

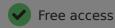






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Research Article

# Effect of Leaves and Fruits of *Moringa oleifera*. on Gastric and Duodenal Ulcers

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#### Abstract

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The effect of different extracts of leaves and fruits of Moringa oleifera. Lam.

(Moringaceae) on gastric and duodenal ulcers was evaluated by using different gastric

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## Introduction

Peptic ulcer is a breach in the gastric and duodenal epithelium associated with acute or chronic inflammation and is the most common gastrointestinal disorder in clinical practice. In spite of established antiulcer drugs, a rational therapy for peptic ulcer remains elusive, and a search for safer potential drugs is being carried out. The use of natural drugs in gastric ulcer has been reported (Sairam et al., 2001).

The development and progression of gastric ulcer depends to some extent on the type of the food consumed by the patient. It has been shown that spicy food, fatty food, or food containing caffeine stimulates acid secretion in the stomach (Crawford, 2003), and high-fiber diets such as potatoes, bananas, peas, beans, and so forth, reduce the development of duodenal ulcers (Maryland Medical Center Programs, 2004).

Previous reports on the incidence of gastric ulcers in the South Asian population reveals that the occurrence is lower due to the type of food consumed by the people of this region; one of the foods that is speculated to protect against ulcers is Moringa oleifera. Lam. (Moringaceae) leaves (Jayaraj et al., 1998). Furthermore, flower bud of Moringa pterygosperma., a synonym of Moringa oleifera. that is widely consumed in Pakistan, has been reported to possess antiulcer activity against aspirin-induced gastric ulcers in rats (Akhtar & Ahmad, 1995). However, the effect of different extracts of leaves or fruits of Moringa oleifera. on gastric and duodenal ulcers is not known. The current study was undertaken to evaluate the effect of fruits and leaves of Moringa oleifera. on

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The shade-dried leaves and fruits of the plant were subjected to Soxhlet extraction, and the extracts thus obtained were subjected to preliminary phytochemical analysis (Mukaherjee, 2002).

## Experimental animals

Albino Wistar rats weighing between 200 and 250 g were used. The institutional animal ethics committee approved the experimental protocol. Animals were maintained under standard conditions in an animal house approved by the Committee for the Purpose of Control, and Supervision on Experiments on Animals (CPCSEA), Government of India.

# Acute toxicity study

The acute oral toxicity study was performed according to the OPPTS (Office of Prevention, Pesticide and Toxic Substance) Up and Down procedure (Health Effect Test Guideline, 2004). The different extracts were suspended using 0.5% sodium carboxy methylcellulose and were administered orally. All the extracts of leaves and fruits were safe at a dose of 5000 mg/kg, p.o., and one-tenth of this dose was selected for evaluation of antiulcer activity.

# Effect on gastric ulcers

## Acetic acid-induced chronic gastric ulcer

Male Wistar rats were fasted for 24 h prior to the experiment. Under light ether anesthesia, a midline epigastric incision was made and the stomach was exposed.

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The PC version of the program was downloaded free from Scion (http://www.scioncorp.com; Scion Image for Windows, release beta 4.0.2). The scale was set at 6.1 pixels per millimeter.

The ulcer index was determined using the formula (Ganguly, 1969)

where X = total mucosal area/total ulcerated area.

The ulcers were given scores based on their intensity as follows: 0 = no ulcer, 1 = superfacial mucosal erosion, 2 = deep ulcer or transmural necrosis, 3 = perforated or penetrated ulcer.

The stomach samples from groups that showed reduction in ulcer index were subsequently processed for histologic examination. Three indices, namely regenerated glandular epithelial width, capillary density in the scar tissue, and volume of collagen content were selected to reflect the rate and quality of ulcer healing (Wang et al., 1999).

## Pylorus ligation-induced ulcers

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The animals were fasted for 36 h with water ad libitum. before pylorus ligation (Shay et al., 1945; Kulkarni, 1999). Normal saline (1 mL/rat, p.o.) was administered twice daily to all the animals during the fasting period (Asad et al., 2003). Under light ether anesthesia, the pyloric portion of the stomach was ligated. The leaf extracts (500 mg/kg, p.o.) were administered intraduodenally immediately after pylorus ligation. Six hours af Inted and the X wk et al., content 1947), p <u>85</u>), and total pro e same method Hea The gas .o.) for 5 days to then treated o.) once

y vehicle.

