Effects of *Matricaria chamomilla* on element status in ethanol-intoxicated rats

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**SUMMARY**

The aim of this study was to investigate the in vivo dose-dependent effects of *Matricaria chamomilla* extract (MCE) on cellular trace and major element concentrations in ethanol-intoxicated rats. Firstly, MCE (0, 25, 50, 100, 200 or 400 mg/kg) or famotidine (20 mg/kg) was administered to male rats (n = 8, in each group) by gavage (0.5 mL) one hour before 80% ethanol gavage (1 mL) then the erythrocyte concentrations of major elements (K+, Ca2+ and Mg2+) and of trace elements (Cu, Zn, Al, Mn, Cr, Se and Ni) were measured by optical emission spectroscopy one hour later and compared to healthy controls (n = 8). The K, Cu, Zn and Al cellular contents did not significantly vary among the different groups. Compared to the healthy controls (untreated and not intoxicated), the famotidine pre-treatment induced significant reductions of the cellular Ca and Mg concentrations and significant increase of the Se content whereas the MCE administration did not significantly alter these biochemical parameters. The Cr and Mn contents tended to increase in rats only intoxicated with ethanol and in rats treated with the lowest and the highest doses of MCE whereas famotidine tended to depress them. Finally, compared to ethanol intoxicated animals, famotidine and MCE (at doses of 100, 200 and 400 mg/kg) pre-treatments have restored the erythrocyte Ni contents which became similar to control values. These results suggest that the MCE treatment, mainly at high doses, may prevent alterations of the mineral status induced by ethanol in rats with the same or even a greater efficiency than the drug reference (famotidine).

**Keywords:** *Matricaria chamomilla*, ethanol, trace elements, major elements, gastric ulcer, famotidine.

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**Introduction**

Gastric mucosal injury is a common disorder of the gastrointestinal (GI) system due to some factors such as ethanol, stress, cigarette smoking, nutritional deficiencies, use of steroidal and non-steroidal anti-inflammatory drugs and reactive oxygen species [2-4, 35], that alter the gastro-duodenal mucosal defence mechanisms.

The gastric mucosal damage induced by high concentrations of ethanol has widely been used in rats to investigate the gastro-protective effects of various medicinal plants [42]. By enhancing lipid peroxidation in gastric mucosa and depleting major antioxidants (superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) enzymes, reduced glutathione and vitamins A, C, and E [16, 20]), ethanol toxicity is mediated throughout production of free radicals, leading to gastric damage.

*Matricaria chamomilla* has been used in Europe for thousands of years. It is a traditional treatment for numerous disorders, including sleep disorders, digestion/intestinal conditions, skin infections/inflammation (including eczema), wound healing, infantile colic, teething pains, and diaper rash. It has been also reported that *Matricaria chamomilla* has moderate antioxidant and antimicrobial activities, and has moderate antioxidant and antimicrobial activities, and a sedative, analgesic, antispasmodic, antimutagenic and cholesterol-lowering activities, as well as some anti-inflammatory effects as well as some antimutagenic and cholesterol-lowering activities, have been also described in vivo [6, 10, 13, 24, 31].

In the United States, chamomilla is also known as an ingredient in herbal tea preparations for which adverse mild sedating effects were reported [31]. The extract of chamomilla flowers contains angelic acid (2-methyl-2-butenolic acid), azulene, chamazulene (1,4-dimethyl-7-ethylazulene), α-bisabol, cineole, matricarin and matricin as major constituents [31]. Some compounds such as apigenin and chamazulene are known to depress the protein expression by partially inhibiting transcription [13, 24]. Recently, it has been demonstrated that some flavonoids from chamomilla exert benzodiazepine-like and phosphodiesterase inhibitory actions, leading to increase of cellular cAMP levels [13]. Several trace elements and major minerals related to numerous enzyme- and hormone systems participate to the control of signalling and metabolism pathways [41].

