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Gastroprotective effects of root bark of *Oroxylum indicum*, vent.

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Abstract

Objective: The present study was designed, to evaluate the gastroprotective effects of root bark of *Oroxylum indicum*, against gastric ulcers. Oxidative stress is considered to be important factors in the pathogenesis of gastric ulcers. Materials and method: The 50% alcoholic extract of root bark of *Oroxylum indicum* and its different fractions viz. petroleum ether, chloroform, ethyl acetate and *n*-butanol were studied (p.o.) against ethanol-induced gastric mucosal damage. Further, *n*-butanol fraction was also studied in Water Immersion Plus Restraint Stress (WIRS) - model. The parameters studied include ulcer index, score for intensity intraluminal bleeding and antioxidant activity. Results: Alcoholic extract (300 mg/kg) and its different fractions (100 and 300 mg/kg) showed significant reduction in gastric ulceration against ethanol-induced gastric damage. Out of all these fractions, *n*-butanol fraction showed significant maximum (99%) inhibition of gastric lesions. In WIRS- model, pretreatment with *n*-butanol fraction showed significant antiulcer and antioxidant activity in gastric mucosal homogenates, where it reversed the increase in ulcer index (UI), lipid peroxidation (LPO) and decrease in superoxidedismutase (SOD), catalase (CAT) and reduced glutathione (GSH) levels induced by stress. Conclusion: The present study reveals significant gastroprotective effect of *n*-butanol fraction against both ethanol and WIRS-induced gastric ulcers in rats. The ulceroprotective activity of *n*-butanol fraction could be mediated through its antioxidant activity, vasodilatation, and gastric cytoprotection.

Key words: *Oroxylum indicum*, stress ulcer, antioxidant activity, *n*-butanol.

1. Introduction

A medicinal plant *Oroxylum indicum*, vent. (Syonakh) belonging to the family Bignoniaceae, used in folk medicine as a cure of various diseases, was selected under the present study. It is an indigenous plant, found in India, Ceylon, Malaysia, China, Philippines and Indonesia [1]. It is used in traditional Ayurvedic medicine to alleviate thirst, rheumatism, dysentery, anorexia, bronchitis, eruptive fevers and dropsy. This plant is used as an astringent, carminative, diuretic, stomachic, antipyretic, aphrodisiac and for respiratory disorders [2]. The plant is reported to possess anti-inflammatory, diuretic, anti-arthritic, antifungal and antibacterial activity [3]. The stem bark and leaves of this plant

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are reported to contain flavonoids namely, baicalein, chrysin, oroxylin-A, and scutellarin [4, 5]. Seeds of this plant are reported to contain ellagic acid [6]. Flavonoid such as baicalein is reported to possess an anti-inflammatory [7], anti-ulcer [8], antioxidant [9], hepatoprotective [10] and immunomodulatory activity [11], while chrysin and baicalein both are reported to have antibacterial, antifungal and antiviral activity [12,13]. Most plant extracts composed of complex phytoconstituents.

Oxidative stress is considered to be one of the important etiological factors in various diseases including gastric ulcers [14]. Ulcers are caused due to imbalances between mucosal offensive and defensive factors of the gastric mucosa. Therefore, in the light of above, the present study was undertaken to evaluate the anti-ulcer effects of different extracts of root bark of *Oroxylum indicum* against ethanol-induced gastric mucosal damage and WIRS-induced gastric ulcer in rats.

2. Materials and methods

2.1 Plant material

The fresh root bark of *Oroxylum indicum* (Family: Bignoniaceae) was collected in the month of January, from Van-aushadhi Ektrikaran Udyan, Ahwa, Dang forest, Gujarat. The authentification of this plant was established by the taxonomist of Gujarat Ayurved University, Jamnagar, India and a voucher specimen (404) deposited in the Department of Pharmacognosy and Phytochemistry, L. M. College of Pharmacy, Ahmedabad, India.

2.2 Extraction of plant material

The root bark was dried and powdered to a 60 mesh size (=250 μm). The powder of the root bark after defatting with petroleum ether (0.32% w/w) was dried, then moistened with ammonia (NH_3) 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 at 4°C in a borosil glass container.

