated through rotary vacuum evaporator to get 7.36% (147.25 g) of DSME and were stored at 4 °C.

2.2. Animal treatment

Twenty-five Sprague-Dawley rats of either sex with weight ranging from 150 g to 200 g were acclimatized for 2 weeks in ordinary cages at a room temperature of 25 ± 3 °C with a 12-hour dark/light cycle. Use of animals for all experimental procedures was conducted in accordance with the guidelines of the National Institutes of Health (Islamabad, Pakistan). The study protocol was approved by the Ethical Committee of Quaid-i-Azam University (Islamabad, Pakistan).

Animals were divided into five groups with five rats in each group. All animal groups were fasted for 12 hours prior to each administration. Rats in Group I were untreated (control) and had free access to food materials. Diclofenac sodium [50 mg/kg body weight (bw)] was intragastrically administered to animals of Groups II, III, and IV once a day for 10 days. However, rats of Groups III and IV were also administered with 200 mg/kg and 400 mg/kg bw of DSME once a day for 10 days. Animals of Group V were treated with 400 mg/kg bw of DSME alone [9].

2.3. Pyloric ligation

Twenty four hours after the last treatment, pyloric ligation was done for 4 hours to collect the gastric juice. The animals were anesthetized, the abdomen was opened by making a small midline incision, and the pyloric stomach was ligated with a thread by avoiding damage to its blood supply. The abdominal wall was closed by interrupted sutures.

2.4. Determination of acid-secretory parameters

The animals did not have access to both food and water during the postoperative period, and were killed after 4 hours of pyloric ligation. The stomach was dissected out along the greater curvature, the gastric juice was drained off and centrifuged at 4000 rpm for 10 minutes. The volume of gastric juice (mL/100 g/4 hours) and pH were estimated. Free acidity and total acidity were estimated according to Card and Marks [10].

2.5. Ulcer index studies

For ulcer index studies, any damage to gastric mucosa, bulging, and/or inflammation were recorded (in millimeter) for each lesion in the stomach [11].