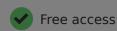






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Research Article

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

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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

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of Oroxylum indicum. and its different fractions on experimentally induced gastric ulcer models.

#### Materials and Methods

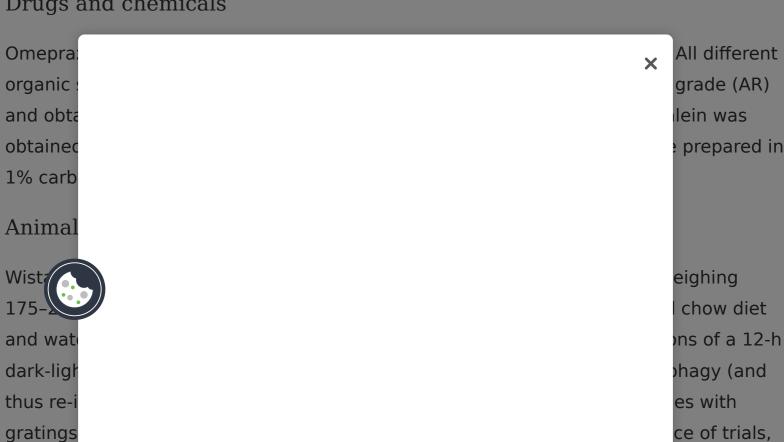
## Procurement of plant material and extraction procedure

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.

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the rats were monitored for growth, health status, and food intake capacity to be certain that they were healthy. Utmost care was taken to ensure that animals were treated in the most humane and ethically acceptable manner. The animals were sacrificed with an overdose of ether anesthesia after the completion of the 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.



estimation of lipid peroxidation (MDA content), endogenous antioxidant enzymes (superoxide dismutase and catalase), and reduced glutathione. The assay for microsomal lipid peroxidation was carried out according to the method of Kiso et al. (1984). The superoxide dismutase (SOD) activity in the samples was determined the 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

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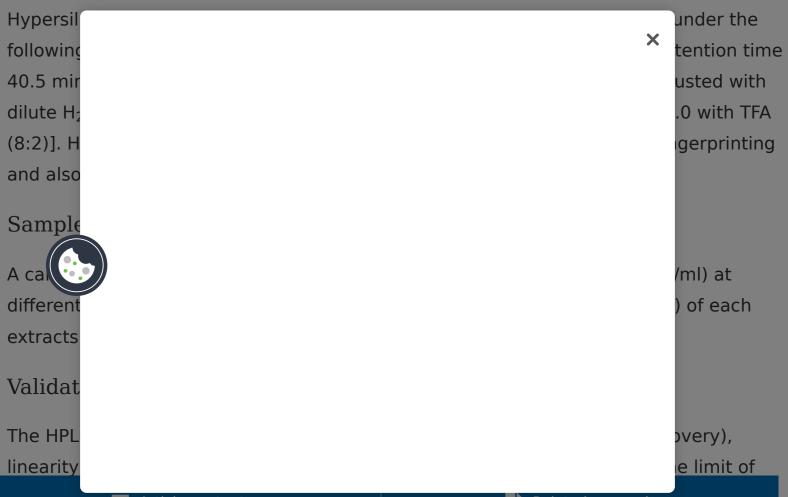
Hydrolysis of n.-butanol: 5 g of n.-butanol fraction was dissolved in water: methanol (9:1) solution hydrolyzed using 2 N HCl by refluxing the mixture for 2 h. After cooling, the ethyl acetate (0.64%) soluble fraction was separated and used for the further studies (TLC, HPLC). TLC co-chromatography was 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  $\times$  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



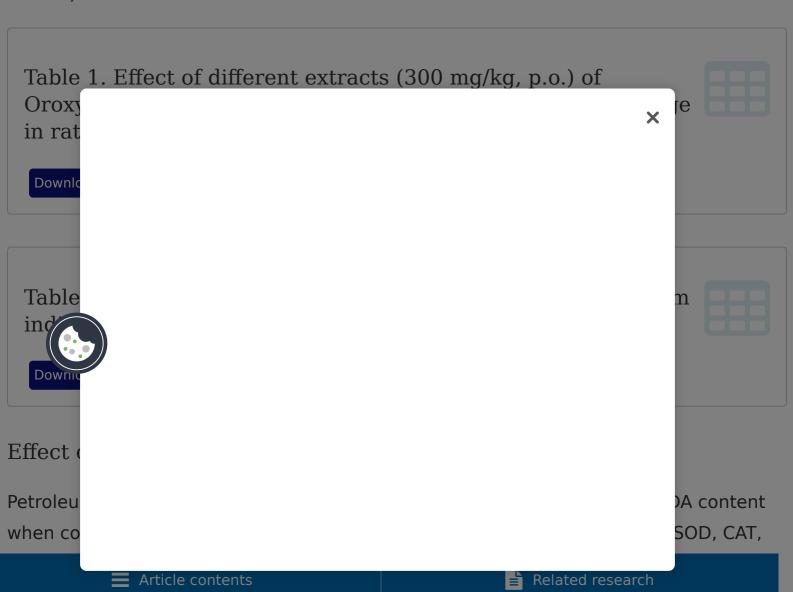
### Statistical analysis

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 (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).



