EVALUATION OF MASTIC, A CRUDE DRUG OBTAINED FROM *PISTACIA LENTISCUS* FOR GASTRIC AND DUODENAL ANTI-ULCER ACTIVITY

MANSOOR S. AL-SAID, A.M. AGEEL, N.S. PARMAR* and M. TARIQ

Departments of Pharmacology & Pharmacognosy and the Research Centre, College of Pharmacy, King Saud University, P.O. Box 2457, Riyadh-11451 (Saudi Arabia)

Summary

The effect of mastic, a concrete resinous exudate obtained from the stem of the tree *Pistacia lentiscus*, has been studied on experimentally-induced gastric and duodenal ulcers in rats. Mastic at an oral dose of 500 mg/kg produced a significant reduction in the intensity of gastric mucosal damage induced by pyloric ligation, aspirin, phenylbutazone, reserpine and restraint + cold stress. It produced a significant decrease of free acidity in 6-h pylorus-ligated rats and a marked cytoprotective effect against 50% ethanol in rats which could be reversed by prior treatment with indomethacin. The protective effect was not seen when it was given intraperitoneally in phenylbutazone and restraint + cold stress models. The reduction in the intensity of ulceration in cysteamine-induced duodenal ulcers was not found to be statistically significant in mastic-pretreated rats. The results suggest that mild antisecretory and a localized adaptive cytoprotectant action may be responsible for its anti-ulcer activity. These observations support the results of an earlier study on the clinical effectiveness of mastic in the therapy of duodenal ulcer.

Introduction

Mastic (saladin) is the concrete resinous exudate obtained from the stem of the tree *Pistacia lentiscus* Linn. (Anacardiaceae) which is cultivated in the Mediterranean areas. Mastic has been used there by traditional healers for the relief of upper abdominal discomfort, gastralgia, dyspepsia and peptic ulcer (Keys, 1976). It has also been used as a masticatory and by dentists for filling carious teeth (Nadkarni, 1954; Bentley and Trimen, 1980; Reynolds,

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*To whom all correspondence should be addressed.

1982). It also is reported to possess stimulant and diuretic properties (Bentley and Triner, 1980). Recently, a double-blind trial of mastic and placebo in the treatment of duodenal ulcer has shown that it produced complete ulcer healing in 70% of patients as compared to 22% of the placebo group (Al-Habbal et al., 1984). Therefore, the present study was undertaken to evaluate its anti-ulcer potential in experimental animals and to provide a rationale for its clinical use.

Methods

Albino rats of either sex of approximately the same age weighing 200–225 g and fed on a standard chow diet were used. They were divided into various groups of 8–10 animals each. The distribution of the animals in groups, the sequence of the trials and the treatment allotted to each group were randomized.

Experimental gastric lesions

The animals were starved for 36 h with access to water ad libitum before subjecting them to one of the following procedures.

Pyloric ligation

The pylorus was ligated according to the method of Shay et al. (1945) under light ether anesthesia with care being taken not to cause bleeding or to occlude blood vessels. Six hours after ligation, the animals were killed by an overdose of ether, the stomach removed, contents collected, measured, centrifuged and subjected to analysis for free and total acidity by titrating against 0.01 N NaOH to pH 3.5 using Toepfer's reagent and to pH 8.0 using phenolphthalein as indicators, respectively. Each stomach was examined for lesions in the forestomach portion and indexed according to severity.

Aspirin

Aspirin ulcers were induced according to the method of Hemmati et al. (1973) . Aspirin (Bayer) was suspended in 1% carboxymethylcellulose in water (20 mg/ml) and administered orally in the dose of 200 mg/kg (10 ml/kg). Four hours after aspirin administration the animals were killed.

Phenylbutazone

The method of Wilhelmi (1974) was followed. Phenylbutazone (Suhrid-Geigy) was administered subcutaneously as an aqueous solution of the sodium salt. Two doses of 100 mg/kg were given at an interval of 15 h. Six hours after the second dose the animals were killed.

Reserpine

The method of Gupta et al. (1974) was followed. Reserpine (CIBA) was administered in a dose of 5 mg/kg i.m. and the animals were killed 24 h later.

Stress ulcers

The method of Levine (1971) was followed with slight modification. The animals were taken off food for 24 h with access to water ad libitum and 1 h after receiving the corresponding treatments, they were placed in metallic restraint cages inside a refrigerator (2—4°C) for 2 h. They were killed at the end of this period.

