Animal Health Prod and Hyg (2026) 15 (2) : 1 - 10

Animal Health, Production and Hygiene

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Research Article

# Effects of Locally Applied Clove and Green Tea Hydrosols on Cyclophosphamide-Induced Oral Mucositis in Rats

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**ABSTRACT**

This study investigated the effect of clove and green tea hydrosol applied locally in a rat model of oral mucositis induced with cyclophosphamide. This study is a preclinical, *in vivo,* experimental, and analytical study. The study was conducted using 40 female Wistar albino rats and were randomly divided into 8 groups. A single high-dose cyclophosphamide injection was administered intraperitoneally for mucositis induction. For mucositis care, the rats received twice daily oral spray clove or green tea hydrosol in the experimental groups. These interventions continued until collection of cardiac blood samples and oral mucosal tissue samples from the right cheek on day 4 or day 8. Serum total antioxidant status (TAS) and total oxidant status (TOS) were determined from the blood samples, and the tissue samples were analyzed for erosion, fibrosis, and inflammatory cell infiltration. Mean serum TAS was higher and TOS was lower in the clove and green tea hydrosol groups. There was also significantly less erosion in the clove and green tea hydrosol groups than in the positive control groups (P=0.003). On day 8, the clove and green tea hydrosol groups had less fibrosis and showed minimal inflammatory cell infiltration. In rats, clove and green tea hydrosols were found to be effective in preventing erosion associated with cyclophosphamide-induced oral mucositis. It is recommended that studies be conducted to investigate the effect of clove and green tea hydrosols on preventing oral mucositis in patients.

***Keywords:*** *Cancer, clove, cyclophosphamide, green tea, oral mucositis*

# Ratlarda Siklofosfamid ile İndüklenen Oral Mukozitte Lokal Olarak Uygulanan Karanfil ve Yeşil Çay Hidrosollerinin Etkileri

**ÖZET**

Bu araştırmada, ratlarda siklofosfamid ile oluşturulan oral mukozit modelinde lokal uygulanan karanfil ve yeşil çay hidrosolünün etkisi araştırılmıştır. Bu çalışma, preklinik, *in vivo* deneysel ve analitik bir araştırmadır. Çalışma, 40 dişi Wistar albino rat kullanılarak yürütülmüş ve ratlar rastgele 8 gruba ayrılmıştır. Mukozit indüksiyonu için ratlara intraperitoneal olarak tek yüksek dozda (300 mg/kg) siklofosfamid uygulanmıştır. Mukozit bakımını sağlamak için deney gruplarındaki ratlara günde iki kez karanfil veya yeşil çay hidrosolü ağız spreyi şeklinde uygulanmıştır. Bu uygulamalara kalp kanı ve sağ yanak mukozasından doku örneklerinin alındığı deneyin 4. gün ve 8. gününe kadar devam edilmiştir. Toplanan kan örneklerinden serum toplam antioksidan seviyesi (TAS) ve toplam oksidan seviyesi (TOS) ölçülmüş, doku örnekleri ise erozyon, fibrozis ve inflamatuar hücre infiltrasyonu açısından incelenmiştir. Karanfil ve yeşil çay hidrosolü uygulanan gruplarda serum TAS ortalama değeri daha yüksek, TOS değeri ise daha düşüktü. Ayrıca pozitif kontrol gruplarına kıyasla, karanfil ve yeşil çay hidrosolü uygulanan gruplarda erozyon istatistiksel olarak anlamlı derecede (P=0,003) daha az gelişti. Sekizinci gün karanfil ve yeşil çay hidrosolü gruplarında fibrozis daha az ve inflamatuar hücre infiltrasyonu minimum seviyedeydi. Ratlarda, siklofosfamide bağlı gelişen oral mukozitte karanfil ve yeşil çay hidrosolünün erozyonu önlemede etkili olduğu saptanmıştır. Karanfil ve yeşil çay hidrosolünün hastalarda oral mukoziti önlemeye yönelik etkisinin çalışmalarla araştırılması önerilmektedir.

