

EFFECT OF AQUEOUS INFUSION FROM *COTINUS COGGYGRIA* LEAVES ON INDOMETHACIN-INDUCED GASTRIC MUCOSAL DAMAGE AND OXIDATIVE STRESS IN RATS

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ABSTRACT

PURPOSE: The Smoke tree (*Cotinus coggygria*) is well known medicinal plant that is used mainly externally by the Balkan folk medicine for its antiseptic and anti-inflammatory properties. There are scarce reports about the internal usage of decoctions from *C. coggygria* leaves against gastric ulcers. Our study was aimed to explore the effect of aqueous infusion from *Cotinus coggygria* leaves (AICCL) on indomethacin-induced gastric mucosal damage in Wistar rats and its possible effect on the gastric oxidative status.

MATERIAL AND METHODS: Three AICCL (1/100, 2/100 and 4/100) were applied by gastric gavage (volume: 10 ml/kg) as a pretreatment 3 days before a single intragastric administration of indomethacin (dose: 100 mg/kg). Gastric ulcer formation was estimated morphometrically and histopathologically 4 h after the indomethacin administration. Malondialdehyde (MDA) in blood serum and stomach was measured as a biochemical marker of lipid oxidation. Gastric necrosis was also evaluated by alkaline phosphatase (ALP) and uric acid (UA) assays.

RESULTS: Morphometrical examinations of stomachs showed that the 2/100 AICCL significantly decreased the ulcer number and area. Histopathological studies demonstrated that AICCL induced a reduction of the depth and severity of indomethacin-induced mucosal lesions. AICCL reduced the elevated by indomethacin gastric MDA, ALP and UA levels.

CONCLUSION: Indomethacin-induced gastric mucosal damage was accompanied by the development of oxidative stress. AICCL-pretreatment alleviated the gastric lesions, and reduced the indomethacin-induced elevation of ALP and UA. It could be suggested that the gastroprotective effect of AICCL was due to its antioxidant properties as evidenced by the decreased gastric MDA levels.

Key words: *Cotinus coggygria*, Indomethacin, Gastric mucosal damage, Oxidative stress, Lipid peroxidation, Antioxidant activity

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INTRODUCTION

Gastric ulcer is a recurrent chronic illness that affects approximately 10% of the world population (1). It is induced by several factors, including infection by *Helicobacter pylori*, emotional stress, smoking, nutritional deficiencies, alcohol consumption and treatment with non-steroidal anti-inflammatory

drugs (NSAIDs) (2, 3). Ulceration occurs when there is an imbalance between protective (mucus secretion, blood flow, prostaglandins, enzymatic and non-enzymatic antioxidants) and aggressive mechanisms in the stomach (acid-pepsin, leukotrienes and reactive oxygen species, ROS) (4). It is well known that ROS, especially the superoxide anion and the hydroxyl radical, play an important role in the pathogenesis of acute experimental gastric lesions induced by NSAIDs (5).

Indomethacin, like other NSAIDs, is known to induce gastric mucosal damage in humans (6) and is considered as an appropriate agent for development of animal models of NSAIDs-induced ulcerogenesis (7). The inhibition of cyclooxygenase (COX) and deficiency of endogenous prostaglandins (PGs) is accepted as a main mechanism implicated in indomethacin-induced gastropathy (8).

The therapy used for treating gastric ulcers includes the control of the *H. pylori* infection, the control of the H⁺/K⁺-ATPase pump and the acid secretion, as well as the damage and inflammation reversal to the mucosa (9). Medications prescribed for the treatment of gastric ulcer are not completely effective and exhibit many adverse reactions in addition of high economic burden (10). This has been the basis for screening of new sources of bioactive compounds, such as herbal extracts.

