The Use of Pistacia Lentiscus Chia Resin versus Omeprazole in Protecting Male Rats Peptic Mucosa against Cold Restraint Stress

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Abstract
Introduction: Peptic mucosal damage induced by acute stress is a serious cause of morbidity and mortality in critically ill patients. The study aimed to investigate the protective, antioxidant and anti-inflammatory effects of pretreatment with Chios mastic gum (CMG), a traditionally consumed herbal resin naturally deriving from the trunk of Pistacia Lentiscus var. Chia compared to Omeprazole, a standard medication used in the prevention and treatment of gastritis, against the effects of cold restraint stress (CRS) in rat gastric and colonic mucosa. Methods: Twenty-one male Wistar rats were randomly assigned to three groups: Control (C), Omeprazole (O), and CMG (M), according to the pre-treatment regime, and were subjected to CRS at 4°C for 3 hours. The gastric and colonic mucosal lesions were histologically assessed. ELISA measured blood concentrations of TNF-α, IL-1β, peroxidase, superoxide dismutase (SOD) and total antioxidant capacity (TEAC). Results: In both groups, O and M, gastric mucosal hyperemia, hemorrhagic infiltration and mucosal oedema, as well as colonic mucosal hyperaemia and hemorrhagic infiltration were significantly reduced compared to the controls (p<0.05). No significant differences were observed between Groups O and M. TNF-α levels were significantly lower in group M compared to Group O (p=0.013). IL-1β levels were significantly depressed in groups M and O compared to control (p≤ 0.001). The activity of both peroxidase and SOD enzymes decreased in group M compared to group O (p= 0.043 and p=0.047 respectively) and the control (p=0.018 and p< 0.001 respectively). Conclusions: The natural Chios mastic gum is a promising nutritional supplement with protective properties to the peptic mucosa against CRS, exerting anti-inflammatory and antioxidant effects.

Keywords: cold restraint stress, colitis, gastric ulcer, oxidative stress, Pistacia Lentiscus Chia, Omeprazole

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INTRODUCTION

Stress gastritis, and less often stress colitis, occurs in critically ill patients following, amongst other matters, trauma, sepsis, shock, multi-organ failure or those undergoing prolonged surgical treatment [1-4]. Lesions of the gastric mucosa may be focal or diffuse, superficial or potentially resulting in ulceration or gastric haemorrhage, which is associated with significant morbidity and mortality.

The predominant theory of the pathogenesis of gastric mucosal damage caused by stress was, until recently, the destruction of the gastric mucosal barrier due to ischemia, which renders the stomach vulnerable to gastric fluids [1-3].

Experimental immobilisation models, involving either exposure to cold [4-8], or water immersion [9-28], are well documented as inducing gastric mucosal lesions in rodents. It has been found that each model produces a stimulus that rapidly activates the sympathetic adrenergic system, reduces blood flow to the gastric mucosa causing local hypoxia and ischemia, epithelial necrosis and hemorrhagic gastric mucosal corrosion [5-28].

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Cold restraint stress (CRS) has been found to induce gastric lesions which are associated with decreased or normal levels of acid secretions [27, 29]. Administration of antacids to neutralise secreted acid does not prevent stress ulcer [30], suggesting that factors other than gastric acid are involved in ulcer formation. Reactive oxygen species (ROS) such as ·O₂⁻ and ·OH are now considered to be major causative factors for mucosal lesions through oxidative damage [21, 22, 27, 31-33].

Colitis, due to stress, is less frequently found in severely ill patients and has not been clinically investigated because colorectal lesions are often due to drug-damage or disorders of the colonic flora caused by medication.

It has been reported that in experimental rodent models, stress caused by immobilisation contributes to the development of colitis [4]. To our knowledge, none of the CRS experimental models has been used to study stress-induced colonic mucosal changes in rodents [4, 5, 29].

