GASTROPROTECTIVE EFFECT OF ETHANOL INFUSION FROM *COTINUS COGGYGRIA* WOOD IN RATS

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ABSTRACT

PURPOSE: The Smoke tree (*Cotinus coggygria*) is a medicinal plant that is traditionally used by the Balkan and Anatolian folk medicine for its antiseptic and anti-inflammatory properties. There are few reports about the internal usage of ethanol extracts from *C. coggygria* wood against gastric ulcer. The aim of this study was to explore the effect of ethanol infusion from *Cotinus coggygria* wood (EICCW) on indomethacin-induced ulcerogenesis in rats and its possible effect on the gastric oxidative status.

MATERIAL AND METHODS: EICCW was applied by oral gavage (volume: 10 ml/kg) as a pretreatment 3 days before a single intragastric administration of indomethacin (dose: 100 mg/kg). Gastric erosions were evaluated histopathologically. Malondialdehyde (MDA) in blood serum and stomach was measured as a biochemical marker of lipid peroxidation. Gastric necrosis was also evaluated by alkaline phosphatase (ALP) and uric acid (UA) assays.

RESULTS: EICCW reduced the elevated by indomethacin gastric MDA, ALP and UA levels. Histopathological studies demonstrated that EICCW induced a reduction of the depth and severity of indomethacin-induced mucosal lesions.

CONCLUSION: Indomethacin-induced gastric mucosal damage was accompanied by the development of oxidative stress. EICCW-pretreatment alleviated the gastric lesions, and prevented the indomethacin-induced elevation of gastric ALP and UA. It could be suggested that the gastroprotective effect of EICCW 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*

INTRODUCTION

The Eurasian smoke tree, sumac or young fustic (*Cotinus coggygria* Scop., Anacardiaceae) is a medicinal plant species, widely distributed from southern Europe, the Mediterranean, Moldova, and the Caucasus to Central China and the Himalayas (1). According to some authors (2,3) the whole plant is poisonous due to the large content of gallotannins (above 25%). However, in the Balkan and Anatolian folk medicine, extracts from *C. coggygria* are applied to treat gingival and throat inflammations, stomach

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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 (1,2,3,4,5). There are few reports about an internal use of ethanol infusions from the wooden parts of the plant to treat gastric ulcer (personally collected data).

Studies on the chemical composition of methanol extracts from *C. coggygria* wood report the presence of numerous polyphenols: sulfuretin, fustin (6), myricetin, quercetin (7), fisetin and methylgallate (8). The antioxidant activity of extracts from *C. coggygria* wood was demonstrated in few studies, suggesting the role of their high polyphenol content (7, 9). Although 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.

Despite significant medical advances, the gastric ulcer is still a common global disease with increasing incidence and prevalence (10). It is induced by several factors, including infection by *Helicobacter pylori*, emotional stress, smoking, nutritional deficiencies, ethanol consumption and treatment with non-steroidal anti-inflammatory drugs (NSAIDs) (11). 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) (12).

Indomethacin is known to induce gastric mucosal damage in humans and is considered as an appropriate agent for development of animal models of NSAIDs-induced ulcerogenesis (13). The non-selective inhibition of cyclooxygenase (COX) and deficiency of endogenous prostaglandins (PGs) is accepted as a main mechanism implicated in indomethacin-induced gastropathy (14). Absolute ethanol is widely used to induce experimental gastric ulcers in rats (15). By increasing mucosal permeability and release of vasoactive products, the absolute ethanol causes gastric mucosal cell necrosis, which leads to ulcer formation (16). It is claimed that ROS play a role in the pathogenesis of acute experimental gastric lesions induced by ethanol or NSAIDs (17, 18). Medications prescribed for the treatment of gastric ulcer are not completely effective and exhibit many adverse reactions in addition of high economic burden (19). This has been the basis for screening of new sources of bioactive compounds, such as plant extracts.

The aim of the present study was to investigate the effect of ethanol infusion from Cotinus coggygria wood (EICCW) on indomethacin-induced ulcerogenesis in Wistar rats and its possible effect on the gastric oxidative status.