The glandular portion of the stomach was then used for estimation of mucin content (Corne et al., <u>1974</u>), total proteins (Lowry et al., <u>1951</u>), antioxidant factors like superoxide dismutase activity (Elstner & Heupel, <u>1976</u>), total tissue sulfhydryl groups (Ellman, <u>1959</u>), and catalase activity (Link, <u>1988</u>).

#### Ethanol-induced ulcers

Male albino rats were fasted for 36 h before administration of 90% ethanol (1 mL/200 g). The leaf extracts (500 mg/kg, p.o.) were administered 1 h before ethanol administration. One hour after ethanol administration, the animals were sacrificed, stomach was isolated, and ulcer index was determined (Brzozowski et al., 1998).

#### Cold restraint stress-induced ulcers

The animals were placed in a restraint cage, and the cage was placed at a temperature of 2°C for 3 h. The leaf extracts (500 mg/kg, p.o.) were administered 30 min prior to subjection of stress. The animals were sacrificed, stomach was isolated, and ulcer index was determined (Vincent et al., <u>1977</u>).

#### Effect on duodenal ulcers

## Cysteamine-induced duodenal ulcers

Duodenal ulcers were induced by administering cysteamine hydrochloride (400 mg/kg, p.o.) twice at an interval of 4 h. Leaf extracts (500 mg/kg, p.o.) were administered 30 min prior to each dose of cysteamine hydrochloride. After 24 h of the first dose of

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The statistical significance was assessed using one-way analysis of variance (ANOVA) followed by Dunnet's comparison test. For comparing nonparametric ulcer scores, ANOVA followed by non-parametric Dunn post test was used. The values are expressed as mean  $\pm$  SEM, and p < 0.05 was considered significant.

## Results

## Acetic acid-induced chronic gastric ulcers

The petroleum ether, acetone, and methanol leaf extracts of Moringa oleifera. showed a significant reduction in ulcer index when compared with control (p < 0.001). The acetone extract of the leaves was most potent; it produced a 79% decrease in the ulcer index. The fruit extracts of Moringa oleifera. and the chloroform extract of the leaves did not show any significant effect on ulcer index (Fig. 1). None of the treatments produced any significant effect on ulcer score (Fig. 2).

Figure 1 Effect of Moringa oleifera. on ulcer index in acetic acid-induced chronic gastric ulcer. All values are mean  $\pm$  SEM,n = 5 to 6. \*p < 0.05, \*\*p < 0.01, \*\*\*p < 0.001 when compared with control group. Cont, control; pet leaf, petroleum ether leaf extract (500 mg/kg p.o.); chcl leaf, chloroform leaf extract (500 mg/kg p.o.); act leaf, acetone leaf extract (500 mg/kg p.o.); meth leaf, methanol leaf extract (500 mg/kg); pet frt, petroleum ether fruit extract (500 mg/kg p.o.); chcl frt, chloroform fruit extract (500 mg/kg p.o.); act frt, acetone fruit extract (500 mg/kg); meth frt, methanol fruit extract



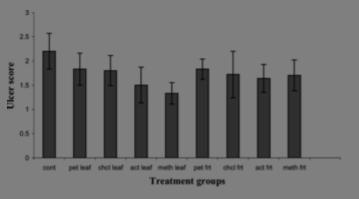
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Figure 2

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compared with control group. Cont, control; pet leaf, petroleum ether leaf extract (500 mg/kg p.o.); chcl leaf, chloroform leaf extract (500 mg/kg p.o.); act leaf, acetone leaf extract (500 mg/kg p.o.); meth leaf, methanol leaf extract (500 mg/kg); pet frt, petroleum ether fruit extract (500 mg/kg p.o.); chcl frt, chloroform fruit extract (500 mg/kg p.o.); act frt, acetone fruit extract (500 mg/kg), meth frt, methanol fruit extract (500 mg/kg p.o.).