Biochemical parameters and bio-element status might alter during ethanol-induced gastric mucosal damage. Plasma or erythrocyte levels have traditionally been used to assess the status of trace and major elements. The present study was designed to investigate the in vivo dose-dependent effect of Matricaria chamomilla extract (MCE) on the trace and major elements concentrations in ethanol-induced gastric mucosal damage in rats after determining the MCE composition itself in trace and major elements. Furthermore, the MCE effect is compared to famotidine (FAM), which is commonly used in the treatment of peptic ulcer as a reference drug.

### Materials and Methods

#### CHEMICALS AND PLANT MATERIAL

Hydrogen peroxide, ethanol, sodium chloride, nitric acid, perchloric acid and Suprapur ICP multi-element standard solutions were purchased from Merck. Famotidine was obtained from Mustafa Nevzat A.G., Turkey and ketamine from Pfizer, Turkey. All other chemicals and reagents used in this study were of analytical grade. Ultra-pure water was used as solvent in all experiments.

The aerial parts of Matricaria chamomilla were collected during May 2005 from the Afyonkarahisar region at an altitude of 1020 m over sea level. The Department of Botany, Science and Arts Faculty, Kocatepe University, Afyonkarahisar, Turkey confirmed the identity of the collected specimens (Herbarium number: Kala1397). A voucher specimen (B3A) has been kept in our laboratory for future reference. After air-drying, the plant was pulverized and extracted on a Soxhlet apparatus using 1 L 37% ethanol and 63% water per 100 g plant material. After extraction, the solvent was recovered and the residue was lyophilized, weighed (yield: 17.7%) and stored at 4°C. The extract was then diluted with distilled water to obtain the different concentrations used for treatment in our experiments.

#### ANIMALS AND PROTOCOL DESIGN

The investigation was conducted in accordance with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication no. 85-23, revised 1996) and approval has been received from our the Animal Ethics Committee of Afyon Kocatepe University.

Sixty-four male Wistar rats weighing 150-200 g were used for the experiment. The rats were fed with standard laboratory chow and tap water ad libitum. The animals were equally divided into eight groups (n = 8) each kept in separate cages. To prevent coprophagy the rats were placed in cages with wire-net floors. Twenty-four hours before the experiment, the rats were fasted giving them access only to water. On the day of the experiment, the rats assigned as controls (Group 1) drank only saline water. Rats from the groups 2 to 7 received 0, 25, 50, 100, 200 and 400 mg/kg chamomilla extract, respectively and in the group 8, the animals were treated with the reference drug, famotidine (20 mg/kg). All drugs were administered by gavage with the same volume (0.5 mL) and 60 min after drug treatment, a single dose of 1 mL ethanol (80%) was administrated to the animals by gavage. One hour after, all rats were injected intraperitoneally with ketamine (100 mg/kg) and blood samples were taken by cardiac puncture into heparin-treated collection tubes. The red blood cells (RBC) were washed three times with phosphate-buffered saline (PBS) pH 7.4 and the erythrocytes were stored in polystyrene plastic tubes at -70°C until the time of analysis.

#### ANALYTICAL PROCEDURES

All the experiments were carried out in plastic containers that were washed in 10% ultrapure grade HNO₃ and then repeatedly rinsed with ultra water. The elements were determined after mineralization of the samples by microwave digestion in a Milestone Start D oven equipped with a Pro 24 High Throughput Rotor and a temperature control unit (Italy). Decomposition of the organic matrix in the erythrocyte samples was performed in cycles of 24 samples. Erythrocyte and Matricaria chamomilla samples of exactly 0.1 g were weighed and placed in high-pressure Teflon vessels, added with a mixture of 3 mL of concentrated HNO₃, 1 mL of H₂O₂ and 0.5 mL HClO₄ (ultrapure, Merck, Germany). The Teflon vessels were then sealed and put into steel bombs, heated in a microwave oven according to the following temperature-time sequences: 90°C/15 min, 120°C/15 min, 140°C/60 min and 150°C/60 min. After cooling to room temperature, the resulting solutions were quantitatively transferred and adjusted in a volumetric flask to 10 mL with 18.2 MΩ.cm ultrapure water (Millipore Direct-Q UV, Japan). The trace and major element concentrations in the digest were determined by inductively coupled plasma-optical emission spectroscopy (ICP-OES; Spectro Genesis, Germany). Accuracy of the analysis was verified by the determination of the mineral content of the ICP multi-element standard obtained from Merck (Germany). The operating conditions of the ICP-OES are given in Table I.