Chios mastic gum (CMG) is a resin naturally produced exclusively in the southern region of the Greek island of Chios, in the Northern Aegean Sea, by pricking the trunk of the shrub Pistacia lentiscus var. Chia. This traditional gum is very popular as a nutritional ingredient, due to its distinct essence, the Eastern Mediterranean and Arab countries. Its beneficial health effects, mainly for gastrointestinal disorders, have been known for more than 2500 years, as documented by Dioscurides and Galen.

Previous studies showed that CMG possesses anti-ulcer activities [2, 3], while antibacterial, antioxidant, and chemopreventive properties of CMG have also been documented [22, 34-38]. Terpenoids are the active compounds of CMG exerting an anti-inflammatory as well as antioxidant effects [36, 37].

Chios mastic gum is effective in the treatment of drug-induced gastroduodenal ulcers and colitis in rats [22, 36, 37], but without any effect as an in vivo monotherapy against Helicobacter pylori [38, 39].

Omeprazole has been a standard prevention and treatment medication for peptic ulcer disease since 1989. Indications include gastroesophageal reflux disease, Zollinger-Ellison syndrome, as well as eosinophilic gastritis. It is also used to prevent upper gastrointestinal bleeding in people who are at high risk but has been associated with certain adverse effects and drug interactions [40, 41].

Given that mastic is a natural product of widespread use in many countries, its potential protective effect on the gastric and colonic mucosa may render it a key target for the pharmacological management of critically ill patients. The prevention of stress-induced changes of the gastric and colonic mucosa via nutritional interventions may significantly reduce morbidity and mortality of critically ill patients.

The present study aimed to investigate the protective, antioxidant and anti-inflammatory action of CMG pre-treatment versus Omeprazole, on the CRS effects induced in the rat gastric and colonic mucosa.

The null hypothesis is that CMG has no protective action on the rat peptic mucosa against CRS.

**Materials and Methods**

Twenty-one male Wistar rats, aged 12-14 months and weighing 320–380 g, were used in this study [42].

The animals were housed in wire cages under standard laboratory conditions. They had been acclimated for two weeks before the experiment in the laboratory. The conditions were 12 h light-dark cycles, 22–25°C room temperature, and 55–58% humidity. The animals were freely fed with a standard laboratory usual pellet diet and water intake.

All experimental procedures in this study were conducted under the Regional State License 1635/15-03-2016 and following the European guidelines of Directive 86/609/EEC on the protection and welfare of animals used for experimental and other scientific purposes.

The sample size was determined by power analysis. The animals were randomly assigned to the following three groups, each of seven animals: Control (C), Omeprazole (O), and CMG (M).

**Preparation of administered substances**

The substances used were:

1. Omeprazole suspension (Omeprazole & SyrSpend® SF Alka Convenience Pack 2 mg/mL, Fargon Hellas-Keritus ICSA, Trikala, Hellas)
2. Chios Mastic natural resin ground in microparticulate form (Chios Mastic Producers Association, Chios, Greece), chemically analysed and suspended in water by shaking.
3. Control group animals received tap water in place of the active agents.
All substances were prepared immediately before use and administered via an orogastric tube.

**The experimental protocol**

Two days before the induced stress, the following was administered to the animals, once a day by orogastric tube.

- Group C, additional water 10 mL;[26].
- Group O, omeprazole 20 mg/kg in water;[21].
- Group M, Chios mastic gum powder 500 mg in micro-particulate form suspended in water [22,23].

On the day before the experiment, in order to avoid coprophagy, the animals were placed in wire-bottomed cages and were deprived of food for 24 hours, but had free access to tap water and received the pre-treatment as mentioned above regimen.

On the day of the experiment, the animals were transferred to Bollmann cages, immobilised and kept at a temperature of 4°C for three hours [8].