2.3 Experimental animals

Wistar albino rats of either sex weighing 150-250 g were selected for the study. Rats were fed a standard rat chow diet and water, which was freely available under standard conditions of a 12 h dark-light cycle, 60 ± 10% humidity and a temperature of 21.5 ± 1°C. Coprophagy was prevented by keeping the animals in cages with gratings on their floor. The distribution of animals in the groups, the sequence of trials and treatment allotted to each group was randomized. Freshly prepared solutions of drugs/chemicals were used through out the study. After completion of the experiments, animals were sacrificed with the use of high dose of ether. This experiment complied with the guidelines of our laboratory for animal experimentation.

2.4 Drugs and chemicals

Omeprazole was obtained from Zydus research centre, Ahmedabad as a gift sample. All different organic solvents used for extraction were obtained from the S.D. Chem. Pvt. Ltd. (Mumbai, India), and were analytical grade (AR grade). Fresh drug solutions were prepared in 1% carboxymethylcellulose (CMC) and were administered orally. Hydrogen peroxide, and Ciocalteu phenol reagent were obtained from S.D. Fine Chemicals Ltd. Trichloroacetic acid, thiobarbituric acid, phosphate buffer, Tris buffer, 5, 5'-dithiobis-2-nitrobenzoic acid (DTNB), bovine serum albumin, and epinephrine were obtained from Sigma-Aldrich (St Louis, MO).

2.5 Methodology

The following models were studied to assess the anti-ulcer property of root bark of *Oroxylum indicum*.

2.5.1 Ethanol- induced gastric mucosal damage [15]

The animals were divided into following groups of six animals each.

Group-I : Animals received only aqueous suspension of 1% CMC as vehicle with respect to the individual ulcerogenic procedure (control).

Group-II : Animals received following treatments: 50% alcoholic extract, petroleum ether, chloroform, ethyl acetate and *n*-butanol extracts (100-300 mg/kg, p.o.).

Group-III : Animals received omeprazole (20 mg/kg, p.o.) 1 h before the ulcerogenic procedure (standard).

Gastric lesions were induced by 1 ml absolute ethanol in 24 h fasted rats as per the method of Robert (1979) [15]. In the treatment group, drug extracts were administrated orally 1 h before the ethanol treatment. Animals were sacrificed 2 h after the ethanol administration, stomachs were removed, opened along the greater curvature, washed with saline and examined for lesion severity by using a 6.4 binocular magnifier. The gastric lesions were measured in terms of ulcer index (UI). Lesions were assessed by two observers unaware of the experimental protocol.

Physical parameter

2.5.5.1 Ulcer index (UI) [16]

Each lesion of the stomach was measured along the greatest length and breath. For circular lesions, the diameter was measured and area calculated. In case of petechies, five of them were considered to be equivalent to 1mm^2 of ulcerated area. The total area of the stomach mucosa and that of ulcerated mucosa were calculated.

Ulcer index = 10 / X,

Where X= (Total mucosal area)/ (Total ulcerated area).

2.5.2 Water immersion plus restraint stress-induced gastric ulceration [17]

The animals were divided into three groups of six animals each as described in earlier model. However, based on the results of ethanol model, the most active fraction, *n*-butanol was given as a part of the treatment in the group - II.

Rats were fasted for 12 h, care being taken to avoid coprophagy. The rats were immobilized in a restrainer and subsequently they were immersed in the water up to xiphoid process for 7 h. The temperature of the water was maintained at 24 ± 1°C. Drug treatment was given orally 30 min prior to the restraint procedure. After 7 h of immobilization and water immersion procedure, the animals were taken out and sacrificed with high dose anesthetic ether. The stomach was removed and the severity of intraluminal bleeding was examined and expressed as score for intensity (SI). After wiping the blood, the ulcer index was determined and stomach tissue was subjected to the estimation of antioxidant parameters. The stomach of each rat in each case was washed with ice-cold saline and a 10% homogenate was prepared in phosphate buffer (10 mM, P^H 7.4). The homogenates were centrifuged at 3,000 rpm at 0°C for 15 minutes using Remi C-24 high speed cooling centrifuge (Japan). The clear supernatant was used for biochemical estimations. The results were compared with that of reference standard omeprazole treated rats.

2.5.5.2 Estimation of free radical generation

The levels of malondialdehyde (MDA) in each sample were estimated (expressed as µmole thiobarbituric acid reactive substances [TBARS]/mg protein) at 535 nm in a Shimandzu UV Spectrophotometer (Shimadzu, Japan) using the methods of Kiso *et al.* [18]. The effects of the *n*-butanol fraction of root bark on the activity of the antioxidant enzymes superoxide dismutase (SOD; in terms of mU/ mg protein), catalase (CAT; as U/min/mg protein) and on the levels of reduced glutathione (GSH; as µmole/mg protein) in the stomach tissue were assayed by the methods of Misra and Fridovich [19], Aebi [20], and Beutler *et al*. [21], respectively. The total protein concentration in each stomach tissue sample was determined by the method of Lowry *et al.* [22].