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Histologic examination of the ulcerated area revealed that there was a significant increase in regenerated glandular epithelium width after treatment with acetone extract of the leaves (p < 0.05) when compared with control. The collagen content in the ulcerated tissue was significantly increased by all the three extracts of the leaves, with acetone extract showing the maximum effect. No significant difference on capillary density in scar tissue was observed after treatment (Table 1).

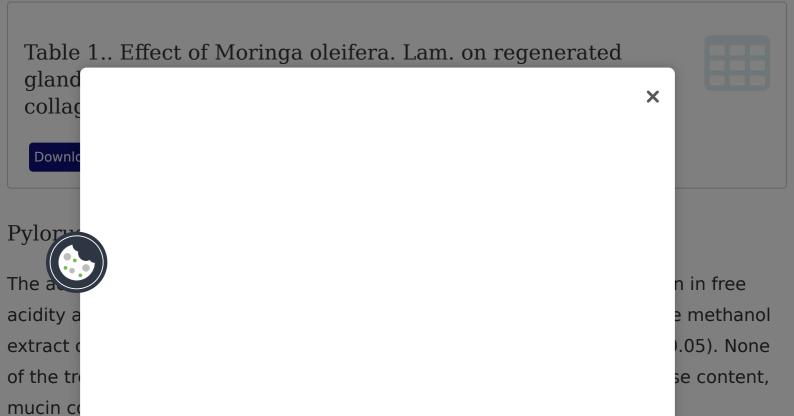


Table 2.. Effect of Moringa oleifera. leaf extracts on free acidity, total acidity, ulcer index, and total hexoses in pylorus-ligated rats



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# Healing of indomethacin-induced gastric ulcers

The acetone and methanol leaf extracts of Moringa oleifera. showed a significant reduction in ulcer index (p < 0.01) and a significant increase in mucus content when compared with control. None of the treatments produced any significant effect on total protein and antioxidant factors like SOD activity, total tissue sulfhydryl group (glutathione), and catalase activity (Table 3).

Table 3.. Effect of Moringa oleifera. leaf extracts on indomethacin-induced ulcers



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# Ethanol-induced gastric ulcers

The acetone and methanol leaf extracts of Moringa oleifera. showed a significant reduction in ulcer index when compared with control (p < 0.05). The petroleum ether extract did not show significant reduction in ulcer index when compared with control (

Table 4).



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potent in reducing ulcer index (Table 4).

## Cysteamine-induced duodenal ulcers

The acetone leaf extract of Moringa oleifera. showed a highly significant reduction in ulcer area when compared with control (p < 0.01). The methanol leaf extracts of Moringa oleifera. showed a significant reduction in ulcer area when compared with that of control (p < 0.05). The petroleum ether leaf extract did not show any significant reduction in ulcer area. None of the treatments produced any significant effect on ulcer score and ulcer index (Table 4).

#### Discussion

induced

The current study dealt with the effect of different extracts of Moringa oliefera. leaves and fruits on the gastric and duodenal ulcers in rats. The extracts that showed ulcerhealing effect in acetic acid-induced gastric ulcers were screened further to determine their effect on gastric cytoprotection and gastric secretion.

Application of glacial acetic acid (0.05 mL) on to the serosal surface of the rat stomach produces deep penetrating gastric ulcers (Okabe et al., 1971). The fruit extracts of the plant did not show any effect on the healing of gastric ulcers induced by acetic acid, whereas the petroleum ether, acetone, and methanol leaf extracts of Moringa oleifera. increased healing of the acetic acid-induced gastric ulcers. The histopathologic carried out to determine the effect of extract

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Ethanol-induced and indomethacin-induced gastric ulcer was employed to study the cytoprotective effect of the extracts. The acetone and methanol extracts of Moringa oleifera. leaves were effective in reducing ulcer index in both these models and significantly increased the mucus content in indomethacin-induced gastric ulcers. However, the leaf extracts of Moringa oleifera. were not effective in altering the antioxidant factors like SOD activity, total tissue sulfhydryl group (glutathione), and catalase activity suggesting that the healing of ulcers or prevention of development of gastric ulcers may not be due to antioxidant effect.