At the end of the experiment, the animals were anesthetized with ketamine 100 mg/kg BW (Ketamin, IFET, Athens, Greece) and xylazine 20 mg/kg BW (Xylamed, Bimeda INC, Oakbrook Terrace, IL, USA). The abdomen of each animal was then opened and 1,5-2,5 mL of blood was obtained from the abdominal aorta for measurement of oxidative stress and inflammation markers by ELISA [12].

The entire stomach and ascending colon were then removed for histological examination. All blood and tissue samples were appropriately encoded in order to ensure blind assessment. The animals were euthanized by an intracardiac infusion of 0.6 mg/kg BW pentobarbital sodium BP20% (Dolethal, Vetoquinol Ltd, UK). The oxidative status was evaluated by determination of the oxidative stress markers peroxidase, total antioxidant capacity in Trolox equivalents (TEAC), and superoxide dismutase (SOD). The anti-inflammatory properties were evaluated by measurement of tumour necrosis factor-alpha (TNF-α) and interleukin-1 beta (IL-1β). The concentration of each marker in serum samples was measured by enzyme-linked immunosorbent assay (ELISA). The oxidative status was evaluated by determination of the oxidative stress markers peroxidase, total antioxidant capacity in Trolox equivalents (TEAC), and superoxide dismutase (SOD). The anti-inflammatory properties were evaluated by measurement of tumour necrosis factor-alpha (TNF-α) and interleukin-1 beta (IL-1β). The concentration of each marker in serum samples was measured by enzyme-linked immunosorbent assay (ELISA).

**Pathological assessment of tissues**

Immediately after removal, the stomachs were inflated by an injecting one mL of 2% formalin then fixed in 2% formalin for ten minutes before being opened along the greater curvature and rinsed with saline to remove the gastric content and clots, before stabilisation in 10% formalin solution. Sections, 5 µm thick, were cut on a microtome (HIRAX M60, Carl Zeiss, Germany) and stained with haematoxylin and eosin (HE).

Gastric mucosal lesions were studied by dissecting microscopy, and the lesion score was calculated.

The damage scores were categorised as follows:

- A one cm segment of each histological section was assessed for:
  - hyperaemia (score: 0–3)
  - loss of epithelial cells (EC) (score: 0–3)
  - oedema in the upper mucosa (score: 0–3)
  - haemorrhagic infiltration (score: 0–3)
  - presence of inflammatory cells (IC) (score: 0–3) [19,21].
- The average score of gastric mucosal lesions in each group of animals was calculated, and the results were statistically analysed.
The ascending colon was immersed and rinsed. Prepared colonic mucosal sections were stabilised with 10% formalin solution; paraffin blocks were formed and stained with HE and Periodic acid–Schiff (PAS). The number of mucin-containing cell-surface cells was quantitated in a region containing ten parallel crypts [4,43].

The results were expressed as the number of mucus-containing cup cells per 100 epithelial cells of the colon [4]. Quantitative measurement of colonic mucin was not conducted.

The colonic mucosal histological damages identified were:
- hyperaemia (score: 0–3) and
- haemorrhagic infiltration (score: 0–3)

The average score of colonic mucosal lesions in each group of animals was calculated, and the results were statistically analysed.

The same pathologist blindly screened all encoded preparations.

Statistical analysis

Statistical analysis of the data was performed using SPSS version 19.0 (IBM, Corp.).

The normality of quantitative variables was tested by the Kolmogorov-Smirnov test.

All serum antioxidant and anti-inflammatory parameters were expressed as mean and standard deviation, [mean(SD)] .

Differences of these parameters between the three groups were assessed by one-way analysis of variance, (ANOVA).

To show the exact differences between the three different experimental groups, multiple comparisons were performed using the Least Significant Difference (LSD) test, without any adjustment of the significance level.

All indices of the pathological assessment of the tissues, hyperaemia, haemorrhagic infiltration, loss of EC, mucosal oedema and presence of IC for the gastric mucosa, and hyperaemia and haemorrhagic infiltration for the colonic mucosa, were expressed as absolute frequencies and percentages (%) when they were considered as qualitative variables, and as Mean(SD) and Median (min-max) values, when they were considered as quantitative variables.