2.5.5.2.1 Estimation of lipid peroxidation (LPO)

The levels of malondialdehyde (MDA, representative of peroxidative damage to cell membranes) were measured by mixing 2 ml of 5% homogenate, (in 0.1 M phosphate-buffered saline [pH 7.4]) with 2 ml of a 28% trichloroacetic acid solution. After thorough mixing, the mixture was then centrifuged at 10,000g at 4°C for 5 minutes and the supernatant was separated for estimation of lipid peroxidation (MDA content). For this, 4 ml supernatant was mixed with 1 ml of 1% thiobarbituric acid solution (TBA), and heated at 100°C for 60 min. The mixture was then cooled to room temperature and the absorbance was measured spectrophotometrically at 532 nm. After accounting for background absorbance using buffer blanks, the total TBARS (TBA-reactive substrate) concentration in each sample was derived from the TBA extinction coefficient C = 1.56 x 10^5 M^{-1} cm^{-1}. The level of lipid peroxidation (MDA content) in each sample was calculated and data were expressed in terms of nmoles of MDA/mg of protein in each sample.

2.5.5.2.2 Estimation of Superoxide dismutase (SOD)

SOD activity in the samples was determined by mixing 0.1 ml of sample with 0.1 ml of EDTA (1 x 10^{-4} M), 0.5 ml of carbonate buffer (pH 9.7), and 1 ml of epinephrine (3 x 10^3 M) (Sigma). The optical density of the adrenochrome was assessed at 480 nm at 30 sec intervals for a total of 3 min. SOD activity was expressed as mU/mg of protein. One unit of activity was defined as the enzyme concentration required to inhibit the chromogen produced, by 50%, in one minute under the defined assay condition.

2.5.5.2.3 Estimation of Catalase (CAT)

Catalase activity in each sample was measured by assessing the decomposition of hydrogen peroxide (H_2O_2) at 240 nm after addition of the whole sample. In a cuvette, 50 µl samples was mixed with 2.95 ml of reaction buffer (0.05 M phosphate buffer [pH 7.0] containing 30 mM H_2O_2) and the absorbance was measured at 15 sec intervals for 3 min. As the optical density measured reflects the peroxide concentration in the cuvette, the activity of catalase in the 3 min period was deduced and expressed as mM H_2O_2 consumed/mg tissue/min.

2.5.5.2.4 Estimation of Reduced glutathione (GSH)

Reduced glutathione (GSH) content in each tissue homogenates was measured after initial precipitation of proteins with 10% chilled trichloroacetic acid. After 30 min incubation, the samples were then centrifuged at 1000 g for 10 min at 4°C. The GSH levels in the

supernatant were then determined by mixing 0.5 ml of the material with 2.0 ml 0.3 M phosphate buffer (pH 7.0) and 0.25 ml DTNB reagent (40 mg/l00 ml in 1% sodium citrate buffer), and then measuring the absorbance at 412 nm. Standard solutions containing different concentrations of GSH were prepared in parallel to generate a standard curve. Results were expressed as μmoles of GSH/mg of protein.

2.5.5.2.5 Estimation of Protein content (PR) [22]

The total protein concentration in each stomach tissue sample was determined by the method of Lowry *et al*. [22] using bovine serum albumin as the standard. Reagent A: 2 % Na_2C0_3 in 0.1 N NaOH solution, Reagent B: 0.5 % $CuSO_4$, $5H_2O$ in l % sodium or potassium tartarate. Solution, Reagent C:Alkaline copper sulfate solution (mixture of 50ml of reagent A and 1ml of reagent B and Reagent D: Dilute Folin phenol reagent. The Ciocalteu phenol reagent was titrated with NaOH solution to a phenolphthalein end-point. On the basis of this titration, the folin phenol reagent was diluted (about 2 fold) to make it 1N in acid. Working standard: It was prepared from human serum diluted 100 to 1000 fold (approximate 700-70 y per ml). This in turn was titrated with standard solution of crystalline bovine serum albumin. The one g is equivalent to 0.97 y of serum protein. Procedure: To a sample of 5-100 y of protein in 0.2 ml in a 3-10 test tube, 1 ml of reagent C was added. Mixed well and allowed to stand for 10 min or longer at room temperature. About 0.10 ml of reagent D was added very rapidly and mixed within a second or two. After 30 min or longer the samples were read in a spectrophotometer (Shimadzu, Japan) at λmax 750nm.