and reduced GSH levels were also observed, and the results were comparable with those of omeprazole treatment (Table 3).

Table 3. Effect of different extracts of Oroxylum indicum. (p.o.) on lipid peroxidation and antioxidant enzymes against ethanolinduced 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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The petroleum ether and n.-butanol fractions showed significant reduction in the protein content of the gastric juice with no change in the carbohydrate content as compared with control group. Whereas, omeprazole treatment caused significant reduction in the TC and PR content, thus suggesting no improvement in the mucin 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.

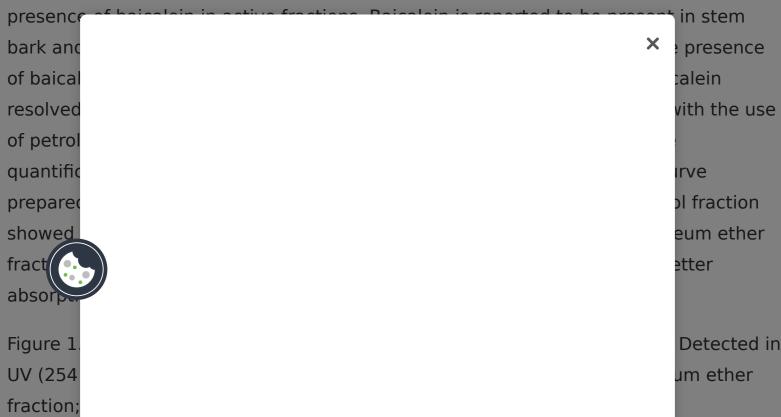


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# Fingerprinting and estimation of baicalein

Based on the results of antiulcer activity, TLC study was aimed at checking the



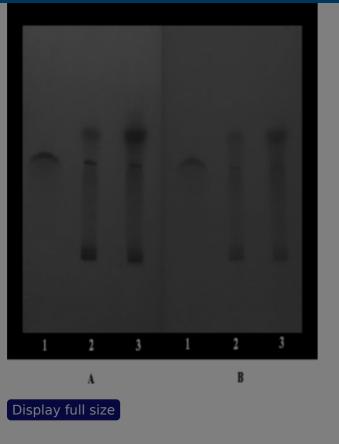


Figure 2. HPLC chromatograms of active fractions and standard baicalein. (a) Standard baicalein, (b) petroleum ether fraction, (c) hydrolyzed n.-butanol fraction.

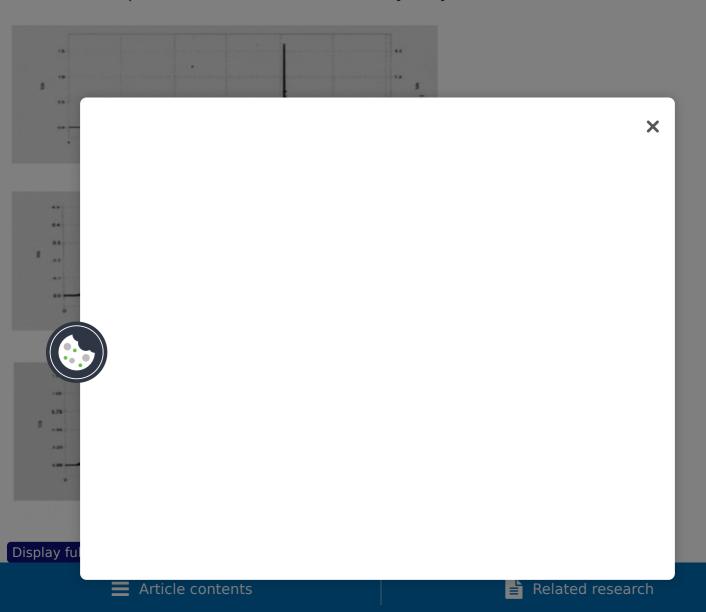
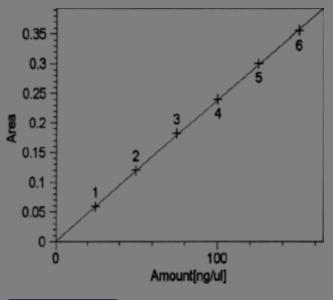
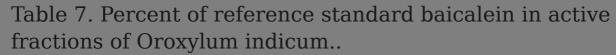


Figure 3. Calibration curve of standard baicalein.