They were analysed using chi-square test, ANOVA and Kruskal-Wallis test, respectively.

All tests were two-tailed, and the significance level was set at α=0.05.

RESULTS

Chios Mastic Gum Analysis

The supplied resin was chemically analysed and was found to contain: natural Polymer – cis-1,4 poly-β-myrcene (25-30 %w/w), mastic oil (2-3%w/w), total mastic extract (70%w/w), acid fraction (38-42%w/w) and neutral fraction (28-32%w/w).

Gastric mucosal response to CRS

Following CRS, rats developed gastric mucosal lesions such as hyperaemia, haemorrhagic infiltration, mucosal oedema, loss of EC and infiltration by IC in all three groups (Figure 1).

The severity of these lesions was affected by the prior administration of either omeprazole or mastic powder.

As shown in Table 1, measurements of hyperaemia, haemorrhagic infiltration, and mucosal oedema were

Fig. 1. Gastric mucosa sections. H E x 200. Mucosal changes in: a. Chios mastic group: minimal hyperaemia and oedema, rare inflammatory cells, b. omeprazole group: mild hyperemia and oedema, minor deposits of inflammatory cells c. control: more extensive hyperemia and oedema and deposits of inflammatory cells, moderate hemorrhagic infiltration and pronounced loss of epithelial cells.
less pronounced in Groups O and M compared to Group C (chi-square test; p<0.05), (ANOVA; p<0.05), (Kruskal-Wallis test; p<0.05), for categorical or continuous variables, respectively (Figure 2). The same applied for the loss of EC. (chi-square test; p=0.084.) There were no statistical differences for any of these parameters, between Groups O and M (LSD test and Mann-Whitney U-test; p>0.05).

No statistically significant differences between the three groups were observed regarding the presence of IC. (p>0.05; chi-square test, ANOVA and Kruskal-Wallis test). Furthermore, the average total histological score was significantly lower in Group O (p<0.001; LSD test and Group M (p<0.001; LSD test) compared to group C. No statistically significant difference was found between Groups O and M (p=0.221; LSD test).