2.6 Statistical analysis

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 [23]. Non-parametric data were evaluated by using wilkoxson's rank sum test [24].

3. Results

3.1 Ethanol-induced gastric mucosal damage

Alcoholic extract (300 mg/kg) and its different fractions (100 and 300 mg/kg) showed significant reduction in gastric ulceration. Out of all these fractions, *n*-butanol fractions showed maximum (99%) inhibition of gastric lesions against ethanol-induced gastric mucosal damage when compared with the control group and results were comparable with that of omeprazole (87%) treated rats. Reduction in the ulcer index was found maximum with the *n*-butanol (99.5%) fraction at 100mg/kg dose level as compared to control group (Table 1).

3.2 Water immersion plus restraint stress-induced gastric ulceration

Severe hemorrhagic gastric-glandular mucosal ulcers were observed in stress-induced control animals. Ulcer index parameter was increased significantly in stressed control animals as compared to non-stressed controls. Pretreatment with *n*-butanol fraction showed significant reduction in ulcer index (0.07 ± 0.002), when compared with the WIRS control group (1.85 ± 0.049) and results were comparable with the omeprazole treated rats (0.04 ± 0.015). Score for intensity of treated group was 1.33 ± 0.33, when compared with the control group (3.83 ± 0.16) and results were comparable with the omeprazole treated animals (0.5 ± 0.219) (Table 2).

Table 1. Effect of different extracts (100-300 mg kg^{-1}; p.o.) of *Oroxylum indicum* on ethanol-induced gastric mucosal damage in rats.

Parameters/Groups	Ulcer index (UI)	% Protection
Control	6.08 ± 0.133	-
Alcohol (50%)(300mg/kg)	0.66 ± 0.065*	89.29
Petroleum ether (300mg/kg)	0.24 ± 0.015*	96.07
Chloroform (300mg/kg)	0.57 ± 0.080*	90.84
Ethyl acetate (300mg/kg)	0.88 ± 0.037*	85.92
Petroleum ether (100 mg/kg)	0.35 ± 0.044*	85.89
n-butanol (300mg/kg)	0.06 ± 0.004*	99.00
n-butanol (100 mg/kg)	0.011 ± 0.003*	99.58
Omeprazole (20 mg/kg)	0.74 ± 0.047*	87.90

All values represent mean ± SEM, n=6 in each group. *$P < 0.05$, when compared with the control group (ANOVA, followed by Tukey's multiple range test), $F_{tab} = 2.15$; F_{cal} (8, 45) = 1182.68 (UI).

Table 2. Effect of n-butanol fraction (100 mg kg^{-1}; p.o.) of root bark of *Oroxylum indicum* on ulcer index in WIRS-induced gastric ulcer model

Group/Parameter	Stress Control	*n*-butanol (100 mg/kg)	Omeprazole (20 mg/kg)
Ulcer index (UI)	1.85 ± 0.049	0.07 ± 0.002*	0.04 ± 0.015*
Score of intraluminal bleeding (SI)	3.83 ± 0.16	1.33 ± 0.33*	0.5 ± 0.219*

All values represent mean ± SEM, n=6 in each group. *$P < 0.05$, when compared with the control group (ANOVA, followed by Tukey's multiple range test), $F_{tab} = 3.68$; F_{cal} (2, 15) = 47.79 (Ulcer index).

Table 3. Effect of n-butanol fraction (100 mg kg^{-1}; p.o.) of root bark of *Oroxylum indicum* on lipid peroxidation and anti - oxidant enzymes on WIRS-induced gastric ulcer model.