The Eurasian smoke tree (*Cotinus coggygia* Scop., Anacardiaceae) is a medicinal plant species with wide distribution from southern Europe, the Mediterranean, Moldova, and the Caucasus to Central China and the Himalayas (11). According to some authors (12, 13) the whole plant is poisonous due to the large content of gallotannins (above 25%). However, in the Balkan folk medicine, decoctions from leaves of *C. coggygia* are applied to treat gingival and throat inflammations, stomach ache, gastric ulcer, diarrhea, nephritis, anthrax, asthma, cardiac and urinal diseases, and even diabetes mellitus, due to their antiseptic, anti-inflammatory, antimicrobial, anti-hemorrhagic, and wound-healing properties (11, 13, 14, 15).

Studies on the chemical composition of leaves infusion report the presence of gallic acid methyl ester and anthocyanins (16), and gallotannins, gallic acid, flavonic glycosides, myrcen, alpha-pinene, camphen,

linalool, and alpha-terpineol (11, 17). Numerous polyphenolic compounds have been isolated, including quercetin, fustin, and taxifolin (18).

The antioxidant activity of extracts from *C. coggygia* leaves was demonstrated in several studies, suggesting the role of high polyphenol content (19, 20, 21, 22). It is noteworthy that the ethanol and aqueous extracts have shown the highest antioxidant activity and the highest content of polyphenols among dozens of investigated Bulgarian medicinal plants (19). Although that the plant seems to be extremely rich in biologically active compounds, it has been somewhat ignored by pharmacological studies because of the traditionally reported toxicity.

The aim of the present study was to investigate the effect of aqueous infusion from *Cotinus coggygia* leaves (AICCL) on indomethacin-induced gastric mucosal damage in rats and its possible effect on the gastric oxidative status.

MATERIAL AND METHODS

Experimental substances

Three aqueous infusions of *Cotinus coggygia* leaves (AICCL) were prepared one hour before each treatment: 1, 2 and 4 g dried material was scalded in 100 ml boiling distilled water for 10 min. 1/100 AICCL is commensurable to a traditional Bulgarian recipe for treatment of gastric ulcer (13).

Chemicals

Indomethacin (Indo) was obtained from Sigma-Aldrich (Germany). It was prepared as a suspension in a vehicle (2 drops of Tween 80 per 5ml of distilled water). All chemicals used for the biochemical analyses and histopathological examinations were of analytical grade and were obtained from Merck (Germany).

Experimental design

Male albino Wistar rats (2-2.5 months old; 220-250g) were kept under the standard conditions of the animal house with 12-h light-dark cycle (light 7:00-19:00) at a temperature 23-25°C. They were fasted 24 h before the indomethacin administration but had free access to water. The cohort comprised of five experimental groups each of eight rats: I. Control (C), II. Indomethacin (Indo), III. 1/100 AICCL+Indo, IV. 2/100 AICCL+Indo, V. 4/100 AICCL+Indo. The rats were orally pretreated by direct stomach

intubation (orogastric cannula) with water (groups I and II, volume: 10 ml/kg) or AICCL (groups III, IV, and V, volume: 10 ml/kg). Three days later, the rats were treated by a single intragastric administration of indomethacin (dose: 100 mg/kg). All procedures concerning animal treatment and experimentation were conducted in compliance with the national laws and policies and in conformity with the international guidelines (EEC Council Directive 86/609, IL 358, 1, December 12, 1987).

Blood serum and tissue preparation

The animals were anaesthetized with diethyl-ether 4 h after the indomethacin treatment. Blood was collected from the sublingual veins in heparinized tubes. Samples were centrifuged at 2000 rpm for 10 min and serum was obtained and stored at 20°C until biochemical analyses of uric acid (UA), malondialdehyde (MDA) and sulphhydryl groups (SH-groups) concentrations. After the animals decapitation the stomachs were removed immediately, opened along the great curvature, gently washed in physiological salt solution, spread over the pad and observed macroscopically for appearance of mucosal lesions. The length of each lesion was measured. In the case of petechia, five of them were considered

as a 1 mm lesion. Mean ulcer number and area were calculated. Stomachs were homogenized in 1:5 w/w 50mM phosphate buffer (pH 7.4) at 4000 rpm for 10 min. The homogenate was centrifuged at 800g rpm for 15 min to discard the sediment and the supernatant was taken for biochemical analyses: activity of alkaline phosphatase (ALP); concentrations of UA and MDA. All manipulations were performed at 4-8°C. MDA, ALP and UA were determined immediately after thawing the samples.