MATERIAL AND METHODS

Infusion preparation

The ethanol infusion of *Cotinus coggygria* wood (EICCW) was prepared following the traditional recipe for coloring high alcoholic beverages: 2g dried material from heartwood was placed in 1L 40% ethanol for 20 days. EICCW was diluted to 20% before each treatment of rats.

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 three experimental groups each of eight rats: I. 20% Ethanol Control; II. 20% Ethanol + Indo; III. EICCW + Indo. The rats were orally pretreated (volume: 10 ml/kg) by direct stomach intubation (orogastric cannula) with 20% Ethanol (groups I and II) or EICCW (group III). 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).

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Blood serum and tissue preparation

The animals were anaesthetized with diethylether 4 h after the indomethacin treatment. Blood was collected from the sublingual veins in heparinized tubes. Samples were centrifuged at 2000g rpm for 10 min and serum was obtained and stored at 20°C until biochemical assays of uric acid (UA), malondialdehyde (MDA) and sulphydryl groups (SH-groups) concentrations. After the decapitation of the animals, the stomachs were removed immediately, opened along the great curvature, gently washed in physiological salt solution and stored at -20°C until further homogenisation in 1:5 w/v 50mM phosphate buffer (pH 7.4) at 4000g 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 in 10% neutral buffered formaldehyde. The further processing included routine techniques for dehydratation, clearing and embedding in paraffin. The prepared sections (3-5 μm) were stained with hematoxyllin/eosin.

Biochemical assays

Membrane lipid peroxidation was monitored by MDA in blood serum and stomach homogenates using the method of Porter (20). Determination of SH-groups was performed spectrophotometrically in serum according to the method of Ellman (21). 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 kit of HUMAN liquicolor (Germany).

Statistical analyses

Data were analyzed by one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison post-test. 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 ± SEM. GraphPad Prism v 5.00 statistical software was used.

RESULTS

Biochemical assays

The gastric MDA and UA levels of 20% Ethanol + Indo-group were higher than the 20% Ethanol Control (Fig. 1A,B) and a significant increase ($P<0.01$) of ALP was registered for 20% Ethanol + Indo group (Fig. 1 C). EICCW decreased significantly ($P<0.05$) the concentration of gastric MDA in comparison with the 20% Ethanol + Indo-group (Fig. 1A). The same decrease was observed in gastric UA and ALP levels (Fig. 1B, C). No significant changes were found in blood serum levels of MDA, UA and SH-groups (Fig. 2).

Histopathological evaluation

In the 20% Ethanol Control group, significant destructive epithelial changes of gastric mucosa were observed. They were localized at the outer 1/3 layer of

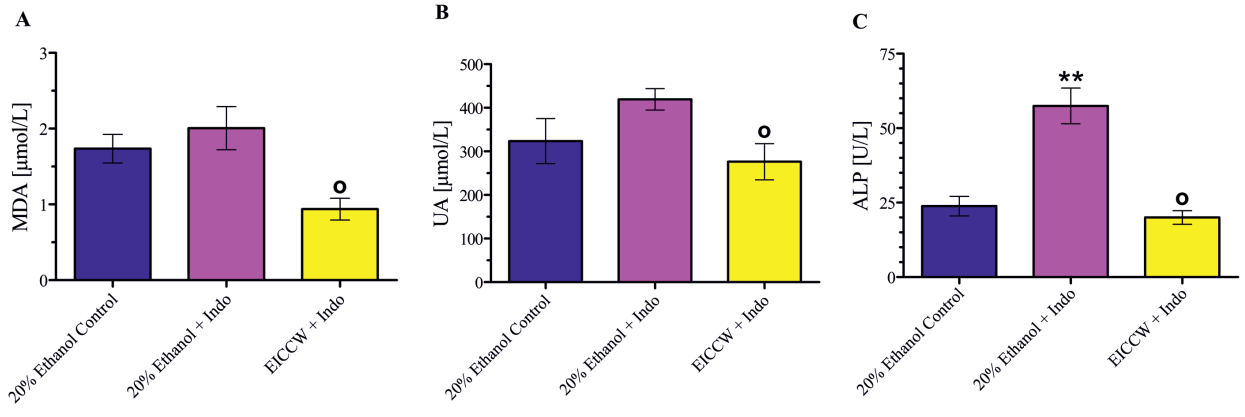

Fig. 1. Results from biochemical analyses of stomach homogenates from rats, treated in a model of indomethacin-induced ulcerogenesis: A. Malondialdehyde (MDA); B. Uric Acid (UA); C. Alkaline phosphatase (ALP).
*Legend: **P<0.01 vs. 20% Ethanol Control; °P<0.05 vs. 20% Ethanol + Indomethacin*