Stress plays an important role in ulcerogenesis. The pathophysiology of stress-induced gastric ulcers is complex. Stress-induced ulcers are probably mediated by histamine release with enhancement in acid secretion and a reduction in mucus production (Brodie & Hanson, 1960; Peters & Richardson, 1983). The petroleum ether, acetone, and methanol extracts of Moringa oleifera. were effective in reducing the ulcers induced by stress. Cysteamine-induced duodenal ulcer in rat is a widely used model of peptic ulcer disease. Cysteamine hydrochloride inhibits the alkaline mucus secretion from the Brunner glands in the proximal duodenum and stimulates gastric acid secretion rate. Gastric emptying is also delayed, and serum gastrin concentration is increased (Parmar & Desai, 1993). The acetone and methanol leaf extracts of Moringa oleifera. were effective in reducing the ulcer area in cysteamine-induced duodenal ulcers.

The acetone and methanol extracts of the leaves were effective in all the tested models of peptic ulcer disease, whereas the petroleum ether was effective only in healing

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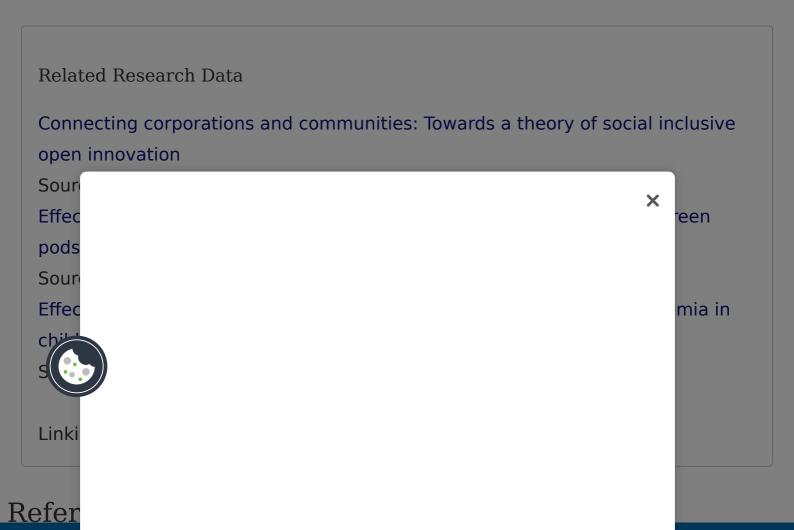
to reduce the development of gastric ulcers (Xiao et al.,  $\underline{1992}$ ). This probably explains the activity of petroleum ether extract.