#### STATISTICAL ANALYSIS

All values were expressed as mean ± standard deviation. The statistical analyses of data were performed using a one-way analysis of variance (ANOVA) and Tukey’s post-test. A value of \( P < 0.05 \) was considered statistically significant.
Results

As reported in the table II, the Matricaria chamomilla Extract (MCE) was enriched in K, Na, and Ca for major elements and B, Zn, Cu, Al, Fe, and Ni for trace elements. As far as major elements were concerned (Table III), there was no statistically significant difference in erythrocyte K concentration among all the treatment groups while the cellular Ca content was significantly depressed in rats treated with famotidine + ethanol (group 8) compared to healthy controls (group 1) ($P < 0.05$) and to rats treated with MCE before ethanol administration ($P < 0.05$ for groups 5 (100 mg/kg), 6 (200 mg/kg) and 7 (400 mg/kg) and $P < 0.01$ for groups 3 (25 mg/kg) and 4 (50 mg/kg)). In the same way, rats from the group 8 exhibited a marked decrease of the erythrocyte Mg content compared to all the other groups except for the group 4 (MCE: 50 mg/kg): the highest mineral concentrations were observed in the groups 2 (only ethanol) and 3 (MCE: 25 mg/kg plus ethanol) (groups 2 and 3 vs. group 8: $P < 0.01$) and the Mg contents in red blood cells were roughly similar in the groups 1, 4, 5, 6 and 7. No statistically significant difference was evidenced between groups for erythrocyte Al, Cu and Zn concentrations (Table IV).

Discussion

Plant extracts have been widely used for treatment of various physiological and pathological processes. In a previous

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**Table I**: ICP-OES apparatus specifications and analytical conditions for the determination of elements.

<table>
<thead>
<tr>
<th>Instrument</th>
<th>Spectro Genesis Fee, Germany</th>
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</thead>
<tbody>
<tr>
<td>Nebulizer</td>
<td>Cross Flow</td>
</tr>
<tr>
<td>Plasma Power</td>
<td>1380 W</td>
</tr>
<tr>
<td>Coolnat Flow</td>
<td>14.00 L/min</td>
</tr>
<tr>
<td>Auxiliary Flow</td>
<td>1.00 L/min</td>
</tr>
<tr>
<td>Nebulizer Flow</td>
<td>1.05 L/min</td>
</tr>
<tr>
<td>Optic Flush</td>
<td>Normal</td>
</tr>
<tr>
<td>Measure Strategy</td>
<td>Best SNR</td>
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<td>Reply</td>
<td>2</td>
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<td>90 s</td>
</tr>
<tr>
<td>Flush Time</td>
<td>40 s</td>
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</tbody>
</table>

**Table II**: Composition (mg/kg) of the *Matricaria chamomilla* Extract (MCE) in trace and major elements. The experiment was performed 3 times and results are expressed as mean ± standard deviation.

**Table III**: Major element contents in erythrocytes from rats treated with MCE (*Matricaria chamomilla* extract, at the doses of 25, 50, 100, 200 and 400 mg/kg) (groups 3-7) or FAM (famotidine, 20 mg/kg) (group 8) one hour before 80% ethanol administration by gavage (1 mL). The rats from the group 1 were healthy controls (no ethanol and no drug treatment) whereas in the group 2, rats received only ethanol. Results are expressed as mean ± standard deviation.