***Anahtar kelimeler:*** *Kanser, karanfil, oral mukozit, siklofosfamid, yeşil çay*

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Received Date: 13.11.2025 - Accepted Date: 09.04.2026
DOI: 10.53913/aduveterinary.1822967

## Introduction

Oral mucositis (OM) is defined as oedema, erythema, and ulceration of the oral mucosa. It most frequently affects the oral cavity, buccal mucosa, lips, soft palate, and sides of the tongue (Negrin and Toljanic, 2021; Oronsky et al., 2018; Razmara and Khayamzadeh, 2019). OM is a common side effect of regional (radiotherapy) or systemic (chemotherapy) cancer treatment. However, its incidence is higher in patients who receive a conditioning regimen in preparation for stem cell transplantation or undergo radiotherapy for head and neck cancer (Carulli et al., 2013; Razmara and Khayamzadeh, 2019; Santosa et al., 2021; Sonis et al., 2004). OM is expected to develop in 68% of autologous stem cell transplant patients and 98% of allogeneic stem cell transplant patients (Babic and Murray, 2019; Baysal and Sarı, 2016).

Cyclophosphamide is an alkylating drug from the nitrogen mustard group that is frequently used in immunosuppressive treatment before stem cell transplantation, and OM is among its common adverse effects (Al-Refai, 2017). Cyclophosphamide causes OM directly by disrupting the proliferation of oral mucosa cells and indirectly by suppressing the bone marrow. OM is reported to be a risk factor associated with shorter survival in patients treated with cyclophosphamide. However, there is no proven agent for OM prophylaxis and the gold standard for its treatment has not yet been established (Al-Refai, 2017; Cidon, 2018; Çıtlak and Kapucu, 2015; Mutluay Yayla, 2017). Therefore, the management of OM remains a current issue, with ongoing research into new treatment methods (İzgü, 2017; Mutluay Yayla et al., 2016).

Cloves and green tea are plants that have been investigated for effectiveness against OM (Carulli et al., 2013; Giacomelli et al., 2015; Kong et al., 2016; Lalla et al., 2020). Clove has been used in the management of OM due to the anti-inflammatory, antibacterial, antimitotic, and antiviral properties of its constituent eugenol (Danthu et al., 2020; Hepokur, 2018; Magalhães et al., 2010; Milind and Deepa, 2011; Rodríguez et al., 2014). An herbal mouthwash containing clove was reported to reduce the duration of grade 2 and higher mucositis and reduce weight loss in patients with radiation-induced OM (Kong et al., 2016). In a study conducted by Lalla et al. (2020) on patients with head and neck cancer treated with radiation, clove-containing mouthwash prevented the development of severe OM and accelerated the healing process.

Green tea contains epigallocatechin gallate, which has strong antioxidant, antiapoptotic, anti-inflammatory, autoantigen inhibitory, and radioprotective properties (Almeida et al., 2014; Aykaç et al., 2014; Trevenzoli et al., 2018). Thus, it was thought to be a potential treatment option for OM, and several studies have yielded promising evidence of its effectiveness against OM (Almeida et al., 2014; Al-Refai et al., 2014; Carulli et al., 2013; Giacomelli et al., 2015). One such study showed that mouthwash containing green tea reduced the incidence, severity, and duration of OM in patients undergoing stem cell transplantation. Its effect was attributed to the antioxidant properties of the green tea, which neutralized the overproduction of reactive oxygen species (ROS) and ulcerative damage (Carulli et al. 2013). Another mouthwash containing green tea was also reported to prevent severe OM in patients undergoing radiotherapy for head and neck cancer (Giacomelli et al., 2015). Studies examining the effect of green tea in animal models of induced OM have reported that it increased epithelial thickness and reduced inflammation and the number of occluded blood vessels in connective tissue in methotrexate-induced mucositis. Green tea was also found to protect against methotrexate-induced OM via its antioxidant, antiangiogenic, and antiapoptotic effects and was described as a natural product that protects the oral mucosa (Almeida et al., 2014; Al-Refai et al., 2014). In addition, green tea was shown to reduce lesion size, enhance clinical improvement, and significantly decrease the number of Candida colonies in patients with denture stomatitis through its antioxidant and anti-inflammatory effects (Ghorbani et al., 2018).