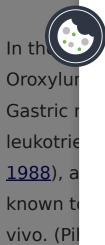
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activity in this model along with alteration in antioxidant enzyme status. Preventive antioxidants, superoxide dismutase (SOD), and catalase (CAT) are the first line of defense against reactive oxygen species (Halliwell, 1995). In addition, reduced 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

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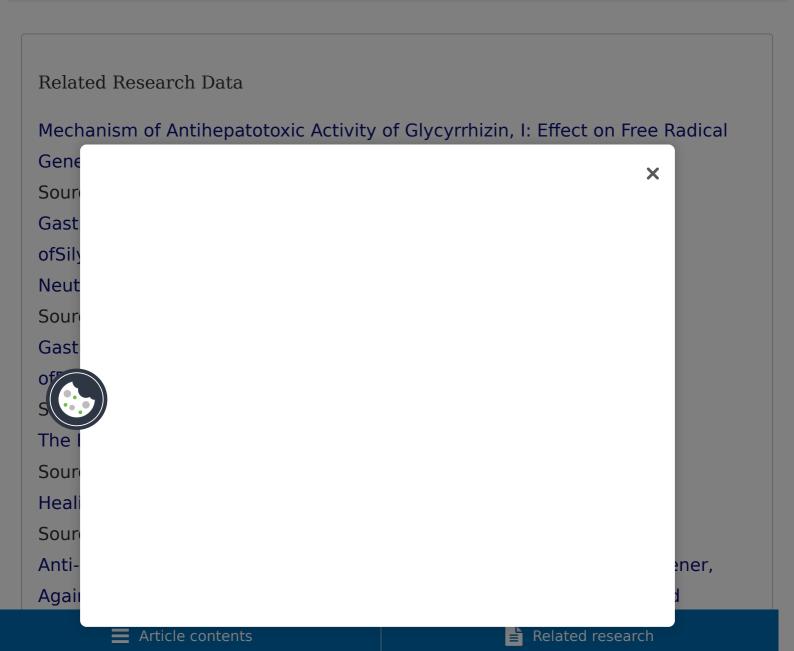
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Conclu

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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Source: Journal of Pharmacy and Pharmacology Studies on the mechanism of ethanol-induced gastric damage in rats Source: Gastroenterology Antioxidant characterization Source: Biochemical Pharmacology Free radicals and lipid peroxidation in ethanol- or aspirin-induced gastric mucosal injury Source: Digestive Diseases and Sciences Lack of Correlation Between Mucus Gel Thickness and Gastric Cytoprotection in Rats Source: Gastroenterology The Role of Superoxide Anion in the Autoxidation of Epinephrine and a Simple Assay for Superoxide Dismutase Source: Journal of Biological Chemistry Ethanol stimulates formation of leukotriene C4 in rat gastric mucosa Source: Prostaglandins PROTEIN MEASUREMENT WITH THE FOLIN PHENOL REAGENT Source: Journal of Biological Chemistry Flavonoids of the leaves of Oroxylum indicum and Pajanelia longifolia Source: Phytochemistry Effec thanol-X Indu Sour Phar neand Sour Cyto Sour Micro ic muco Sour **Anal** Sour

