Evaluation of antioxidant and antimutagenic activities of aluminum chloride

Purpose
Hemostatic agents are used to control hemorrhage and the gingival crevicular fluid for dental applications. In this study; the antimutagenic and antioxidant activities of aluminum chloride (AlCl₃), a topical hemostatic agent used especially in the fields of dermatology and dentistry, were determined. To our knowledge, this is the first study that investigates these properties.

Materials and Methods
The antioxidant activity was determined by DPPH free radical scavenging and β-carotene-linoleic acid bleaching assays. The antimutagenic activity was evaluated with the Ames Salmonella microsome mutagenicity test using Salmonella typhimurium TA98 and TA100 strains.

Results
The total antioxidant activity of AlCl₃, determined by β-carotene bleaching assay was found to be 25.59 ± 2.55% and the DPPH scavenging activity of AlCl₃ was determined as 17.49 ± 3.07%. AlCl₃ showed not any mutagenicity at the tested concentrations by the AMES test used S. typhimurium TA98 and TA100. This drug demonstrated antimutagenic effects at the test concentrations and the strongest antimutagenic activity was observed on 1.25 mg·mL⁻¹/plate concentration of AlCl₃.

Conclusion
AlCl₃ showed potent antimutagenic and antioxidant activities and these properties are significant for dentistry and dermatology.

Keywords: AlCl₃; Ames; oxidative stress; DPPH; hemostatic

Introduction
Mutations and rearrangements in DNA may give rise to development of many degenerative diseases such as atherosclerosis, autoimmune diseases, Alzheimer’s disease, some types of diabetes, and aging (1,2). In cancer initiation and other stages of the carcinogenic process, mutation in somatic cells plays a key role (3).

Reactive oxygen species (ROS) made oxidative stress in the organism and these radicals caused oxidation of biomolecules and eventually cellular damage. The damage that ROS brings about on DNA, protein and lipids result in tissue injury (4,5). During oxidative stress lots of ROS are revealed and they are one of the most common reasons of intracellular DNA modifications (6,7). Oxygen radicals react with DNA and this interaction lead to oxidative damage of DNA (8). Following the reactions formed with free radicals, base changes in nucleic acid and chain breaks in DNA take place. If this change is not repaired, mutation and mutagenic DNA forms will be formed (9).

Cancer incidence may be reduced if mutation rates are decreased. The
best way to decrease mutation rates in human beings is to prevent exposure to mutagenic and carcinogenic agents (10). The natural antimutagens, using control of mutagenity, can prevent cancer and other diseases caused by genotoxic agents (11,12).

Aluminum chloride is a topical agent used for hemostasis. It is formulated as AlCl₃ and has acidic character. It can be formulated in concentrations of 20-40% in water, alcohol, ether or glycerol as a protein coagulant (13). The 25% buffered aluminum chloride solution is marketed under the brand name Frenna AC Solution (Dharma Research, Inc., US).

Since there is a significant amount of protein in the blood, its protein coagulant property makes this agent a potent hemostatic agent (13). When blood is exposed to AlCl₃ a chemical reaction occurs between blood proteins and hydrochloric acid (HCl) that is believed to be formed by hydrolysis of AlCl₃ (13,14). This causes coagulation of the tissues, vasoconstriction, thrombus and occlusion of small blood vessels, tissue damage and thus activation of the extrinsic coagulation pathway, protein precipitation and coagulation (14,15).

The literature has reported use of this material especially in the fields of dermatology and dentistry (13). In dermatology, AlCl₃ is applied to bleeding areas with a cotton-tipped applicator after the wound is dried as much as possible. The tip is applied on the wound with a slight pressure and a twisting motion perpendicular to the skin. It is known to be used after curettings and shave and punch biopsies (15). It is also used as a successful contrast enhancer to differentiate between normal and cancer cells in Mohs micrographic surgical technique (16). In dentistry, it has been put to use in dental surgeries to obtain hemostasis and also as a medicament solution for gingival retraction cords to obtain proper impressions in the field of prosthetic dentistry (13,17). Furthermore, AlCl₃ is the most popular topical treatment applied to patients with Frey Syndrome who present with complaints about hyperhidrosis (18).

AlCl₃ is a preferred agent because it is an inexpensive, easy to use, easily stored material that does not require preparation (15). Despite these advantages, it has side effects such as painful paresthesia, burning sensation, tissue irritation and delayed wound healing; thus it must be applied with caution (15,19).

This study is aimed to determine the antimutagenic activity of AlCl₃. The H₀ hypothesis of this study is that there is no difference between the different concentrations of AlCl₃ on the antimutagenic and antioxidant activities. To the best of our knowledge, this property has not yet been studied and this will be the first to be reported in the literature.