To date, limited studies have tested the use of cloves and green tea in OM, and these studies have used cloves and green tea in combination with other herbal and nutritional supplements (Carulli et al., 2013; Giacomelli et al., 2015; Kong et al., 2016; Lalla et al., 2020). Therefore, this study was conducted to investigate the effect of locally applied clove and green tea hydrosol in a cyclophosphamide-induced OM model in rats.

This study hypothesized that locally applied clove and green tea hydrosols would have preventive effects in a cyclophosphamide-induced oral mucositis model in rats, and that their effects would differ.

## Materials and Methods

This preclinical, *in vivo* experimental and analytical study was conducted in the Experimental Animals Unit of Aydın Adnan Menderes University Faculty of Medicine with approval from the Animal Experiments Ethics Committee (64583101/2021/117).

*Cyclophosphamide and Hydrosols Used in the Study*

The endoxan 1 g (Eip Eczacıbaşı Pharmaceutical Marketing Inc.) used in the study (1069.9 mg cyclophosphamide monohydrate equivalent to 1 g cyclophosphamide) was obtained from the Aydın Adnan Menderes University Research and Training Hospital Pharmacy.

Clove and green tea hydrosols were used for oral care. Hydrosols are also known as floral water, herbal distillates, or aromatic waters and are obtained by distilling different parts of plants with water and steam. Hydrosols generally contain the condensed water-soluble constituents of the essential oil and consist of the hydrophilic components of the plant. In the literature, hydrosols have been reported to be a well-tolerated, feasible, cost-effective, safe, and non-invasive method



**Table 1.** Library matching results of the clove hydrosol and green tea hydrosol

| Retention Time (min) | Compound Name | Match (%) | Relative Percentage (%) |
|---|---|---|---|
| **Clove Hydrosol** | | | |
| 12.1 | 1,8-Cineole | 99 | 0.17 |
| 22.0 | Eugenol | 98 | 88.92 |
| 23.6 | Caryophyllene | 99 | 7.94 |
| 24.5 | α-Humulene | 99 | 1.64 |
| 25.8 | 2,4-Di-Tert-Butyl-phenol | 95 | 0.12 |
| 26.2 | Trans-Calamenene | 87 | 0.20 |
| 26.9 | Dimethyl (2'-Methyl-2'-Propen-1'-YL) Phosphonate | 49 | 0.12 |
| 27.6 | 1,Z-5,E-7-Dodecatriene | 70 | 0.09 |
| 27.7 | Aromadendrene | 92 | 0.82 |
| **Green Tea Hydrosol** | | | |
| 2.0 | Isobutanol | 76 | 1.00 |
| 2.2 | Ethanoic Acid | 86 | 2.76 |
| 2.4 | Cyclopentanol | 37 | 1.37 |
| 3.0 | Isopentanol | 72 | 1.00 |
| 3.1 | 2-Methyl-1-Butanol | 86 | 2.07 |
| 12.1 | 1,8-Cineole | 97 | 2.11 |
| 14.4 | Vetiverol | 74 | 1.83 |
| 16.1 | 5-Methyl-2-(1-Methylethyl)-(2s-Trans) Cyclohexa-none | 98 | 10.06 |
| 16.4 | L-Menthone | 95 | 5.79 |
| 16.7 | 3-P-Menthanol | 91 | 43.37 |
| 22.0 | Eugenol | 98 | 19.41 |
| 29.3 | γ-Muurolene | 86 | 3.10 |
| 29.9 | Bisabolone Oxide | 83 | 6.14 |

(Mutluay Yayla et al., 2016). Therefore, we preferred to use the hydrosol form of clove and green tea for the prevention and treatment of OM in this study. Clove and green tea hydrosols were obtained from Uludağ Agro Medical Aromatic Plants Cosmetic Pharmaceutical Food and Tourism, Ltd. According to the test/analysis report No. 2206050 from Bursa Technical University Central Research Laboratory, the chemical compositions of clove and green tea hydrosols were analyzed using HS-GC-MS, and the identified compounds along with their relative percentages are summarized in Table 1.

