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Research Article

Antiulcer Activity of the Root Bark of *Oroxylum indicum*. Against Experimental Gastric Ulcers

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Abstract

The current study was undertaken to investigate the effect of the root bark of Oroxylum indicum. alcohol extract troleum ether, ch ethanol-induced ifferent fractions ation. Out of all the s showed max sal damage. The resu ethanol-induced meprazole showed extent of lipid per long with

glutathione levels (GSH), when compared with the control group. In 6-h pylorus-ligated animals, active fractions of drug at 100 mg/kg showed significant reduction in the ulcer index. Furthermore, in the pylorus-ligation model, significant reduction ($p < 0.05$) was observed in total acidity, total acid output, pepsin activity, and pepsin output, along with a significant rise in the total carbohydrate to protein ratio (reflecting mucin activity) when compared with the control group. TLC studies revealed the presence of baicalein in the petroleum ether and hydrosylate in n.-butanol fraction. Fingerprinting of both the active fractions was developed by performing HPLC analysis. Baicalein was found to be a major flavonoid present both in petroleum ether and n.-butanol hydrosylate. The mechanism of its antiulcer activity could be attributed to a decrease in gastric acid secretory and antioxidant activities leading to gastric cytoprotection. This activity could be linked to the presence of baicalein in the root bark of the plant.

Keywords: Antioxidant activity | antiulcer activity | gastric ulcer | Oroxylum indicum

Introduction

Oroxylum indicum. Vent. (Bignoniaceae), commonly known as Syonakh, has been selected for the current study. This plant is used as an astringent, carminative, diuretic, stomachic, and aphrodiasic and is valued for stimulating digestion, curing fevers, coughs and other respiratory disorders (John, 2001). Oroxylum indicum. is used as one of the important ingredients in the commonly used Ayurvedic preparation “dasamula.” It is also yarista, narayan alwha, and so forth tory, diuretic, 1995). The stem ba s, namely, chryc Seeds of this p ver, the root bark of C ological activity. hol extract of Oroxy gastric ulcer models.

Materials and Methods

Procurement of plant material and extraction procedure

The fresh root bark of Oroxylum indicum. was collected in January 2005 from Vanaushadhi Ektrikaran Udyan, Ahwa, Dang Forest, Gujarat, India. The authentification of this plant was established by the taxonomist of Gujarat Ayurved University, Jamnagar, India, and a voucher specimen (404) was deposited in the Department of Pharmacognosy and Phytochemistry, L. M. College of Pharmacy, Ahmedabad, India. The root bark was sun-dried and powdered to 60 mesh. The powder of root bark after defatting with petroleum ether (0.32% w/w) was dried and then moistened with ammonia solution and extracted with chloroform (0.78% w/w), ethyl acetate (1.52% w/w), and n.-butanol (1.68% w/w), successively. The dried fractions were stored in an air-tight borosil glass container until further use.

Drugs and chemicals

Omeprazole was obtained from Zydus Research Centre, Ahmedabad, India. All different organic solvents and reagents used for the current study were of analytical grade (AR) and obtained from S.D. Chem. Pvt. Ltd. (Mumbai, India). The standard baicalein was obtained from Sigma-Aldrich (St. Louis, MO, USA). Fresh drug solutions were prepared in 1% carboxy methylcellulose (CMC) and were administered orally.

Animals

Wistar albino rats (Zydus Cadila Limited, Ahmedabad, India) of either sex weighing 175–225 l chow diet and wat ons of a 12-h dark-ligh phagy (and thus re-i es with gratings ce of trials, and t utions of drugs ed with universit dy period, the rats to be certain t ls were treated i were

experiments. The stomachs were removed, opened along the greater curvature, washed with saline, and examined using a 6.4 binocular magnifier. Lesions were assessed by two unbiased observers.

Methodology

The animals were divided into following groups of six.

- Group I (control): Rats received only aqueous suspension of 1% CMC vehicle with respect to the individual ulcerogenic procedure.
- Group II (drug treatment): Rats received the following treatments: 50% alcohol extract, petroleum ether, chloroform, ethyl acetate, and n.-butanol extracts (100–300 mg/kg, p.o.).
- Group III: Rats received standard omeprazole (20 mg/kg, p.o.) 1 h before the ulcerogenic procedure.