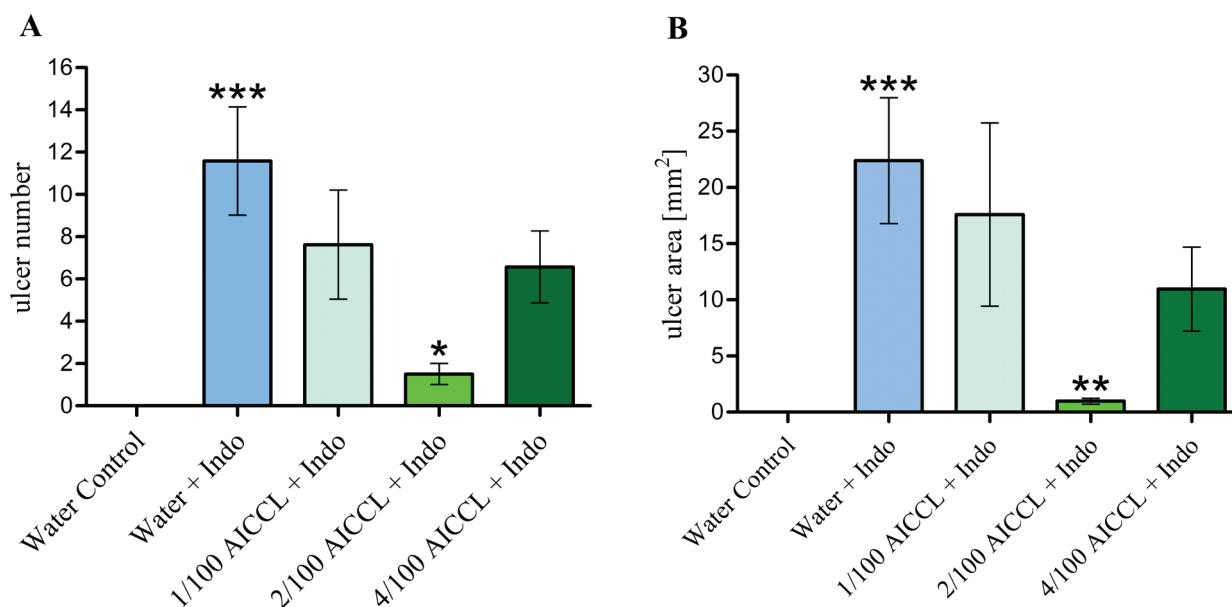
Histopathological study

Pieces of the stomachs were fixed immediately after morphometrical examination in 10% neutral buffered formaldehyde solution. Fixed tissues were embedded in paraffin, cut into sections and placed on microscope slides. Staining of the slides with hematoxylin-eosin was used for the histopathological examination which was performed under light microscopy and documented by microphotocamera.

Biochemical assays

Membrane lipid peroxidation was monitored by MDA in blood serum and stomach homogenates using the method of Porter (23). Determination of SH-groups was performed spectrophotometrically in se-

Fig. 1. Effect of AICCL-pretreatment on the number (A) and area (B) of gastric mucosal lesions in rats with indomethacin-induced ulceration.



*** $P < 0.001$ vs. water control; ** $P < 0.01$ vs. Indo; * $P < 0.05$ vs. Indo

rum according to the method of Ellman (24). Activity of ALP in stomach homogenates was measured by the standard kit of BioSystems S.A. (Spain). UA levels in blood serum and homogenates were measured by the standard kits of HUMAN liquicolor (Germany).

Statistical analyses

Data were analyzed by one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison posttest. Each two independent groups were compared also by Student's t-test. A value of $P < 0.05$ was considered as statistically significant. Data are expressed as mean \pm SEM. GraphPad Prism v 5.00 statistical software was used.