*Experimental Procedure*

Animal studies in the literature recommend limiting the number of experimental animals to between 5 and 10 per study group for ethical reasons (Özsoy and Yıldırım, 2012). In accordance with this recommendation, the number of animals used in this study was determined as 5 per group. The study was conducted with 40 female Wistar albino rats aged 6-8 weeks old and weighing 200-250 g, obtained from Aydın Adnan Menderes University Faculty of Medicine Experimental Animals Production and Research Laboratory. Female rats were selected for this study for both logistical and experimental standardization reasons, due to their greater availability in laboratory stocks and the lack of reported significant sex-related differences in oral mucositis models. The rats were housed in transparent cages and provided standard rat food and tap water ad libitum during the study. The cages were kept in dedicated rooms with 12 h/12 h light-dark cycle, temperature of 22±2 °C, and humidity of 50-55%. After the adaptation period, the rats were randomly divided into 8 groups (n=5 rats in each) (Table 2).

On day 0 of the experiment, each rat was weighed using a Kern brand electronic precision scale with dynamic weighing feature and 8000-g capacity. Rats in the negative control group were administered an intraperitoneal (IP) injection of 0.9% sodium chloride (NaCl), while the rats in all other groups received a single IP injection of 300 mg/kg cyclophosphamide to induce OM (Al-Refai, 2017; Saleh et al., 2019). The cyclophosphamide solution was prepared at the time of administration, protected from light, and injected immediately.

**Table 2.** Summary of the study groups

| Group | n | Oral Mucositis Induction (day 0) | Oral Care (applied twice daily) | Sample Collection |
|---|---|---|---|---|
| 4th day NG | 5 | None | 0.9% NaCl | Day 4 |
| 8th day NG | 5 | | | Day 8 |
| 4th day PG | 5 | 300 mg/kg intraperitoneal cyclophosphamide | 0.9% NaCl | Day 4 |
| 8th day PG | 5 | | | Day 8 |
| 4th day CLV | 5 | | Clove hydrosol | Day 4 |
| 8th day CLV | 5 | | | Day 8 |
| 4th day GT | 5 | | Green tea hydrosol | Day 4 |
| 8th day GT | 5 | | | Day 8 |

NG:Negative control group, PG:Positive control group, CLV:Clove hydrosol group, GT: Green tea hydrosol group.

**Table 3**. Histopathological scoring (Oğuz et al., 2023)

| Score | Erosion | Fibrosis | Inflammatory Cell Infiltration |
|---|---|---|---|
| 0 | None | None | None (0) |
| 1 | Minimal | Minimal | Minimal (1-5) |
| 2 | Moderate | Moderate | Moderate (6-10) |
| 3 | Severe | Severe | Severe (>10) |

Oral application was administered according to group allocation twice daily (morning and evening), starting on day 0 and continuing until the rats were sacrificed and cardiac blood and oral mucosal tissue samples were obtained on day 4 (groups 1-4) or day 8 (groups 5-8). Oral care consisted of 0.9% NaCl (negative and positive control groups), clove hydrosol, or green tea hydrosol. An average of three sprays (1 spray = 0,15 mL) were administered to each animal at each application to ensure contact with all surfaces of the mouth.