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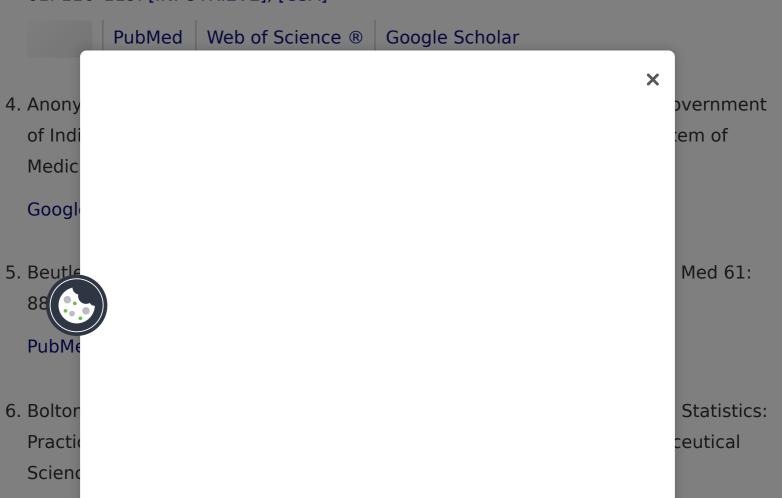
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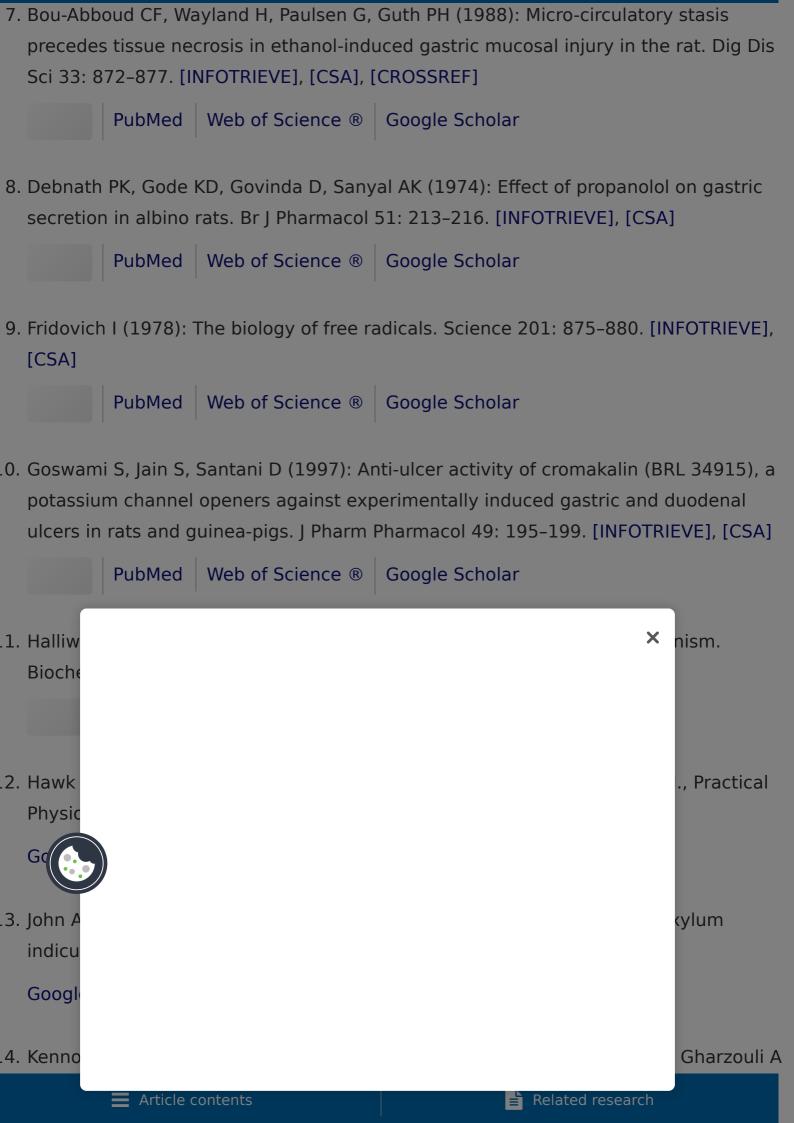