Table 1. Gastric mucosal lesions in the three groups

| Parameter                  | C            | O            | M            | p value | Multiple comparisons |
|----------------------------|--------------|--------------|--------------|---------|----------------------|
|                           | C vs O       | C vs M       | O vs M       |         |                      |
| Hyperemia                  |              |              |              | 0.001a  |                      |
| Negative                  | 0 (0.0)      | 2 (28.6)     | 1 (14.3)     |         |                      |
| Low                       | 0 (0.0)      | 5 (71.4)     | 6 (85.7)     |         |                      |
| Moderate                  | 6 (85.7)     | 0 (0.0)      | 0 (0.0)      |         |                      |
| High                      | 1 (14.3)     | 0 (0.0)      | 0 (0.0)      |         |                      |
| Mean value (SD)           | 2.14 (0.38)  | 0.71 (0.49)  | 0.86 (0.38)  | <0.001b | <0.001c              |
| Median value (min-max)     | 2 (2 – 3)    | 1 (0 – 1)    | 1 (0 – 1)    | <0.001d | 0.001e               |
|                           |              |              |              |         | 0.001e <0.001        |
| Hemorrhagic infiltration  |              |              |              |         | 0.530e               |
| Negative                  | 0 (0.0)      | 7 (100.0)    | 6 (85.7)     |         |                      |
| Low                       | 4 (57.1)     | 0 (0.0)      | 1 (14.3)     |         |                      |
| Moderate                  | 3 (42.9)     | 0 (0.0)      | 0 (0.0)      |         |                      |
| High                      | 0 (0.0)      | 0 (0.0)      | 0 (0.0)      |         |                      |
| Mean value (SD)           | 1.43 (0.54)  | 0.0 (0.0)    | 0.14 (0.38)  | <0.001  | <0.001 <0.001        |
| Median value (min-max)     | 1 (1 – 2)    | 0 (0 – 1)    | 1 (0 – 1)    | <0.001  | 0.001 0.002          |
|                           |              |              |              |         | 0.317                |
| Loss EC                   | 0.084        |              |              |         |                      |
| Negative                  | 0 (0.0)      | 4 (57.1)     | 3 (42.9)     |         |                      |
| Low                       | 5 (71.4)     | 3 (42.9)     | 4 (57.1)     |         |                      |
| Moderate                  | 2 (28.6)     | 0 (0.0)      | 0 (0.0)      |         |                      |
| High                      | 0 (0.0)      | 0 (0.0)      | 0 (0.0)      |         |                      |
| Mean value (SD)           | 1.29 (0.49)  | 0.43 (0.54)  | 0.57 (0.54)  | 0.014   | 0.006 0.019          |
| Median value (min-max)     | 1 (1 – 2)    | 0 (0 – 1)    | 1 (0 – 1)    | 0.026   | 0.015 0.030          |
|                           |              |              |              |         | 0.606                |
| Oedema                    | 0.017a       |              |              |         |                      |
| Negative                  | 0 (0.0)      | 5 (71.4)     | 4 (57.1)     |         |                      |
| Low                       | 7 (100.0)    | 2 (28.6)     | 3 (42.9)     |         |                      |
| Moderate                  | 0 (0.0)      | 0 (0.0)      | 0 (0.0)      |         |                      |
| High                      | 0 (0.0)      | 0 (0.0)      | 0 (0.0)      |         |                      |
| Mean value (SD)           | 1.00 (0.0)   | 0.29 (0.49)  | 0.43 (0.54)  | 0.012   | 0.005c 0.020c        |
| Median value (min-max)     | 1            | 0 (0 – 1)    | 0 (0 – 1)    | 0.020d  | 0.007e 0.023e        |
|                           |              |              |              |         | 0.591e               |
| Infiltration IC           | 0.466        |              |              |         |                      |
| Negative                  | 3 (42.9)     | 5 (71.4)     | 3 (42.9)     |         |                      |
| Low                       | 4 (57.1)     | 2 (28.6)     | 4 (57.1)     |         |                      |
| Moderate                  | 0 (0.0)      | 0 (0.0)      | 0 (0.0)      |         |                      |
| High                      | 0 (0.0)      | 0 (0.0)      | 0 (0.0)      |         |                      |
| Mean value (SD)           | 0.57 (0.54)  | 0.29 (0.49)  | 0.57 (0.54)  | 0.507   | 0.317 1.000          |
| Median value (min-max)     | 1 (0 – 1)    | 0 (0 – 1)    | 1 (0 – 1)    | 0.483   | 0.298 1.000          |
|                           |              |              |              |         | 0.298               |
| Average histological score, mean (SD) | 6.43 (1.13) | 1.71 (0.95) | 2.57 (1.62) | <0.001  | <0.001 <0.001        |

a chi-square test; b ANOVA; c LSD test; d Kruskal-Wallis test; e Mann-Whitney Test U-test. C: Control; O: Omeprazole M: Mastic Gum.
Effects of omeprazole and mastic gum on colonic mucosa

In all three groups of animals, cold restraint of rats at 4°C for three hours produced mucosal changes, as histologically observed in sections of the ascending colon.

Mucosal changes such as hyperaemia and haemorrhagic infiltration were observed in examined sections. The response of the colonic mucosa to the stimulus of stress was calculated by the number of mucin-containing Goblet cells (Figure 3) [43].

Measurements of hyperaemia and haemorrhagic infiltration of colonic mucosal lesions were less pronounced in Groups O and M compared to Group C. (p=0.001 and p=0.002, respectively; Chi-square test) or (p<0.001; ANOVA) and (p<0.001; Kruskal-Wallis test;) (Table 2 and Figure 4).