*Sample Collection and Evaluation*

Mucositis symptoms are reported to develop an average of 4-8 days after the initiation of chemotherapy and regress within two weeks after the conclusion of chemotherapy (Al-Refai, 2017; Göktuna, 2017; Saleh et al., 2019). Therefore, study data for mucositis assessment were collected on days 4 and 8. The rats were weighed again before sample collection. Total antioxidant status (TAS) and total oxidant status (TOS) were determined from cardiac blood samples (1 mL on average) obtained from the rats while under anaesthesia. Intraperitoneal injection of ketamine (80 mg/kg) and xylazine (10 mg/kg) was used for anesthesia in all groups. After centrifuging, the serum was transferred to an Eppendorf tube, stored at -20 °C, and sent to the laboratory under appropriate conditions for analysis. In the laboratory, commercially available kits (Relassay, Türkiye) were used for TAS and TOS measurement. The TAS assay is based on decolorization of the radical cation of ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) by antioxidants. The test has excellent precision values of less than 3%. The results are expressed in mmol Trolox equivalent/L (Erel, 2004). In the TOS assay, oxidants in the sample oxidize the ferrous ion–o-dianisidine complex to ferric ion. The oxidation reaction is enhanced by glycerol molecules abundant in the reaction medium. The ferric ion forms a coloured complex with xylenol orange in an acidic medium. This colored product can be measured spectrophotometrically and is associated with the total amount of oxidant molecules present in the sample. The assay is calibrated with hydrogen peroxide, and the results are expressed in micromolar hydrogen peroxide equivalents (μmol $H_2O_2$ equivalent/L) per liter (Erel, 2005).

Tissue samples were excised from the right cheek of the sacrificed rats immediately after the cardiac blood sample was taken under anaesthesia. The tissue samples were placed in formalin and delivered under appropriate conditions to the A Medical Pathology Laboratory for histopathological examination. In the laboratory, the samples were fixed in 10% neutral formalin for 72 hours and placed in tissue cassettes. Routine processing of the samples (dehydration by graded ethanol series, clearing with xylene, and embedding in paraffin blocks) was performed by a fully automated tissue processor. From these blocks, tissue sections 5-6 microns thick were obtained in the microtome, stained with haematoxylin-eosin, and examined and imaged under



**Table 4.** Comparison of mean TAS values of the groups on day 4 and day 8[1]

| Groups | n | Mean ± SE | F | P |
|---|---|---|---|---|
| **Day 4** | | | | |
| NG | 5 | 1.80±0.08 | 3.180 | 0.053 |
| PG | 5 | 1.04±0.29 | | |
| CLV | 5 | 1.63±0.18 | | |
| GT | 5 | 1.63±0.09 | | |
| **Day 8** | | | | |
| NG | 5 | 1.91±0.04[a] | 4.800 | 0.014 |
| PG | 5 | 1.45±0.05[b] | | |
| CLV | 5 | 1.73±0.11[ab] | | |
| GT | 5 | 1.59±0.11[ab] | | |

[1]F:One-way ANOVA, NG:Negative control group, PG:Positive control group, CLV:Clove hydrosol group, GT: Green tea hydrosol group. Day 8 partial η2=0.374. [a,b,c] Different superscript letters indicate significant differences among the means (P<0.05).

**Table 5.** Comparison of mean TOS values of the groups on day 4 and day 8[1]

| Groups | n | Median (min-max) | Mean Rank | $X^2$ | P |
|---|---|---|---|---|---|
| **Day 4** | | | | | |
| NG | 5 | 10.53 (9.04-11.94) | 5.80 | 5.263 | 0.154 |
| PG | 5 | 12.18 (10.60-25.55) | 12.40 | | |
| CLV | 5 | 11.87 (10.87-13.74) | 13.80 | | |
| GT | 5 | 10.90 (10.57-12.46) | 10.00 | | |
| **Day 8** | | | | | |
| NG | 5 | 10.61 (10.34-11.43) | 7.40 | 2.572 | 0.463 |
| PG | 5 | 11.33 (10.59-11.71) | 11.40 | | |
| CLV | 5 | 11.22 (10.94-15.32) | 13.20 | | |
| GT | 5 | 10.74 (10.15-13.52) | 10.00 | | |

[1]$X^2$: Kruskal Wallis test, NG:Negative control group, PG:Positive control group, CLV:Clove hydrosol group, GT: Green tea hydrosol group. (P<0.05).

