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 (AlCl$_3$), 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 AlCl$_3$, determined by β-carotene bleaching assay was found to be 25.59 ± 2.55% and the DPPH scavenging activity of AlCl$_3$ was determined as 17.49 ± 3.07%. AlCl$_3$ 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 AlCl$_3$.

Conclusion
AlCl$_3$ showed potent antimutagenic and antioxidant activities and these properties are significant for dentistry and dermatology.

Keywords: AlCl$_3$; 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
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 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 hemostasis. 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
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
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.

Türkçe Öz: Alüminyum klorit antioksidan ve antimutagenik aktivite-lerinin değerlendirilmesi. Araç; Hemostatik ajanlar, kanamayı ve dişetin oluşu sivvisini kontrol etmek amacıyla diş hekimliği uygulamalarında kullanılmaktadır. Bu çalışmada; özellikle dermatoloji ve diş hekimliği inde topikal hemostatik ajan olarak kullanılan alüminyum klorit (AlCl₃) antimutagenik ve antioksidan aktiviteleri belirlenmiştir. Edindilen bilgilerle göre bu çalışma bu aktivitelerin araştırıldığı ilk çalışma ol büyük önem ve öneme sahiptir. Gereç ve yöntem: Antioxidan aktivite DPPH serbest radikal giderici ve β-karoten- linoleik asit asit ağırtma deneyi ile belirlenmiştir. Antimutagenik aktivite Ames Salmonella/mikrozomal mutantjen testi ile Salmonella typhimurium (TA98) ve TA100 suşları kullanılarak değerlendirilmiştir. Bulgular: AlCl₃’ün β-karoten- linoleik asit ağırtma deneyi ile belirlenen toplam antioksidan aktivitesi %25.59±2.55 olarak belirlenmiştir. DPPH serbest radikal giderici aktivitesi 17.49±3.07 olarak tespit edilmiştir. AlCl₃’ün test konsantrasyonlarında, Salmonella typhimurium (TA98) ve TA100 suşları ile yapılan Ames testine göre herhangi bir mutagenik etkiye sahip olmadığını belirtmiştir. Bu ilaç test konsantrasyonlarında antioksidan etkisi göstermiştir ve en güçlü antimutagenik aktivite 1.25 mg/ml puanti konsantrasyonunda tespit edilmiştir. Sonuç: AlCl₃ antimutagenik ve antioksidan aktivite göstermiştir ve bu özellikler diş hekimliği ve dermatoloji için önem arz etmektedir. Araştırma Kellime-leri: AlCl₃; Ames; oksidatif stres; DPPH; hemostatic

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