Both hyperaemia and haemorrhagic infiltration were similar between Groups O and M (p>0.05; LSD test and Mann-Whitney U-test). Furthermore, the average total histological score was significantly lower in Group O (p<0.001;LSD test) and Group M (p<0.001;LSD test) compared to Group C. No statistically significant difference was found between Groups O and M (p=0.119; LSD test). No statistically significant differences between the three groups were observed concerning the number of colonic Goblet cells (p=0.370;ANOVA) Table 2.

Antioxidative and anti-inflammatory properties of Omeprazole and mastic resin

Table 3 contains the results for the comparison of the anti-inflammatory and the oxidative stress markers between the three groups, both altogether and in pairs (Figure 5).

Statistically significant differences of peroxidase levels were observed between the three groups (p=0.039; ANOVA).

Post-hoc analysis revealed that 27.2% lower peroxidase levels were observed in Group M compared to Group C (p=0.018; LSD test) and by 23.6% in Group O (p=0.043;LSD test).

Fig. 2. Graphic for statistic comparison of gastric mucosal lesions between the three groups.

Fig. 3. Mucin containing mucosal Goblet Cells in histologic sections of the ascending colon, presenting as pink flower petals. Periodic acid–Schiff (PAS) X 200. a. after pre-treatment with CMG, b. after pre-treatment with Omeprazole and c. in the control group.
Peroxidase levels were not significantly different in groups C and O (p=0.666; LSD test).

No statistically significant difference of TEAC levels was observed between the three groups (p=0.277; ANOVA).

Analysis of variance revealed statistically significant differences in SOD levels between the three groups (p<0.001). Post-hoc analysis using LSD test revealed that, compared to group C, SOD levels were by 33.3% lower in Group O (p<0.001) and 44.2%, lower in group M (p<0.001). Moreover, SOD levels were significantly lower in Group M compared to Group O, by 16.3%, (p=0.047).

Regarding the anti-inflammatory properties, analysis of variance showed statistically significant differences in TNF-α and IL-1β levels between the three groups (Table 3).

TNF-α levels were 34.6% lower in Group M compared to Group C (p=0.100) and significantly lower, by 45.7%, compared to Group O (p=0.013).

Compared to Group C, lower IL-1β levels by 44.9%, were observed in Group O (p=0.001) and by 63.9%, in Group M (p<0.001).

Moreover, there was a 34.6% lower level of IL-1β in Group M compared to Group O (p=0.120), but this difference did not reach the statistical significance.

### Table 2. Mucosal lesions observed in the ascending colon in the three groups

| Groups | C | O | M |
|--------|---|---|---|
| Hyperemia | 0 (0.0) | 3 (42.9) | 0 (0.0) |
| Low | 1 (14.3) | 4 (57.1) | 7 (100.0) |
| Moderate | 3 (42.9) | 0 (0.0) | 0 (0.0) |
| High | 3 (42.9) | 0 (0.0) | 0 (0.0) |
| Mean value (SD) | 2.29 (0.76) | 0.57 (0.54) | 1.00 (0.0) |
| Median value (min-max) | 2 (1 – 3) | 1 (0 – 1) | 1 |

| Hemorrhagic infiltration | 0.002 |
|---|---|
| Negative | 0 (0.0) | 7 (100.0) | 5 (71.4) |
| Low | 3 (42.9) | 0 (0.0) | 2 (28.6) |
| Moderate | 4 (57.1) | 0 (0.0) | 0 (0.0) |
| High | 0 (0.0) | 0 (0.0) | 0 (0.0) |
| Mean value (SD) | 1.57 (0.54) | 0.0 (0.0) | 0.29 (0.49) |
| Median value (min-max) | 2 (1 – 2) | 0 | 0 (0 – 1) |
| Average Histological Score, mean (SD) | 3.86 (1.22) | 0.57 (0.54) | 1.29 (0.49) |
| Number of Goblet cells | 50.54 (3.56) | 52.71 (2.84) | 52.51 (2.83) |