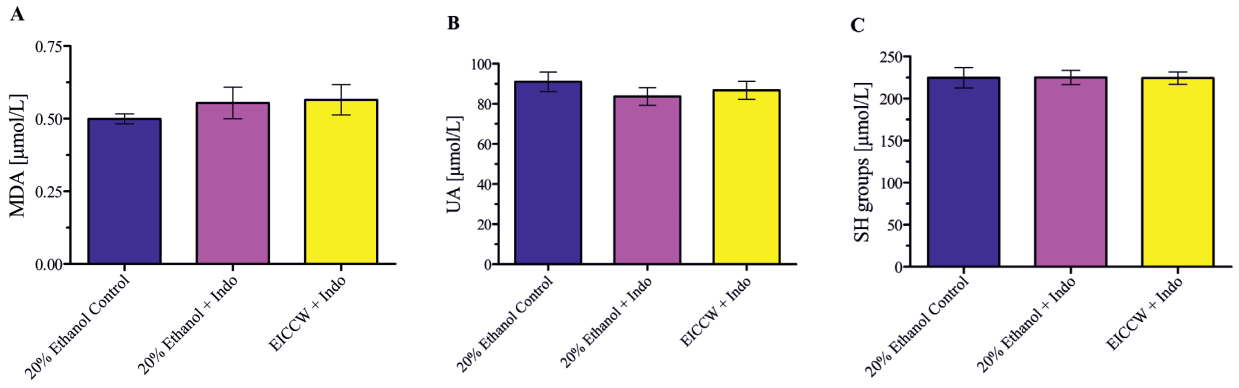

Fig. 2. Results from biochemical analyses of blood serum from rats, treated in a model of indomethacin-induced ulcerogenesis: A. Malondialdehyde (MDA); B. Uric Acid (UA); C. Sulphydryl groups (SH-groups).

the mucosa, but erosions were not found (Fig. 3A). In the 20% Ethanol + Indo group, there were multiple erosions, reaching to the inner 1/3 mucosal layer. The erosions were filled with blood and necrotic epithelial cells (Fig. 3B). In the group, pretreated by EICCW before the indomethacin administration, the gastric erosions were more superficial and the necrotic areas were smaller. Focal epithelial desquamation and small bleeding were only observed in some cases (Fig. 3C).

endogenous PGs synthesis due to the non-selective inhibition of COX (14). Some authors (22) concluded that the complete inhibition of COX leading to decrease in PGE_2 content probably consumed a much longer time and did not occur 6 h after a single dose. In regard to these findings, we may suppose that the inhibition of the PGs secretion is not the main pathobiochemical mechanism of ulcerogenesis in the current experimental model. Another possible mechanism of ulcerogenesis could be the increased muco-