**Table 6.** Comparison of erosion grades in the groups on days 4 and 8[1]

| Groups | n | Median (min-max) | Mean Rank | $X^2$ | P | partial η2 |
|---|---|---|---|---|---|---|
| **Day 4** | | | | | | |
| NG | 5 | 0.00 (0-0) | 4.00[b] | 12.493 | 0.006 | 0.38 |
| PG | 5 | 1.00 (1-2) | 15.60[a] | | | |
| CLV | 5 | 1.00 (0-1) | 11.20[a] | | | |
| GT | 5 | 1.00 (0-1) | 11.20[a] | | | |
| **Day 8** | | | | | | |
| NG | 5 | 0.00 (0-0) | 4.60[c] | 13.737 | 0.003 | 0.69 |
| PG | 5 | 2.00 (1-3) | 17.00[a] | | | |
| CLV | 5 | 1.00 (0-1) | 9.40[bc] | | | |
| GT | 5 | 1.00 (1-1) | 11.00[b] | | | |

[1]$X^2$:Kruskal-Wallis test, NG:Negative control group, PG:Positive control group, CLV:Clove hydrosol group, GT: Green tea hydrosol group.[a,b,c] Different superscript letters indicate significant differences among group medians (P<0.05).

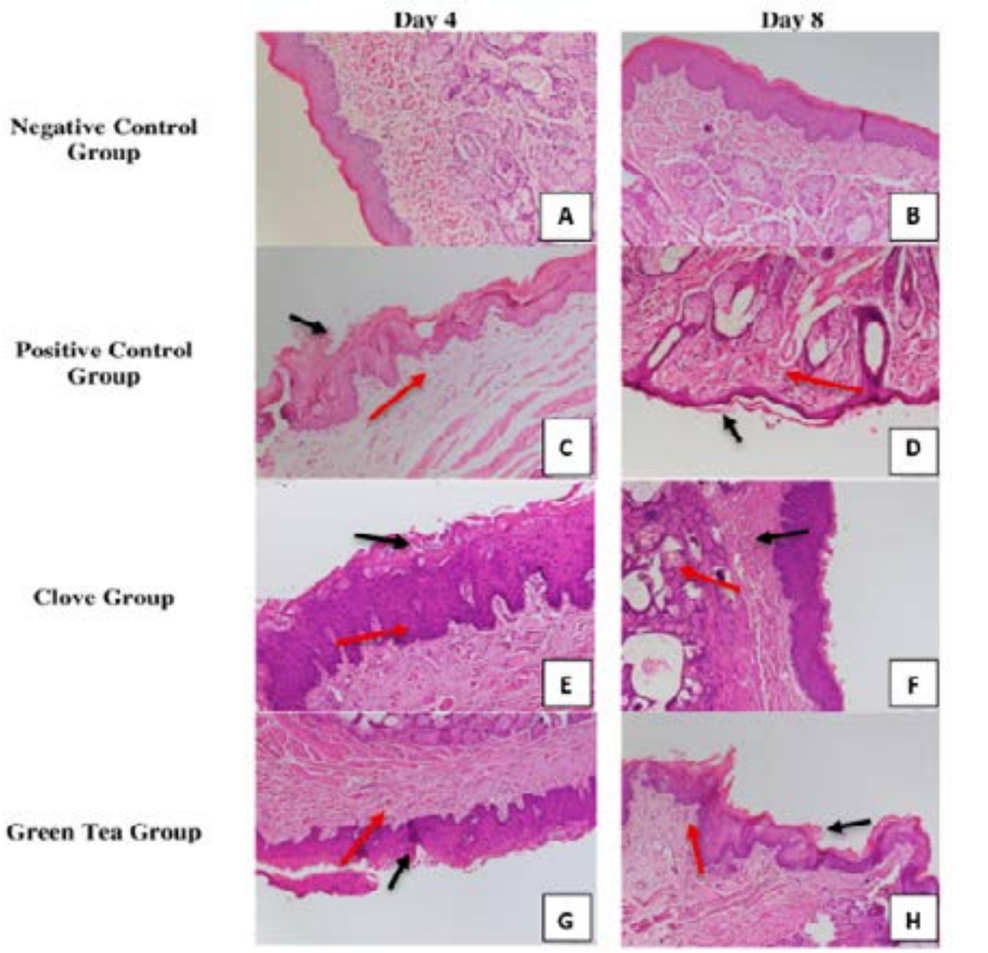


**Figure 1.** Histopathological evaluation of oral mucosal tissues stained with haematoxylin–eosin (H&E) on days 4 and 8. Negative control (A, B) shows normal histological architecture, while positive control (C, D) demonstrates moderate to severe epithelial erosion and inflammatory changes. Clove (E, F) and green tea (G, H) hydrosol groups exhibit minimal epithelial disruption with limited inflammation. Arrows highlight epithelial erosion (black) and areas suggestive of inflammatory cell infiltration (red). Fibrosis was not clearly identifiable. Scale bar = 100 µm.

independent variable. Post hoc analysis among the groups revealed a significant difference between the negative and positive control groups (P=0.002). The comparison of TOS values between the groups is presented in Table 5. No statistically significant differences in TOS levels were observed among the groups on either day 4 or day 8 (P>0.05). The lowest mean serum TOS values were observed in the negative control group, followed by the green tea group.

*Histopathological Evaluation*

At the end of day 4 and day 8 of the experiment, oral mucosal tissue samples were excised from the right inner cheek of the rats after cervical dislocation, and histopathological examination was performed by staining with haematoxylin-eosin (Figure 1). The stained samples were evaluated for erosion, fibrosis, and inflammatory cell infiltration.