*p value for Group C vs O, C vs M, O vs M: ANOVA; LSD test; *chi-square test; ANOVA; *LSD test; *Kruskal-Wallis test; *Mann-Whitney Test U-test. C: Control; Omeprazole M: Mastic Gum.*

Fig. 4. Graphic of statistic comparison of colonic mucosal lesions in the three groups
**Discussion**

Numerous studies have documented that CRS induces gastric mucosal lesions as a result of oxidative damage caused by the significant generation of ·OH [13, 22, 28-30].

In the present study, CMG was tested vs Omeprazole as a protection of the gastric and colonic mucosa against CRS induced by a mild severity protocol. The antioxidant and anti-inflammatory effects of the two agents were also evaluated.

Omeprazole is a selective and irreversible proton pump inhibitor, which suppresses stomach acid secretion by specific inhibition of the H^+K^+-ATPase system found at the secretory surface of gastric parietal cells [40, 41]. Biswas et al. (2003) have demonstrated that Omeprazole not only acts by decreasing gastric acid secretion [44].

It blocks the stress-induced increased generation of ·OH and associated lipid peroxidation and protein oxidation, indicating that its antioxidant role plays a major part in preventing oxidative damage. Omeprazole also

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**Table 3. Serum antioxidant and anti-inflammatory markers in the three groups**

|                      | C               | O               | M               | p value | Multiple comparisons |
|----------------------|-----------------|-----------------|-----------------|---------|---------------------|
|                      | μM Peroxidase/mg of protein | 0.151 (0.039) | 0.144 (0.027) | 0.110 (0.016) | 0.039 | 0.666 | 0.018 | 0.043 |
|                      | mM TEAC/mg protein | 0.024 (0.006) | 0.028 (0.004) | 0.031 (0.009) | 0.277 | 0.386 | 0.114 | 0.450 |
|                      | SOD U/mg protein | 0.156 (0.012) | 0.104 (0.016) | 0.087 (0.016) | <0.001 | <0.001 | <0.001 | 0.047 |
|                      | pg TNFa/mg protein | 0.999 (0.437) | 1.202 (0.396) | 0.653 (0.264) | 0.040 | 0.322 | 0.100 | 0.013 |
|                      | pg IL-1β/mg of protein | 4.399 (1.450) | 2.426 (0.762) | 1.587 (0.291) | <0.001 | 0.001 | <0.001 | 0.120 |

*a comparison between groups (one-way ANOVA); b pair-wise comparisons between groups (LSD test). C: Control; O: Omeprazole; M: Mastic Gum. TEAC: Trolox Equivalent antioxidant capacity, SOD: Superoxide dismutase.
prevents stress-induced DNA fragmentation caused by 
·OH in vitro [44]. However, serious side effects associ-
ated with Omeprazole may include Clostridium difficile 
colitis, an increased risk of pneumonia or bone frac-
tures. Moreover, acting as an inhibitor of the enzymes 
CYP2C19 and CYP3A4, Omeprazole may alter the 
absorption and plasma levels of anticoagulants, anti-
depressants, analgesics and some antibiotics [40, 41].

Consistent with previous experimental findings, hist-
ological changes of the gastric mucosa were observed 
in the herein study, such as hyperaemia, haemorrhagic 
infiltration, loss of EC, mucosal oedema and presence 
of IC in all three groups of animals, while true ulcers 
were not detected. The beneficial preventive effect of 
either Omeprazole or mastic gum compared to the 
control group was more pronounced for hyperaemia, 
haemorrhagic infiltration, loss of EC and oedema and 
almost non-existent for the presence of IC. However, 
no statistically significant effect was evident when com-
paring Omeprazole vs mastic gum for any of the five 
histological parameters.