### Materials and Methods

**Frenna AC solution**

The sample of AlCl₃ was provided as Frenna AC solution (25% AlCl₃) (Dharma Research Inc., US). The concentrations of 0.025, 0.25, 1.25 and mg·mL⁻¹/plate of AlCl₃ prepared with distilled water were used in the mutagenity and antimutagenicity tests. For antioxidant activity measurements, 125 mg·mL⁻¹ concentration prepared with distilled water was used.

**Microbial strains**

The mutagenicity and antimutagenic activity of AlCl₃ was determined with Ames Salmonella/ microsome mutagenicity assay. In this test the mutant strains S. typhimurium TA98 and TA100, histidine dependent Salmonella strains were used (20).

**Determination of DPPH radical scavenging activity**

The radical scavenging activity of AlCl₃ was determined by DPPH free radical method (21). AlCl₃ was used at 125 mg·mL⁻¹ concentration. Ascorbic acid (5 mg·mL⁻¹) and α-tocopherol (5 mg·mL⁻¹) were used as positive controls.

**The β-carotene bleaching assay**

The antioxidant activity of AlCl₃ was also determined by the β-carotene bleaching assay (22). AlCl₃ was used at 125 mg·mL⁻¹ concentration; ascorbic acid (5 mg·mL⁻¹) and α-tocopherol (5 mg·mL⁻¹) were used as positive controls. Antioxidative activity of the AlCl₃ was compared with the positive controls ascorbic acid and α-tocopherol.

**Mutagenic and antimutagenic activity**

Mutagenic and antimutagenic activities of AlCl₃ were examined using the plate incorporation method (23) detailed by Sarac and Sen (24). Known mutagens 4-nitro-o-phenylenediamine (4-NPD) (3 µg/plate) and sodium azide (NaN₃) (8 µg/plate) were used as positive controls for S. typhimurium TA98 and S. typhimurium TA100, respectively. After determining the cytotoxic doses of AlCl₃, the subcytotoxic doses (0.025, 0.25, and 1.25 mg·mL⁻¹/plate concentrations) were studied for the activity assay. The antimutagenic activity (%) was calculated using the following equation:

\[
\text{Inhibition} \%(\%) = \left( \frac{A-B}{A} \right) \times 100
\]

A: The number of revertants per plate in the positive control,
B: The number of revertants per plate in the presence of mutagen and AlCl₃.

40% or more inhibition was determined as strong; 25-40% inhibition as moderate, and 25% or less inhibition was determined as low/none antimutagenic activity (11,25).

**Statistical analysis**

All tests were carried out in triplicates. Data were presented as mean ± SD. Dose dependent antimutagenic activity of AlCl₃ is also evident from the correlation and regression analyses, the F- and t-tests were used. SPSS 16.0 (SPSS, Inc, Chicago, IL, USA) and Microsoft Excel 2010 were used for the statistical evaluations. All hypotheses were tested at 0.05 significance level.

**Results**

This study evaluated the antioxidant and antimutagenic properties of AlCl₃, a buffered hemostatic agent, used in the fields of dermatology and dentistry. H₀ hypothesis was accepted. DPPH assay was used to determine the free-radical-scavenging activity of AlCl₃ (Table 1). The radical scavenging activity of AlCl₃ was less than the
activity of α-tocopherol and ascorbic acid. According to the results, AlCl₃ has moderate scavenging activity (17.49%). Total antioxidant activity of AlCl₃ was evaluated using the β-carotene bleaching assay (Table 2). The total antioxidant activity of AlCl₃ was found to be lower than that of ascorbic acid and α-tocopherol. The antioxidant activity results showed that AlCl₃ has moderate total antioxidant activity (25.59%).

In the Ames test, firstly, the cytotoxicity of AlCl₃ on S. typhimurium TA 98 and TA 100 was evaluated and the minimum cytotoxic dose was determined as 2.5 mg·mL⁻¹/plate concentration. In the mutagenicity and antimutagenicity tests the subcytotoxic doses of AlCl₃ (1.25, 0.25, and 0.025 mg·mL⁻¹/plate concentrations) were used. The AlCl₃ at the tested concentrations showed no mutagenic effects in the Ames test (data not shown).

The antimutagenic activity of AlCl₃ against 4-NPD and NaN₃ was determined with the same strains (Table 3). 0.025, 0.25, and 1.25 mg·mL⁻¹/plate concentrations were used; and AlCl₃ was effective at 1.25 mg·mL⁻¹/plate concentration on TA98, and at 0.25 and 1.25 mg·mL⁻¹/plate concentrations on TA100. The strongest antimutagenic activity was determined at 1.25 mg·mL⁻¹/plate concentration on S. typhimurium TA98.