**Table IV**: Composition (mg/kg) of the *Matricaria chamomilla* Extract (MCE) in trace and major elements. The experiment was performed 3 times and results are expressed as mean ± standard deviation.
study, we have investigated the possible anti-hyperglycemic and antioxidant activities of the *M. chamomilla* in streptozotocin-induced diabetic rats. It was demonstrated that treatment with different doses of *M. chamomilla* significantly reduced postprandial hyperglycemia and the streptozotocin-induced oxidative stress, and augmented the efficiency of the various antioxidant systems [6]. In the present study, we investigated the in vivo dose-dependent effect of *chamomilla* extract on the erythrocyte trace- and major elements concentrations in ethanol-induced gastric mucosal damage in rats. In our earlier studies, we have demonstrated that ethanol has induced gastric lesions in rats [3, 7]. Ethanol is a commonly used as an ulcerogenic agent and when given by gavage to rats, it produces severe gastric hemorrhagic lesions. The mechanism of ethanol-induced gastric lesions is varied, including the depletion of mucus mucus content, damaged mucosal blood flow and mucosal cell injury [3].

Several factors such as increased vascular permeability, gastric motility and vagal activity, decreased gastric blood flow [30]. Moreover, local prostaglandins act as gastric mucosa protectors by promoting cell proliferation and mucus secretion and by reducing HCl secretion [39]. Various compounds are known to induce peptic ulcer in rats: some of them like indomethacin or acetylsalicylic acid inhibit the prostaglandin synthesis and others like hydrocholoric acid or ethanol promote the radical oxygen species (ROS) production in the gastro-intestinal tissue [30, 39]. The absolute ethanol leads to intense damage of the gastric mucosa, inducing multiple hemorrhagic red bands or patches of different sizes along the long axis of the stomach [25]. The potency of 40% ethanol often consumed does indeed induce hemorrhagic gastric ulcer in rats [28]. The gastro-toxicity results from the solubilisation of the mucus constituents coupled to the decrease of the electrical potential difference in the mucosa leading to Na⁺ and K⁺ accumulation into the lumen. In parallel, histamine was locally released and this mediator stimulates the K⁺/H⁺ antiports: the H⁺ ions are consequently expelled from gastric cells into the lumen with an efficiency which is proportional to the K⁺ concentration gradient and the proton accumulation leads to the chemical activation of pepsinogen into pepsin [15, 37, 38]. The gastric H⁺/K⁺-ATPase of the parietal cell is responsible for acid secretion in the stomach and is the main target in the pharmacological treatment of acid-related diseases. Potassium competitive acid blockers compete with K⁺ for binding to the H⁺/K⁺ antiports and consequently limit the proton secretion [19]. The lack of variations in the cellular K⁺ concentration among the experimental groups might suggest that the acid secretion was not altered.

Anti-acid drugs, mainly aluminium-magnesium preparations, are regularly and heavily consumed by the most peptic ulcer patients [32] despite the risk for bone aluminium accumulation [32]. However, no significant alteration of erythrocyte Al content was evidenced in the present study whatever the dispensed treatment. LIANG et al [21] have investigated the potential effect of Mg adjuvant treatment coupled with esomeprazole on active duodenal ulcer diseases in humans and they concluded that both treatments presented the same efficiency and the same safety. In the present study, the erythrocyte Mg concentrations were depressed by the famotidine treatment compared to ethanol intoxicated animals whereas they tended to decrease with the MCE treatment, mainly with high doses and were similar to values recorded in healthy controls. A variety of pathological conditions are associated with low Mg levels. Hypocalcemia and hypokaliemia frequently accompany magnesium deficiency [33] (Table III).

Both *in vitro* and *in vivo* studies have shown that the radical oxygen species, primarily superoxide and hydroxyl radicals, play important roles in the pathogenesis of acute gastric mucosa injury [2-4, 11, 35, 36]. Furthermore, increase of lipid peroxidation and ROS in blood result in alteration of the cellular homeostasis leading to membrane damage. Antioxidant enzymes such as Mn- and Cu/Zn SOD, CAT and GPx play an important role in the mechanisms of defence against free radical damage [41]. The variations of the erythrocyte element contents as increases of the erythrocyte antioxidant enzyme activities might be peripheral responses of the organism to increased ROS production in gastric injury.