Ethanol-induced gastric mucosal damage

Gastric lesions were induced by 1 ml absolute ethanol in 24-h fasted rats as per the method of Robert (1979). In the treatment group, drug extracts in 1% CMC solution were administered orally 1 h before the administration of ethanol. Animals were sacrificed 2 h after the ethanol administration, and gastric lesions were measured in terms of ulcer index (UI) determined by the method of Goswami et al. (1997). Each lesion of the stomach was measured along the greatest length and breath. For circular lesions, [illegible]es, five of them we[illegible]al area of the stom[illegible]ndex = 10/X, where X [illegible] drug administ[illegible]luated. The stomach[illegible]ff, washed with[illegible]ffer (10 mM, pH 7.4). [illegible]using Remi C-24 hig[illegible]l for the estimati[illegible]ymes (superox[illegible]or [illegible]

method of Mishra et al. (1973). Catalase (CAT) activity was measured according to the method of Aebi (1974). The reduced glutathione (GSH) was determined by the method of Beutler et al. (1963). The protein concentration in all samples was determined by the method of Lowry (1951).

Pylorus-ligation (PL) model

Rats fasted for 24 h were anesthetized with ether, and a portion of abdomen was opened by a small midline incision below the xiphoid. The pylorus portion of the stomach was lifted and ligated (with care being taken not to occlude blood vessels) by the method of Shay et al. (1945). The stomach was closed with interupted sutures. Six hours after the pylorus ligation, animals were sacrificed. The stomach was dissected and the contents collected, measured, centrifuged, and subjected to biochemical analysis described below. Parameters investigated include: a ulcer index (UI) as described earlier, b acid secretory parameters, and c mucoprotective parameters. Acid secretory parameters include measurement of volume of gastric secretion, total acidity determined by titrating against 0.01 N sodium hydroxide to pH 8.0 using phenolphthalein as an indicator (Hawk et al., 1954), and total acid output (product of total acidity and volume of gastric secretion). Further, pepsin activity was determined using hemoglobin as a substrate, according to the modified method of Debnath et al. (1974). Total carbohydrates (TC) (Nair, 1976), total protein content (PR) (Lowry et al., 1951), mucin activity (TC/PR), and gastric mucus content (g) (Alarcon et al., 1993) were considered as a measure of the mucoprotective parameters.

Fingerprinting and estimation of flavonoid

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studies (TLC, HPLC). TLC co-chromatography was performed on the petroleum ether fraction, the hydrolyzed n.-butanol fraction, and standard baicalein.

Method of TLC analysis

Ten microliters of each sample solution was spotted on the TLC plate (precoated with silica gel 60 F_{254}, thickness 0.2 mm, 20 × 20 cm) (E Merck, Darmstadt, Germany) along with a standard solution of baicalein. Chromatogram was developed using chloroform:ethyl acetate:formic acid (10:8:2) as a mobile phase and visualized using natural product poly ethylene glycol (NP/PEG) reagent. On the basis of the TLC study, an HPLC method was developed for the quantification of baicalein in both the active fractions and development of fingerprints of the same.

HPLC analysis

Chemicals: Methanol (HPLC grade), acetonitrile (HPLC grade), water (HPLC grade), trifluoroacetic acid (TFA) (analytical grade), baicalein (Sigma-Aldrich, Powai, Mumbai). HPLC was performed on a Shimadzu 2010 C (Tokyo, Japan), equipped with a C-18 Hypersil BDS column (250 × 4.6 mm, 5 µm). The instrument was operated under the following conditions: UV visible detector 254 nm, flow rate of 1.0 ml/min, retention time 40.5 min, injection volume 10 µl, and mobile phase A, water (pH = 2.70 adjusted with dilute H_2SO_4), mobile phase B, acetonitrile [diluent methanol: water pH = 3.0 with TFA (8:2)]. HPLC analysis of both the fractions was carried out for developing fingerprinting and also to verify the presence of baicalein in these extracts.

Sample

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The results were expressed in terms of mean±SEM. The significance of difference between mean values for the various treatments was tested using one-way analysis of variance test (ANOVA test) followed by Tukey's multiple range tests (Bolton, 1997) wherever applicable to assess statistical significance of difference between the groups.

Results

Ethanol-induced gastric mucosal damage

Alcohol extract and the different fractions (300 mg/kg) showed a significant reduction in the ulcer index when compared with the control group, and results were comparable with the omeprazole-treated rats (Table 1). Reduction in the ulcer index was found to be maximum with both the n.-butanol (99.5%) and petroleum ether (96.0%) fractions at 100 mg/kg dose level as compared with control and omeprazole (99.5%) treatment (Table 2).

Table 1. Effect of different extracts (300 mg/kg, p.o.) of Oroxylum indicum. on ethanol-induced gastric mucosal damage in rats.

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Table 3. Effect of different extracts of Oroxylum indicum. (p.o.) on lipid peroxidation and antioxidant enzymes against ethanol-induced gastric mucosal damage.