The antimutagenic activity of AlCl₃ increased dose dependently. AlCl₃ has moderate antimutagenic activity in higher test concentrations. The results showed no significant difference between the different doses of AlCl₃ in increasing the number of revertant colonies.

### Discussion

The oxidation caused by toxic ROS on cellular structures such as DNA, lipids, proteins, carbohydrates and other biological molecules may result in DNA mutations and/or damage target cells or tissues which frequently leads to cell senescence and death (26,27). Natural antioxidants acquired from herbs and spices play a role in inhibition or prevention of the destructive consequences of oxidative stress (28).

Ames test is a short-term bacterial reverse mutation test specifically designed to investigate new substances and drugs that can cause damage genetically and lead to gene mutations (20). The Salmonella strains used in Ames test have different mutations in the histidine operon and each mutation is designed to respond to mutagens that have various mechanisms of action (20,23).

Genetically transferred metabolic disorders, various human diseases with age related and cancer, are resulted by mutations (29). The best way to decrease mutation rate in human beings is to lessen exposure to mutagenic and carcinogenic agents (10).

Cancer can be defined as an excessive multiplication of cells, which when followed by a cell invasion in the tissue surrounding it, spreads to other parts of the body. One of the chief characteristics of cancer is consistent cell proliferation, which disrupts the balance of the cell life cycle (30). Usually, cancer occurs when a mutation takes place in a cell and later it undergoes transformation turning into a malignancy of different stages by an acquisition (in a sequence) of further mutations (31).

Oral cancer stands fifth among the most commonly suffered cancer forms around the world; it is a life shattering disease (32). Oral cancer can be described as the cancer of pharynx and mouth, tongue, lips, palate, alveolar mucosa, floor of the mouth, tonsils, salivary glands, buccal mucosa, gingiva, and oropharynx (33). Cancer potential may be minimised if the mutation rate is decreased. An effective way to control this mutation rate is by avoiding exposure to the ingestion of carcinogens and mutagens (10).

The use of antimutagenic and anticarcinogenic agents in daily life is the most effective method to prevent cancer and genetic diseases (10). The control of cellular mutagens by

### Table 1: Free radical scavenging activity (%) of AlCl₃.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Activity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AlCl₃</td>
<td>17.49 ± 3.07</td>
</tr>
<tr>
<td>α-tocopherol</td>
<td>92.95 ± 0.54</td>
</tr>
<tr>
<td>Ascorbic acid</td>
<td>96.59 ± 0.06</td>
</tr>
</tbody>
</table>

### Table 2: Antioxidant activity (%) of AlCl₃ in β-carotene bleaching assay.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Antioxidant activity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AlCl₃</td>
<td>25.59 ± 2.55</td>
</tr>
<tr>
<td>Ascorbic acid</td>
<td>55.08 ± 2.95</td>
</tr>
<tr>
<td>α-tocopherol</td>
<td>91.99 ± 0.61</td>
</tr>
</tbody>
</table>

### Table 3: The antimutagenic activity of AlCl₃.

<table>
<thead>
<tr>
<th>Test items</th>
<th>Concentration</th>
<th>Number of revertants</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>TA98</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mean ± SD</td>
</tr>
<tr>
<td>Negative control</td>
<td></td>
<td>5.66 ± 0.57</td>
</tr>
<tr>
<td>4-NPD† (µg/plate)</td>
<td>3</td>
<td>378 ± 29.66</td>
</tr>
<tr>
<td>NaN₃† (µg/plate)</td>
<td>8</td>
<td>757 ± 33.2</td>
</tr>
<tr>
<td>AlCl₃ (mg·mL⁻¹/plate)</td>
<td>1.25</td>
<td>231.33 ± 24.19</td>
</tr>
<tr>
<td></td>
<td>0.25</td>
<td>300.33 ± 20</td>
</tr>
<tr>
<td></td>
<td>0.025</td>
<td>370.66 ± 4.04</td>
</tr>
</tbody>
</table>

†4-NPD and NaN₃ were used as positive controls.
natural antimutagenic agents can help prevent the mutations that eventually result in cancer and other diseases caused by genotoxic agents (11,12). Even though the antimutagenic activity of a drug does not definitely indicate that it is anticarcinogenic, it may certainly be considered a sign of anticarcinogenicity (34).

**Conclusion**

According to analysis performed within the present study, AlCl₃ was found to exhibit antimutagenic and antioxidant activity in vitro. The results showed that AlCl₃ was safe at the tested concentrations, and may represent an easily attainable antioxidant and antimutagenic source for dental applications. Moreover, the antioxidant and antimutagenic activity of AlCl₃, used as a hemostatic agent in dermatology and dentistry, has potential characteristics to provide prophylaxis against oxidations and mutations to an extent.

**References**

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