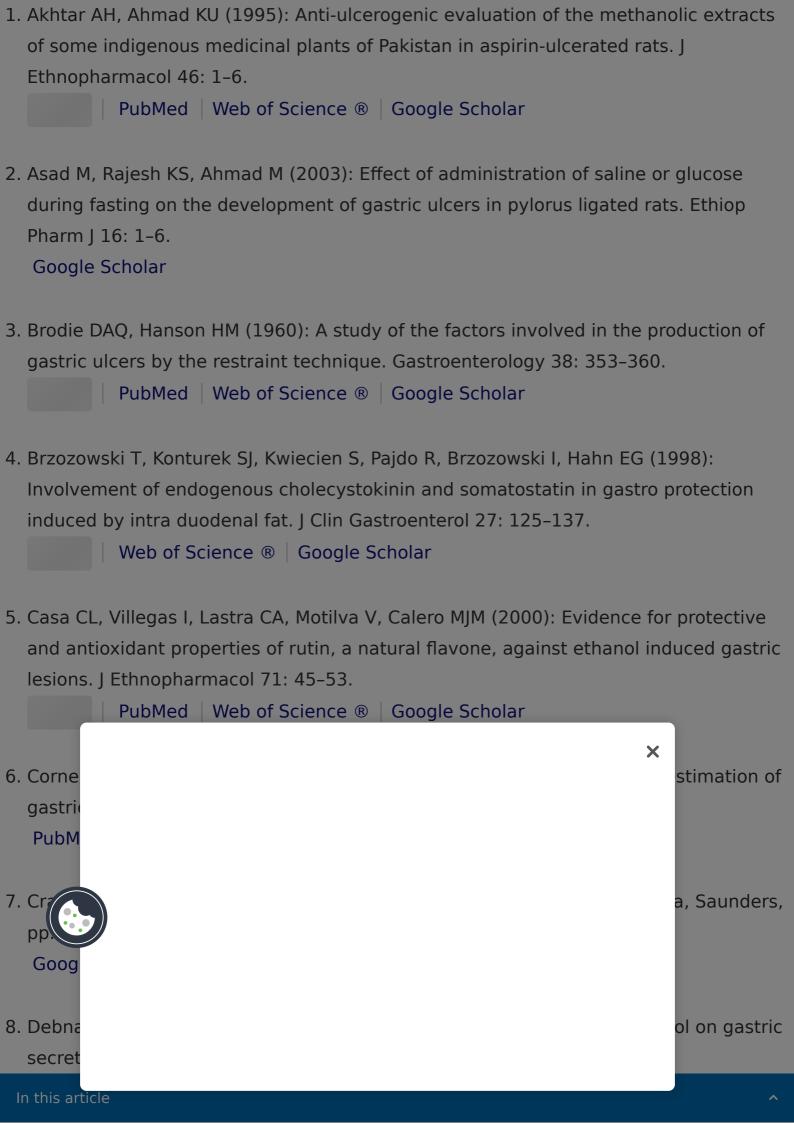
The results of the current study suggest that consumption of the leaves of Moringa oleifera. may be beneficial in healing of ulcers in patients suffering from peptic ulcer disease.