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Pylorus-ligation gastric ulcer model

The petroleum ether, n.-butanol fractions, and omeprazole pretreated rats showed significant reduction in the ulcer index when compared with the control group (Table 4).

Table 4. Effect of active fractions (100 mg/kg, p.o.) of Oroxylum indicum. on pylorus-ligated gastric ulcer model.

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Effect on acid secretory parameters

Both the active fractions of drug and omeprazole treatment showed significant decrease in the volume of gastric secretion along with significant increase in the gastric pH, as compared with control group. They also showed significant reduction in total acidity, total acid output, pepsin activity, and pepsin output as compared with control group (Table 5).

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activity. Therefore, TC:PR ratio (mucin activity) was significantly increased by both fractions. Furthermore, the gastric mucus content was found increased in petroleum ether and n.-butanol fractions pretreated animals as compared with control group. Omeprazole treatment also showed significant rise in mucus content of gastric mucosa (Table 6).

Table 6. Effect of active fractions of Oroxylum indicum. (100 mg/kg, p.o.) on mucoprotective parameters in pylorus-ligated gastric ulcer model.

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Fingerprinting and estimation of baicalein

Based on the results of antiulcer activity, TLC study was aimed at checking the presence of baicalein in active fractions. Baicalein is reported to be present in stem bark and leaves of Oroxylum indicum.. Our observations on TLC support the presence of baicalein, a major flavonoid (Fig. 1). Further, the authentic sample of baicalein resolved at 0.42 retention time (R_t), and nearly the same R_t was observed with the use of petroleum ether and hydrolyzed n.-butanol fractions (Fig. 2; Table 7). The quantification and validation of baicalein was done by using a calibration curve prepared under same HPLC conditions (Fig 3; Table 8). Hydrolyzed n.-butanol fraction showed 12% of baicalein as compared with that of 5% of baicalein in petroleum ether fraction. etter absorpti

Figure 1. Detected in UV (254 um ether fraction;

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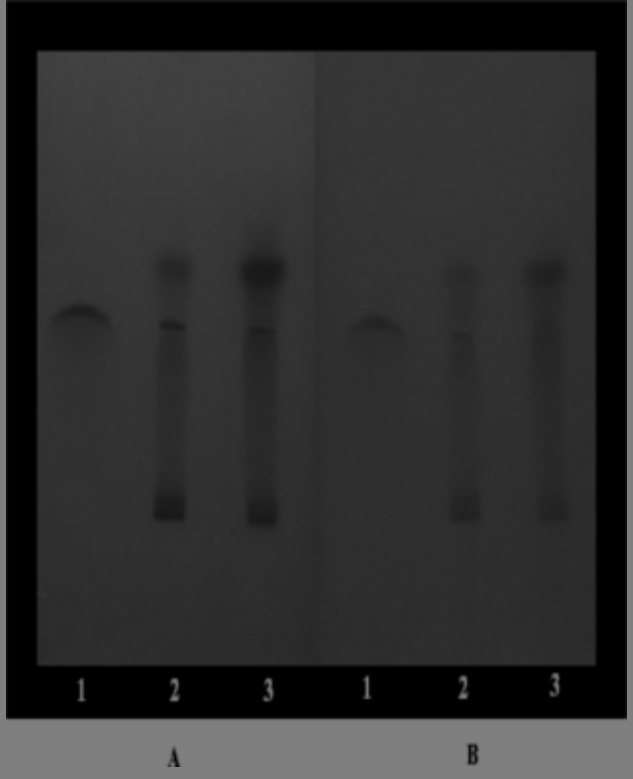

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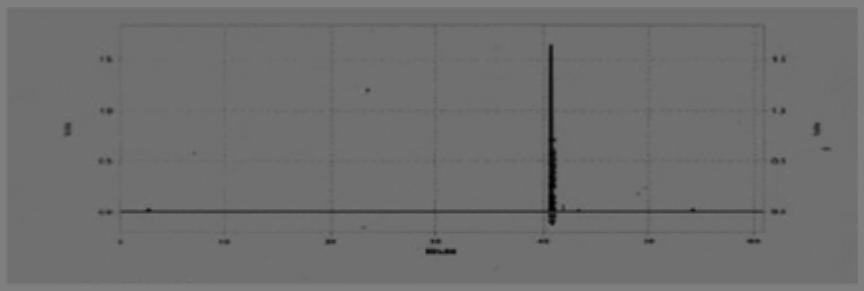
Figure 2. HPLC chromatograms of active fractions and standard baicalein. (a) Standard baicalein, (b) petroleum ether fraction, (c) hydrolyzed n.-butanol fraction.