RESULTS

Morphometric evaluation of gastric mucosal damage

No mucosal lesions were detected in rats from water control group. Indomethacin induced multiple gastric mucosal lesions in the glandular part of the stomach, most often 1-4 mm² in size. The mean ulcer number in the Indo-group was 11.58 (Fig. 1A), and the mean ulcer area was 22.39 mm² (Fig. 1B). Pretreatment with 2/100 AICCL significantly reduced the ulcer number by 87% (Fig. 1A), and the ulcer area by 96% (Fig. 1B), respectively.

Fig. 2. Microscopic appearance (X100) of a rat stomach following the application of water (A), water plus indomethacin (B), and 2/100 AICCL plus indomethacin (C)

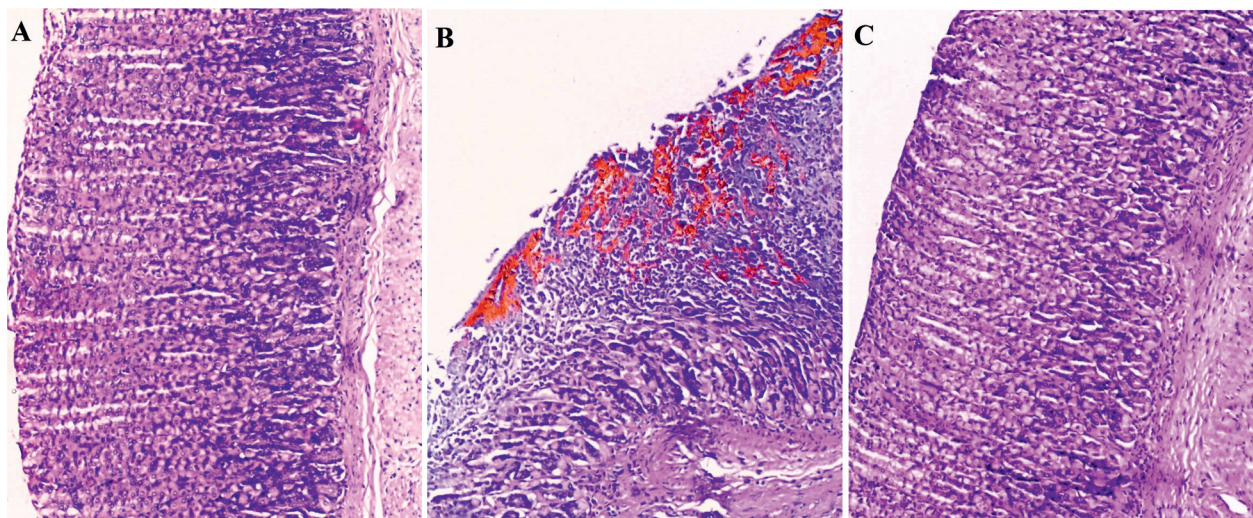


Table 1. Results from biochemical analyses of stomach homogenates and blood serum from rats, treated in a model of indomethacin-induced ulcerogenesis

Biochemical markers		Control (Water)	Indomethacin (Indo+Water)	1/100 AICCL + Indo	2/100 AICCL + Indo	4/100 AICCL + Indo
Stomach	MDA [$\mu\text{mol/L}$]	1.216 \pm 0.10	^{ooo} 3.285 \pm 0.32	^{**} 1.462 \pm 0.23	^{**} 1.369 \pm 0.16	^{**} 1.660 \pm 0.11
	UA [$\mu\text{mol/L}$]	225.7 \pm 37.9	^{ooo} 447.1 \pm 19.0	^{**} 210.7 \pm 38.6	^{**} 312.5 \pm 39.5	^{**} 247.4 \pm 40.2
	ALP [U/L]	19.17 \pm 1.53	^{oo} 47.28 \pm 5.68	^{**} 18.23 \pm 2.28	^{**} 18.31 \pm 2.53	^{**} 20.90 \pm 3.68
Blood	MDA [$\mu\text{mol/L}$]	0.507 \pm 0.02	0.541 \pm 0.03	0.483 \pm 0.02	0.530 \pm 0.02	0.441 \pm 0.02
	UA [$\mu\text{mol/L}$]	105.9 \pm 4.50	94.13 \pm 5.32	89.10 \pm 4.22	83.91 \pm 4.82	83.17 \pm 3.60
	SH-groups [$\mu\text{mol/L}$]	225.6 \pm 9.08	220.8 \pm 7.47	230.5 \pm 9.55	232.0 \pm 14.54	224.1 \pm 12.86