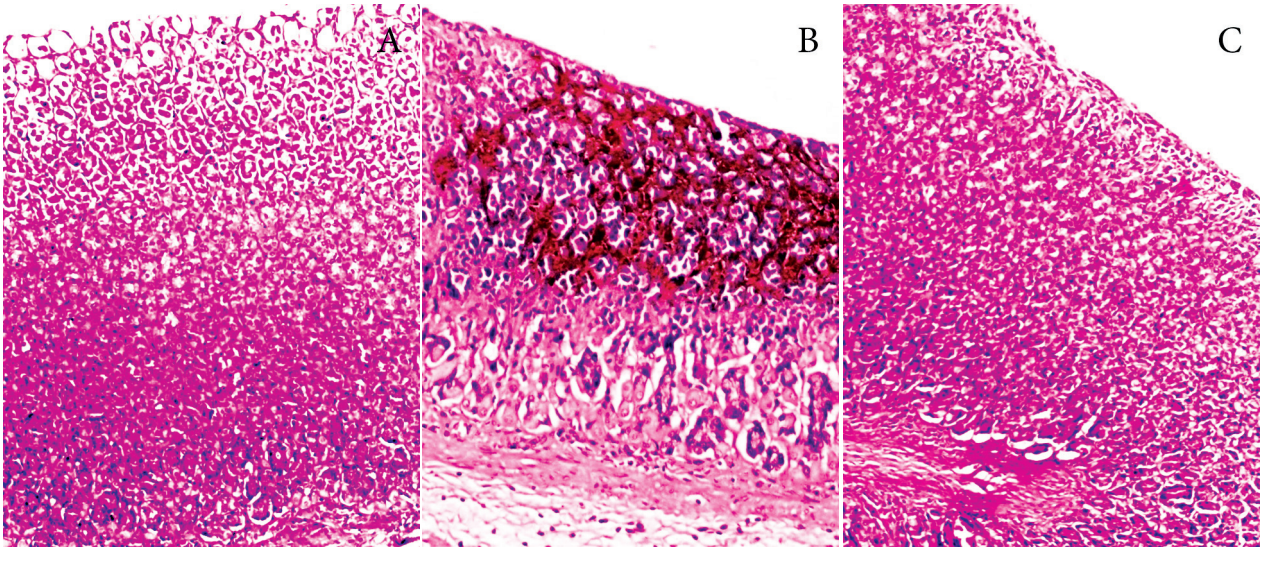

Fig. 3. Microscopic appearance of a rat stomach following the administration of: A. 20% Ethanol; B. 20% Ethanol + Indo; C. EICCW + Indo

DISCUSSION

It is generally accepted that the ulcerogenic activity of NSAIDs is related to their ability to inhibit sal permeability and release of vasoactive products, caused by the ethanol (15,16,17). The presented results showed that the pretreatment with 20% Etha-

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nol for 3 days did not induce erosions, and only destructive epithelial changes of gastric mucosa were observed (Fig. 3A). On the other hand, indomethacin in combination with 20% Ethanol caused deep multiple erosions, bleeding and necrosis (Fig. 3B).

There are data that ROS are involved in the development of mucosal damage by NSAIDs or ethanol (17,18), and that they increase lipid oxidation, an important cause for cellular membranes damage. In our study, the involvement of lipid peroxidation in indomethacin-induced gastric mucosal damage was evidenced by the increase of gastric MDA levels (Fig. 1A) in agreement with the results obtained by some other authors (23), who studied the peroxidation of lipids in a similar experimental model. They reported that the concentration of MDA in the gastric mucosa and blood was significantly increased 4 h after intraperitoneal administration of indomethacin. In this study, no significant changes were found in blood serum levels of MDA, UA and SH-groups (Fig. 2). Most probably, the intragastric administration of indomethacin could not change the oxidative status of the blood within the next 4 hours. The presented results demonstrated that the EICCW significantly reduced the oxidative stress (Fig. 1A) and the related histomorphological signs of indomethacin-induced gastric mucosal damage (Fig. 3C). One possible mechanism of this effect is the antioxidant activity of EICCW (7,9), due to its polyphenol content, predominantly of sulfuretin, fustin, methylgallate and myricetin (6,7,8). Another possible mechanism of the gastroprotective effect of EICCW could be the anti-histamine activity demonstrated for some flavonoids (24).

Alkaline phosphatase (ALP) activity has been reported as a biochemical marker in bone, liver and gastrointestinal lumen diseases (25). The release of this enzyme is related to the mechanisms of tissue necrosis (26). The results from the biochemical analyses showed that indomethacin increased significantly ($P<0.01$) the ALP activity in gastric tissue (Fig. 1C). The pretreatment with EICCW decreased significantly ($P<0.05$) the gastric ALP levels (Fig. 1A), suggesting a protective effect of *Cotinus coggygria* wood infusion that was also confirmed histopathologically (Fig. 3C), and by the significantly lower ($P<0.05$) gastric UA concentrations of EICCW + Indo group (Fig. 1B). Higher UA levels in 20% Ethanol + Indo group could be associated with the degradation of nucleotides due to elevated necrosis caused by Indomethacin.

CONCLUSIONS

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

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