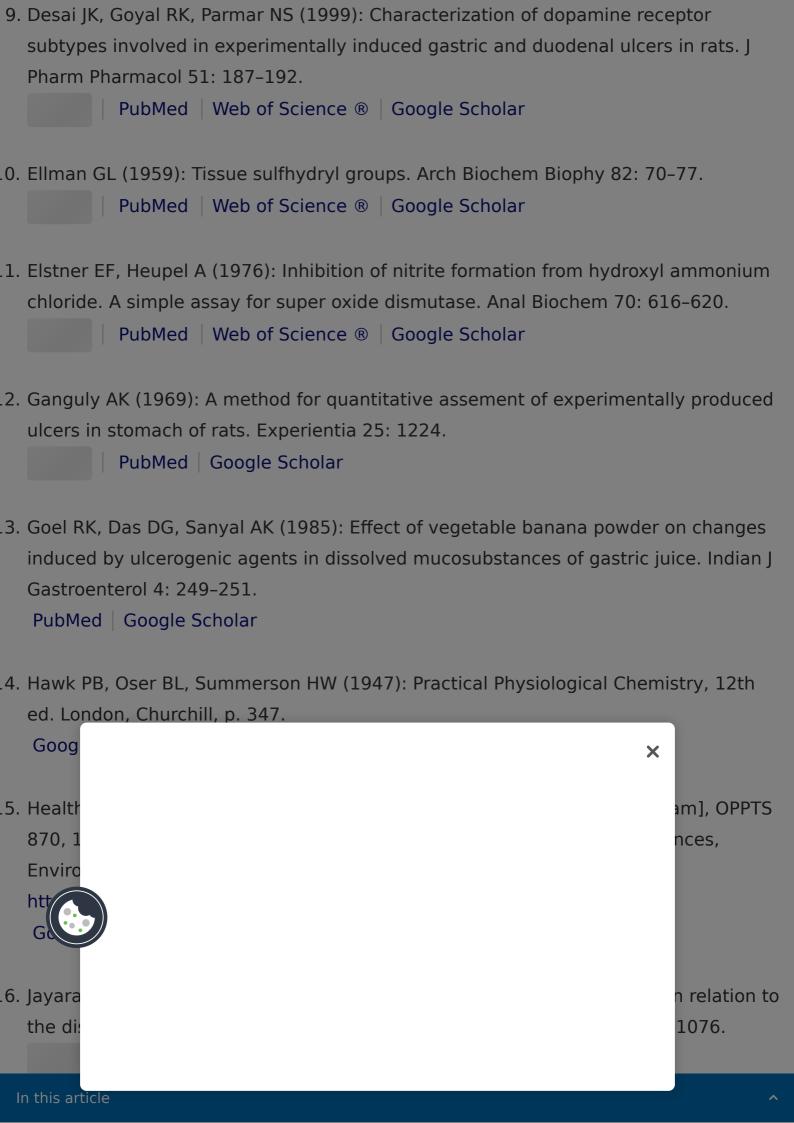
# Acknowledgment

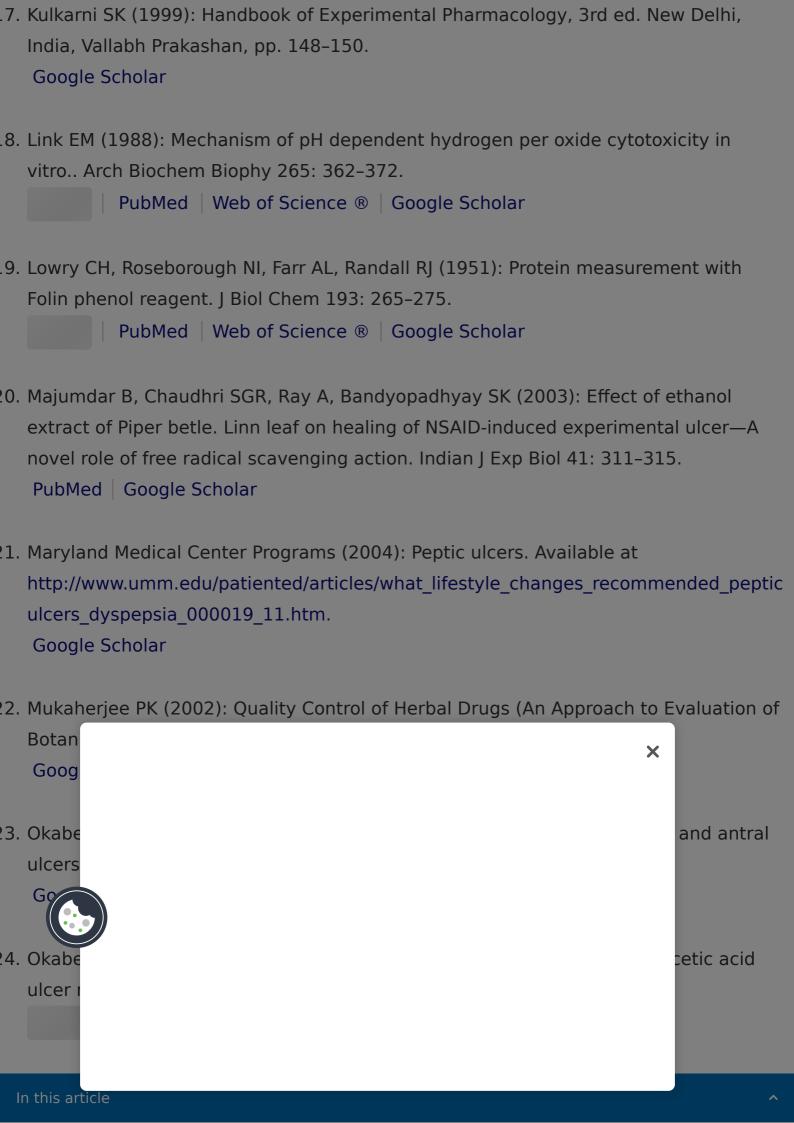
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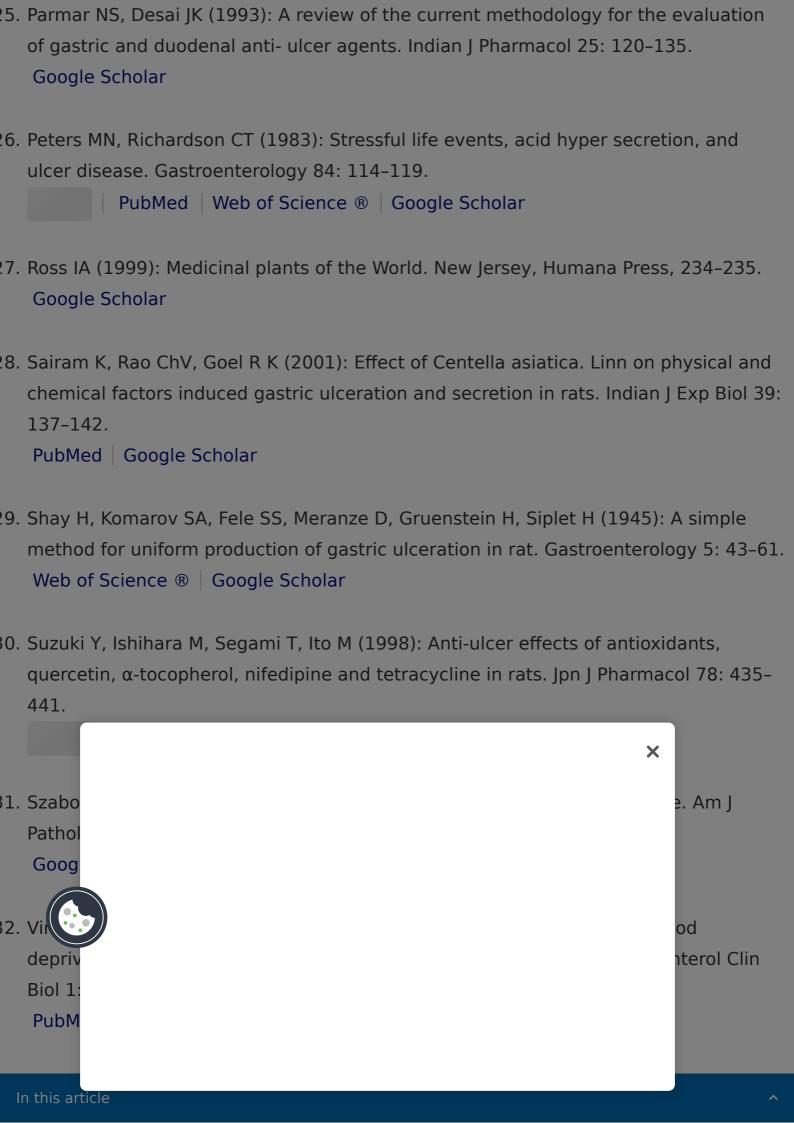