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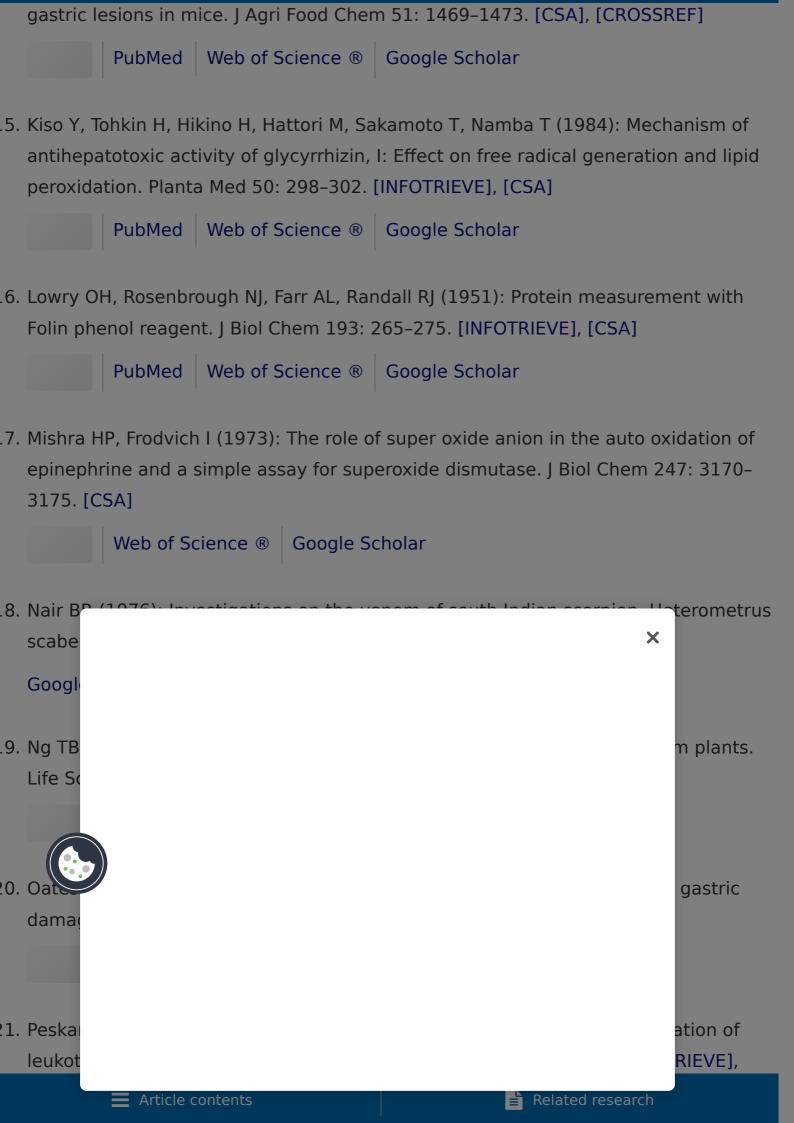
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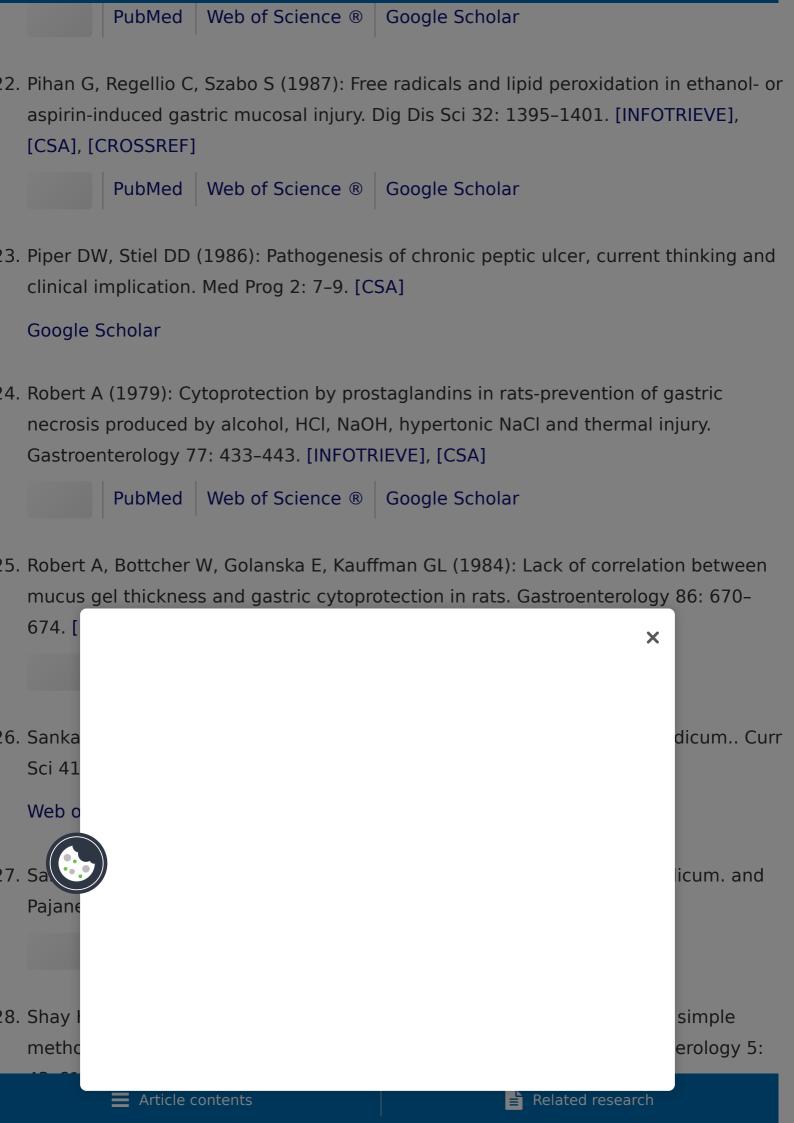
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