Cytoprotection

The method of Robert et al. (1979) was followed. One milliliter of 50% ethanol was administered to rats starved for 36 h with free access to water. The animals were killed 1 h after the administration of ethanol. Indomethacin was used in a dose of 10 mg/kg s.c. 1 h before ethanol as prostaglandin biosynthesis inhibitor in one of the groups.

Experimental duodenal lesions

Cysteamine-induced ulcers

The method described by Szabo (1978) was followed. Female Wistar albino rats, weighing 180—200 g were used. Food and water were available ad libitum throughout the study. Duodenal ulcers were induced by two oral administrations of cysteamine hydrochloride, 400 mg/kg, in aqueous solution at an interval of 4 h. All the animals were killed 48 h after the first dose of cysteamine.

General procedures

Mastic was administered finely suspended in corn oil (100 mg/ml) at a dose of 500 mg/kg p.o. (5 ml/kg) 30 min before the administration of an ulcerogenic agent (twice in case of phneylbutazone and cysteamine) and the beginning of stress procedures. In Shay rats it was administered immediately after pylorus ligation. It was also administered intraperitoneally in two groups in order to assess its effectiveness after parenteral administration. Control animals received an equivalent volume of the vehicle in each group.

The animals were killed at the end of specified periods using anaesthetic ether and their stomach and duodenum were excised. The duodenum was opened along its anti-mesenteric side and the stomach along the greater curvature, their contents were rinsed off with saline and the linings examined with a 6.4X binocular magnifier. Lesions were assessed by two observers unaware of the experimental protocols.

Gastric lesions induced by all the procedures used in this study were multiple in each stomach. They were evaluated singly according to their dimensions and severity, and scored using a scale of 0 (no visible ulcers) to 10 (deep lesions with diameter greater than 8 mm in each stomach). The scores for each single lesion were then summed up so that the total score per stomach could exceed the value of 10 (Tariq et al., 1985).

The duodenal ulcers were scored for intensity, using a scale of 0 to 3, where 0 = no ulcer, 1 = superficial mucosal erosion, 2 = deep ulcer or transmural necrosis, 3 = perforated or penetrated (into the pancreas or liver) ulcer (Szabo, 1978). The ulcer index is the sum of the arithmetic mean of the intensity in a group and the ratio of positive/total multiplied by 2, e.g. 2.1 + (9/10 × 2).

The results refer to the average lesion scores ± S.E.M. Statistical analysis of the severity of gastric ulcers was done by Student's t-test.

Results

In the Shay rats, oral administration of mastic immediately after pylorus ligation led to a significant decrease in the ulcer index and free acidity of the stomach contents at the end of the 6-h period. The ulcers were mainly located in the forestomach and only few hemorrhagic spots were observed in the glandular stomach (Table 1).

Administration of aspirin, phenylbutazone and reserpine, and subjecting the animals to cold + restraint resulted in the production of gastric mucosal damage mainly in the glandular segment of the stomach in 100% of the animals. The majority of these lesions were gastric erosions, i.e. superficial hemorrhagic mucosal lesions not penetrating the muscularis mucosae. Approximately 20—25% animals in the phenylbutazone and reserpine groups showed true ulcers. Pretreatment with mastic administered orally was effective in reducing the intensity of ulceration in all four groups (Table 2); however, parenteral administration failed to reduce the intensity of ulceration in the phenylbutazone-treated and cold + restraint stressed rats.

The lesions induced by 50% ethanol were grouped in patches of varying size, usually parallel to the major axis of the stomach. Treatment with mastic significantly reduced the severity of these lesions. This cytoprotective effect of mastic was reversed by pretreatment with indomethacin (Table 2).

TABLE 1

EFFECT OF MASTIC ON THE VOLUME OF GASTRIC SECRETIONS, FREE AND TOTAL ACID PRODUCTION AND THE DEGREE OF ULCERATION IN 6-h PYLORUS-LIGATED (SHAY) RATS

Treatment	N	Oral dose (mg/kg)	Mean ± S.E.M.			
			Volume of gastric contents (ml)	Free acid (mEquiv./l)	Total acid (mEquiv./l)	Ulcer index
Control	8	—	8.75 ± 0.90	33.8 ± 2.9	49.7 ± 3.8	33.2 ± 1.8
Mastic	6	500	8.20 ± 1.68	18.2 ± 2.8*	44.6 ± 4.6	17.5 ± 2.2**

Significance relative to control data: *$P < 0.01$ and **$P < 0.001$.