Legend: ^{ooo} $P < 0.001$ vs. Water Control; ^{oo} $P < 0.01$ vs. Water Control; ^{**} $P < 0.01$ vs. Indomethacin

Histopathological study

The microscopic appearance of the gastric mucosa of the control rats was normal (Fig. 2A). The experimental lesions evoked by indomethacin were manifested by multiple erosions comprising about 2/3 of the mucosal layer thickness. The defects were filled with hemorrhages and epithelial cell necroses (Fig. 2B). In the rats pretreated with 2/100 AICCL before the indomethacin administration, the gastric erosions were more superficial and in some cases only bleeding and focal desquamation of the epithelium were found (Fig. 2C).

Biochemical assays

All results from biochemical measurements are presented in Table 1. The gastric MDA levels of Indo-group were increased by 270% versus the control. In the groups pretreated with AICCL before the indomethacin administration, the concentration of MDA decreased significantly ($P < 0.01$) in comparison with the Indo-group. The same tendency was observed in UA and ALP levels in the stomach. No significant changes were found in blood serum levels of MDA, UA and SH-groups.

DISCUSSION

The indomethacin-induced gastric lesions could be caused by several mechanisms. It is generally accepted that the ulcerogenic activity of NSAIDs is related to their ability to inhibit endogenous PGs synthesis due to the non-selective inhibition of COX (8). Some authors (25) concluded that the complete inhibition of COX leading to decrease in PGE₂ content probably consumed a much longer time and did not occur 6 h after a single dose. In regard to these findings, we might suppose that in the current experimental model the inhibition of the PGs secretion is not the main pathobiochemical mechanism of ulcerogenesis.

There are data that ROS are involved in the development of mucosal damage by NSAIDs (5), and that they increase lipid peroxidation, an important cause for cellular membranes damage. In our study, the involvement of extensive lipid peroxidation in indomethacin-induced gastric mucosal damage was evidenced by the accumulation of MDA in gastric mucosa (Table 1). Similar are the results obtained by some other authors (26), who studied the peroxidation of lipids in the same experimental model. In

their study, the amount of MDA in the gastric mucosa was significantly increased 4 h after indomethacin administration. Presented results demonstrated that the AICCL reduced the oxidative stress and the related histomorphological signs of indomethacin-induced gastric mucosal damage. One possible mechanism of this effect is the antioxidant potential of AICCL (19), due to the polyphenols in the infusion, predominantly high gallic acid content (11, 16, 17). Another possible mechanism of the gastroprotective effect of AICCL might be the anti-histamine activity demonstrated for some flavonoids (27).

ALP activity has been reported as a biochemical marker in bone, liver and gastrointestinal lumen diseases (28). The release of this enzyme is related to the mechanisms of tissue necrosis (29). The results from biochemical analysis (Table 1) showed that indomethacin increased significantly the levels of ALP in gastric tissue. The pretreatment with AICCL decreased significantly gastric ALP levels, suggesting a strong protective effect of *Cotinus coggygria* infusions that was confirmed histopathologically (Fig. 2). The absence of gastric lesions in AICCL-pretreated groups was also confirmed by the significantly lower gastric UA concentrations. Higher UA levels in Indo-group could be associated with the degradation of nucleotides due to elevated necrosis caused by indomethacin.

CONCLUSIONS

Our study demonstrated a strong protective effect of aqueous infusion from *Cotinus coggygria* leaves against indomethacin-induced gastric ulceration. Based on results discussed above, we suggest that the most probable mechanism of this beneficial effect is the significant decrease of lipid peroxidation, due to antioxidant properties of plant investigated.

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