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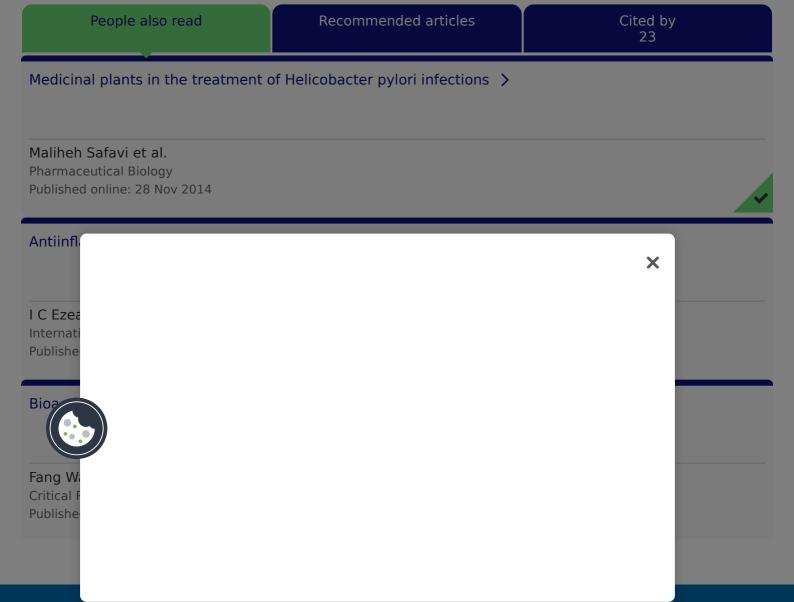
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