GÖTZ et al [14] reported that in gastritis due to Helicobacter pylori the Mn-SOD activity and concentration increased for minimizing gastric mucosal damage due to free radicals produced in response to *H. pylori* infection, whereas the
other Cu/Zn SOD isoenzyme slightly decreased. In agreement with that, erythrocyte Cu and Zn concentrations were not significantly altered by drug treatment (famotidine or MCE) and/or by ethanol administration, whereas Mn concentrations were significantly increased in rats receiving only ethanol and in animals pre-treated with the lowest (25 mg/kg) and the highest (400 mg/kg) MCE doses, suggesting the Mn-SOD up regulation in erythrocytes in these groups. In addition, zinc supplementation has been shown to have a preventive effect on experimentally induced acute gastric lesions in rats, probably throughout the inhibition of gastric mucosal mast cell degranulation and the stabilization of lysosomal membranes [8, 9]. However, ITO et al. [17] reported that zinc chloride (49.0 mg/kg, given two times a day by the oral route) was ineffective against the healing of acetic acid-induced gastric ulcers in rats and Frommer [12] also reported that zinc sulphate (220 mg, three times a day, orally) accelerated the healing of gastric ulcers in humans.

Selenium is an essential element which improves the activity of the seleno-enzymes, particularly of GPx, and prevents oxidative lesions in cells and tissues in vivo [18]. Büyükoğlu et al. [4] observed a decrease of the GPx activity in rats with ethanol-induced gastric lesions treated with an anti-ulcer and antioxidant drug, the dantrolene sodium. In the present study, contrary to the famotidine administration, the pre-treatment with MCE at doses superior to 50 mg/kg has induced significant reductions of the erythrocyte Se content compared to rats intoxicated with ethanol.

It has been found that ROS and the consequent lipid peroxidation are involved in the pathogenesis of ethanol-induced gastric lesions and gastrointestinal damage, and that calcium is an important mediator in these events. The oxygen-derived free radicals and extent of lipid peroxidation increase in hypoxic tissue resulting in drastic changes at the cellular level causing plasma membrane damage, intracellular calcium accumulation (which plays a role in free radical-mediated lipid peroxidation), cell death, exfoliation and epithelial erosion. An increase of the intracellular calcium concentration activates some calcium-dependent enzymes and worsens the oxidative damage [5, 22, 34]. Accordingly, the erythrocyte Ca content was reduced in the FAM treated group compared to healthy control (P < 0.05) but values recorded in MCE treated groups remained similar to those obtained in controls, suggesting that the alteration of the cellular calcium concentrations has not occurred in these conditions.

Furthermore, the trivalent chromium may act as an antioxidant. It was reported that intraperitoneal administration of Cr (III) protected rodents from acute CCl4 toxicity [29]. The authors pointed out that microsomal lipid peroxidation was depressed in mice 24 hours after Cr (III) administration, suggesting its role as a radical scavenger [40]. In this way, cellular Cr content tended to increase in MCE pre-treated groups, mainly at the lowest dose (p < 0.001) compared to famotidine treated animals.

Urease is a nickel-requiring enzyme that catalyses the hydrolysis of urea into CO2 and NH4+ and was essential for the H. pylori pathogenesis [23, 26, 27]. Indeed, urease-negative mutants fail to colonize various animal models and nickel transporters, such as NixA, are required for complete urease activity in both H. pylori and in an E. coli infection models [1, 26]. The erythrocyte Ni content was markedly depressed in ethanol intoxicated rats compared to healthy controls (P < 0.05) whereas the pre-treatments with famotidine or MCE (mainly at doses above 100 mg/kg) prevented the erythrocyte Ni depletion.

In conclusion, the MCE pre-treatment of rats appears to prevent the ethanol induced alterations of some minerals such as Ca, Mg, Mn, Se and Ni, directly or indirectly implicated in the antioxidant systems with a similar or even a higher efficiency than the reference famotidine drug, suggesting the potential interest of MCE in the cure of gastric peptic ulcers. Nevertheless, further studies are required for morphologically evaluating the evolution of the ulcer diseases.

References