Parameter/ Group	LPO (µmole/mg protein)	SOD (munits/mg protein)	CAT (units/min/mg protein)	Reduced GSH (µmole/mg protein)
Control	0.060 ± 0.007	0.577 ± 0.012	0.992 ± 0.003	0.313 ± 0 .002
Stress Control	0.070 ± 0.004	0.154 ± 0.007	0.724 ± 0.051	0.034 ± 0.006
Omeprazole (20 mg/kg)	0.052 ± 0.001*	0.178 ± 0.010	0.841 ± 0.033	0.051 ± 0.006*
n-Butanol (100 mg/kg)	0.040 ± 0.004*	0.190 ± 0.007*	0.914 ± 0.049*	0.052 ± 0.004*

All values represent mean ± SEM, n=6 in each group. All values represent mean ± SEM, n=6 in each group. $^{+}P < 0.05$, when compared with the control group (Unpaired student '*t*'- test) and * $P < 0.05$, when compared with the control group (ANOVA, followed by Tukey's multiple range test), $F_{tab} = 3.68$; F_{cal} (3, 20) = 15.136(LPO), 4.756(SOD), 4.382(CAT), 2.978 (Reduced GSH).

WIRS-control animal showed significant increase in UI alongwith increase in LPO (0.060± 0.007) and decrease in SOD (0.577 ± 0.012), CAT (0.992 ± 0.003) and reduced GSH (0.313± 0 .002) levels. Pretreatment with *n*-butanol fraction showed significant decrease in LPO (0.040 ± 0.004) and increase in SOD (0.190 ± 0.007) and CAT (0.914 ± 0.049) levels alongwith reduced GSH (0.052 ± 0.004) levels (Table 3).

4. Discussion

In the present study, it is suggested that root bark of *Oroxylum indicum* possesses significant antiulcer activity in rats. Gastric mucosal damage induced by ethanol is reported to be due to mucosal leukotriene release [25], sub mucosal venular constriction [26] and eventual injury [27]. Reactive oxygen species are also known to be involved in the pathogenesis of ethanol-induced gastric mucosal injury *in vivo* [28]. This causes damage to the cell and cell membranes [29]. Oral administration of necrotizing agents, ethanol, ethanol-HCl, etc. stimulates release of prostaglandins from the stomach to prevent gastric lesions through adaptive cytoprotection [30]. All the extracts selected under study showed protection in gastric mucosal injury as evident from the reduction in the ulcer index. However, *n*-butanol fraction showed maximum protection in gastric mucosal injury. The mechanism of this action could be related to prostaglandin-induced vasodilatation and thereby leading to gastric cytoprotection.

The experimental stress ulcer may be considered equivalent to clinical stress ulcer, which occurs after surgery, head injury or shock. An acute gastric hemorrhagic lesion in the glandular stomach characterizes a stress ulcer [31]. The *n*-butanol fraction of *Oroxylum indicum* showed significant decrease in the ulcer index at 100mg/kg dose in a WIRS-induced gastric ulcer model. The specific pathophysiologic mechanism involved in stress-induced ulcers could be ultimate multifactorial impairment of mucosal defense system. An increase in gastric acid secretion, reduction of gastric mucus and alteration in the microvasculature of the gastric mucosa play a major role in the pathogenesis of stress-induced ulcers [32, 33]. Other possible mechanisms include vagal over-activity [34] and increased mast cell degranulation during stress [35]. Stress ulcers are medicated by brain gut axis and complex neural mechanism [36]. Stress causes an ischemic condition in the gastric mucosa by activation of parasympathetic and sympathetic nervous system resulting in vasoconstriction, which in turn causes free radical generation. Further, stress has been found to inactivate mucosal prostaglandin synthetase by accumulating H_2O_2, which in turn inhibits the synthesis of prostaglandins known to favour the generation of reactive oxygen species causing lipid peroxidation and leading to membrane fluidity.

This is turn increases the influx of Ca^{+2} ions, resulting in reduced membrane integrity of surface epithelial cells and thereby generating gastric ulcers [36, 37]. In WIRS-induced gastric ulcer model, antioxidant enzymes levels were significantly altered in experimentally induced stress, which could be due to the fact that the experimental stress is of acute nature, where the system tends to defend itself from the oxidative damage [38].

Thus, the anti-ulcer activity n-butanol fraction of root bark of *Oroxylum indicum* in stress ulcer model could be correlated significantly with that of antioxidant mechanisms. In addition to this, there could be inhibitory effect towards central component involved in the stress leading to vasodilatation.

5. Conclusion

Our results suggest a positive correlation of free radical-induced oxidative stress and gastric mucosal damage-induced by ethanol and stress. The n-butanol fraction of root bark of *Oroxylum indicum* was found to be the most potent one, produced significant anti-ulcer potential. The mechanism of its action could be due to mainly anti-oxidant activity, vasodilatation and gastric cytoprotection.

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