TABLE 2

EFFECT OF MASTIC ON EXPERIMENTALLY-INDUCED GASTRIC AND DUODENAL
ULCERS IN RATS

Treatment	N	Dose (mg/kg × route)	Lesion score (mean ± S.E.M.)
Aspirin			
Control	8	—	22.75 ± 1.20
Mastic	8	500 × 1, p.o.	12.62 ± 0.92**
Phenylbutazone			
Control	8	—	24.50 ± 2.28
Mastic	8	500 × 2, p.o.	13.75 ± 1.59*
Mastic	8	500 × 2, i.p.	21.25 ± 1.78
Reserpine			
Control	8	—	32.75 ± 1.16
Mastic	8	500 × 1, p.o.	18.00 ± 2.06**
Stress			
Control	9	—	12.33 ± 0.83
Mastic	8	500 × 1, p.o.	4.87 ± 0.48**
Mastic	6	500 × 1, i.p.	12.80 ± 1.22
50% Ethanol			
Control	10	—	52.4 ± 3.1
Mastic	6	500 × 1, p.o.	21.5 ± 2.8**
Indomethacin + mastic	6	10 × 1, s.c. 500 × 1, p.o.	54.5 ± 3.3***
Cysteamine			
Control	10	—	3.80 ± 0.20
Mastic	9	500 × 2, p.o.	3.33 ± 0.25

Significance relative to respective control data: $*P < 0.01$ and $**P < 0.001$.
Significance relative to mastic-treated group: $***P < 0.001$.

Administration of cysteamine hydrochloride caused mortality in 5% of
the rats in 24 h. Those rats which died had perforated duodenal ulcers.
Cysteamine treatment produced duodenal ulcers in 90% of all the rats.
Usually two ulcers were produced close to the pylorus, the larger on the
anterior and the smaller on the posterior wall of the duodenum. They were
elongated, extending longitudinally down the duodenum and could easily
be measured. The mean total lengths in the control and treated groups were
13.6 ± 2.2 mm and 12.1 ± 1.9 mm, respectively. Pretreatment with mastic
was not found to be effective in reducing the intensity of duodenal ulcera-
tion induced by cysteamine. The slight reductions in the average lesion
scores and in the average total lengths were statistically not significant
(Table 2).

Discussion

The results of our study show that orally administered mastic was highly effective in preventing the gastric lesions induced by pyloric ligation, aspirin, phenylbutazone, reserpine, 50% ethanol and cold + restraint stress. Since these methods produce ulceration of the gastric mucosa by a variety of mechanisms including the breaking of mucosal barrier (Davenport, 1967), inhibition of mucosal cells (Bucciarelli et al., 1968), stimulation of gastric secretion (Emas and Fyro, 1965) and increased turnover of histamine, serotonin and catecholamines (Hemmati et al., 1973; Gupta et al., 1974; Daas et al., 1977; Parmar et al., 1984), it appears that mastic acts by a non-specific protective mechanism to nullify the deleterious effects of these ulcerogenic drugs and stress.

A localized protective action on the gastric mucosa seems probable since systemic administration of mastic proved ineffective in preventing the phenylbutazone and stress-induced gastric ulcers. This thesis is also substantiated by the "adaptive cytoprotection" shown against ethanol lesions which was reversed by the prostaglandin biosynthesis inhibitor indomethacin. Indomethacin has been shown to antagonize the cytoprotective effect of mild irritants in rats (Konturek et al., 1982).

The severity of duodenal ulcers, though slightly reduced, was not statistically significant after oral pretreatment with mastic. Cysteamine-induced duodenal ulcers are considered to be due to a long lasting hypersecretion of gastric acid (Szabo et al., 1977; Kirkegaard et al., 1980) which may be partly due to increased plasma levels of gastrin (Lichtenberger et al., 1977a) not sufficient to produce ulcers on its own. In fact, hypersecretion of acid, disturbed gastroduodenal motility, hypergastrinaemia and decreased mucosal resistance have all been implicated in the pathogenesis of cysteamine-induced duodenal ulceration (Lichtenberger et al., 1977b; Ishii et al., 1976). It is possible that higher and more frequent dosing with mastic may reveal some duodenal anti-ulcer activity in this model. Even the standard H_2-antihistaminic cimetidine has been found less effective against cysteamine-induced duodenal ulceration in rats (Szabo et al., 1979).

In conclusion, the observations made in this study corroborate the results of the clinical report on mastic (Al-Habbal et al., 1984) and suggest a local anti-ulcer effect for this drug. Further studies are warranted on the mode of action and the specific active principles responsible for this gastroprotective effect.

Acknowledgement

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