These results suggest that CMG acts as efficiently 
as Omeprazole in the prevention of hyperaemia and 
haemorrhagic infiltration, oedema and loss of EC in 
the gastric mucosa of rats caused by CRS.

Hypoaemia and haemorrhagic infiltration of the 
colonic mucosa were less pronounced after omeprazole 
or mastic gum administration compared to the control 
group, but not significantly different between the two 
agents. Based on the results, CMG prevents hyperae-
emia and haemorrhagic infiltration of the colonic mu-
cosa caused by CRS.

Colonic mucin- containing cup cells that were quan-
titatively evaluated synthesise and secrete mucins, 
high molecular weight glycoproteins, which form a 
protective layer throughout the gastrointestinal (GI) 
tract. This layer acts as an effective barrier as shown by 
changes in mucins in inflammatory conditions of the 
GI tract, by the altered Goblet cell response in germ-
free animals, and by the enhanced mucus secretion 
seen in response to infections [4, 43]. Quantitative and 
qualitative evaluation of the mucin was not evaluated 
in the present study.

Triantafyllou et al. (2011) have demonstrated that 
the anti-inflammatory activity of CMG is associated 
with its antioxidant activity [37]. Pro-inflammatory 
cytokines stimulate superoxide production by NADPH 
oxidases, thus providing feed-forward activation of in-
flammatory pathways. This superoxide production is 
inhibited by mastic gum. It is suggested by recent stud-
ies that CMG acts as a peroxisome proliferator-activat-
ed receptor (PPAR) modulator [45]. PPARs are nuclear 
receptors that control cellular functions via transcrip-
tion at the level of gene expression implicated in the 
pathways of metabolic syndrome, inflammation, ath-
erosation, and cancer [36, 45].

In the present study, it was evident that the mastic 
gum affected the antioxidant enzymes peroxidase and 
SOD.

Administration of mastic gum significantly de-
creased the activity of both enzymes compared to the 
levels observed by the administration of Omeprazole. 
SOD, but not peroxidase, was also significantly reduced 
for Omeprazole compared to the control group.

Regarding the anti-inflammatory properties of 
CMG, the inhibition of pro-inflammatory cytokines 
such as prostaglandin E2 (PGE2) and nitric oxide 
(NO) is mainly attributed to the inhibition of iNOS and 
COX-2 protein expression by activated macrophages 
and the scavenging of NO [46].

Also, in studies on experimental colitis, CGM ad-
ministration resulted in a significant reduction of in-
flammatory markers as TNF-α, IL-6, and ICAM-1 and 
downregulated NF-jB p65 [37, 38]. Papalois et al. [36] 
have demonstrated that Chios mastic as a whole rather 
than its acidic, neutral components or oleanolic acid, 
exerts an anti-inflammatory effect on the colonic mu-
cosa in trinitrobenzene sulfonic acid (TNBS)-induced 
colitic in rats via NF-jB regulation.

Mastic gum, in the present study, demonstrated 
lower values for the pro-inflammatory cytokine TNF-α 
compared to Omeprazole and for IL-1β compared to 
the control group.

**Conclusions**

Peptic mucosal damage induced by acute stress is a 
serious cause of morbidity and mortality in critically 
ill patients. In this study, a mild CRS protocol induced 
peptic mucosal alterations, which were decreased in 
animals pre-treated with either CMG or Omeprazole. 
Furthermore, CMG appeared to significantly reduce 
hyperaemia, haemorrhagic infiltration, oedema and 
loss of epithelial cells to the gastric mucosa as well as 
hyperaemia and haemorrhagic infiltration to the col-
onic mucosa. At the same time, its antioxidative and 
anti-inflammatory properties, tested by various mark-
ers, were found comparable to those of Omeprazole.
It is concluded that Chios mastic resin is effective against CRS-induced peptic mucosal damage in rats.

The study showed promising results of the use of Chios mastic gum as a nutritional supplement and its incorporation in therapeutic research protocols in clinical practice.

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