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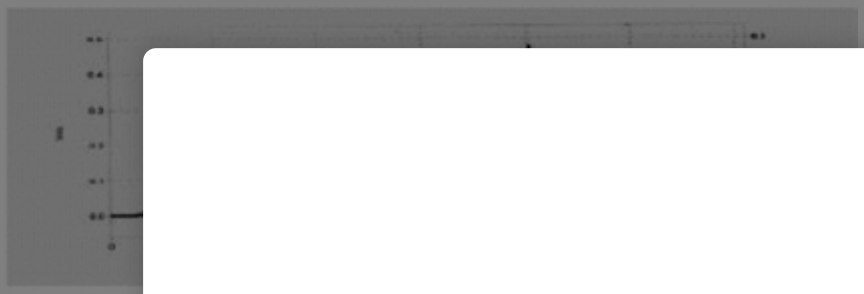

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Figure 3. Calibration curve of standard baicalein.

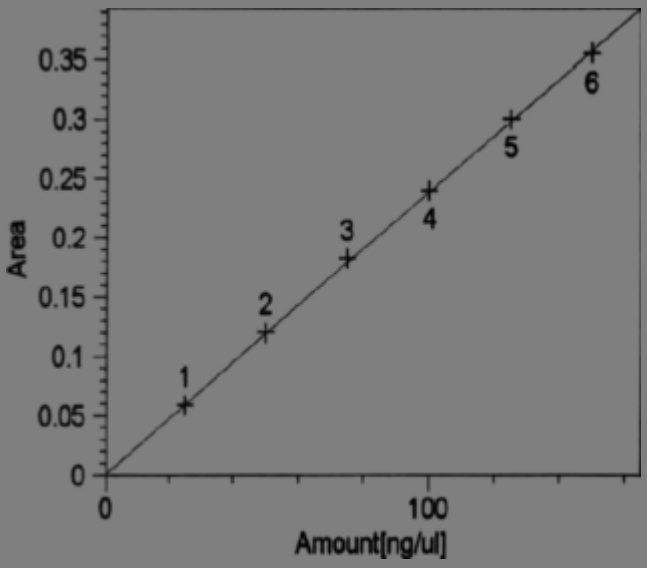

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Table 7. Percent of reference standard baicalein in active fractions of Oroxylum indicum..

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Table 8. Validation parameters of baicalein by HPLC.

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activity in this model along with alteration in antioxidant enzyme status. Preventive antioxidants, superoxide dismutase (SOD), and catalase (CAT) are the first line of defense against reactive oxygen species (Halliwell, 1995). In addition, reduced glutathione (GSH) is a major low-molecular-weight scavenger of free radicals in the cytoplasm and an important inhibitor of free radical–mediated lipid peroxidation (Piper & Stiel, 1986). It was observed in our study that the drug pretreatment resulted in significant reduction in MDA content, along with significant rise in SOD, CAT, and reduced GSH levels, suggesting their efficacy in preventing free radical–induced damage. The mechanism of antiulcer activity in this model, therefore, can be attributed to the free radical scavenging activity of this drug that in turn might lead to gastric cytoprotection.

Gastric acid and pepsin are important factors for the formation of ulcers in pylorus-ligated rats (Shay et al., 1945). Increased synthesis of nucleic acids and metabolism of carbohydrates and other compensatory mechanism could also be responsible for the ulceration due to pylorus ligation (Robert et al., 1984). We observed significant reduction in total acidity and pepsin activity along with significant increase in the gastric pH in drug-treated animals. Besides, there was a significant rise in mucin activity and mucus content. Therefore, it is suggested that the fractions suppressed the gastric damage caused by aggressive factors and cause increase in defensive factors in terms of gastroprotection.

Preliminary screening using TLC revealed the presence of baicalein as one of the flavonoids in both petroleum ether and hydrolyzed n.-butanol fractions. RP-HPLC analysis [illegible] the baicalein in both t[illegible]ivity (Ng et al., 200[illegible] Thus, the antiulce[illegible]ed to baicalei[illegible]re, it is evident[illegible]otection than[illegible]se mechan[illegible]

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It is concluded that both the n.-butanol and petroleum ether fractions of Oroxylum indicum. possess significant antiulcer activity. There was an inhibitory effect on acid secretory mechanisms and free radical scavenging activity and a significant rise in gastric mucin activity. Further, with the help of HPLC-based profiling techniques, the antiulcer activity could be linked to a significant extent to the presence of baicalein in both fractions.

Acknowledgment

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