Comparison of Erosion: Day 4 samples showed normal mucosa with no erosion in the negative control group and minimal erosion in the clove and green tea hydrosol groups (4 of 5 samples). Moderate erosion was observed in the positive control group. The difference in erosion grade among the groups was statistically significant (KW=12.493, P=0.006) (Table 6).

In day 8 samples, no erosion was observed in the negative control group, while minimal erosion was detected in 4 samples in the clove hydrosol group and in all samples in the green tea hydrosol group. The positive control group exhibited moderate (3 samples) to severe (1 sample) erosion. In day 8 samples, the degree of erosion differed significantly between the positive control group and the other groups (KW=13.737, P=0.003). There was no statistically significant difference between the minimal erosion in the clove hydrosol group and the absence of



**Table 7.** Comparison of fibrosis grades in the groups on days 4 and 8[1]

| Groups | n | Median (min-max) | Mean Rank | $X^2$ | P |
|---|---|---|---|---|---|
| **Day 4** | | | | | |
| NG | 5 | 0.00 (0-0) | 10.00 | 3.000 | 0.392 |
| PG | 5 | 0.00 (0-0) | 10.00 | | |
| CLV | 5 | 0.00 (0-0) | 10.00 | | |
| GT | 5 | 0.00 (0-1) | 12.00 | | |
| **Day 8** | | | | | |
| NG | 5 | 0.00 (0-0) | 6.50 | 6.333 | 0.096 |
| PG | 5 | 1.00 (0-1) | 14.50 | | |
| CLV | 5 | 0.00 (0-1) | 10.50 | | |
| GT | 5 | 0.00 (0-1) | 10.50 | | |

[1]$X^2$:Kruskal-Wallis test, NG:Negative control group, PG:Positive control group, CLV:Clove hydrosol group, GT: Green tea hydrosol group. (P<0.05).

**Table 8.** Comparison of inflammatory cell infiltration grades in the groups on days 4 and 8[1]

| Groups | n | Median (min-max) | Mean Rank | $X^2$ | P |
|---|---|---|---|---|---|
| **Day 4** | | | | | |
| NG | 5 | 0.00 (0-0) | 7.00 | 5.637 | 0.131 |
| PG | 5 | 1.00 (0-1) | 13.00 | | |
| CLV | 5 | 1.00 (0-1) | 13.00 | | |
| GT | 5 | 0.00 (0-1) | 9.00 | | |
| **Day 8** | | | | | |
| NG | 5 | 0.00 (0-1) | 7.50 | 6.840 | 0.077 |
| PG | 5 | 1.00 (1-1) | 15.50 | | |
| CLV | 5 | 0.00 (0-1) | 9.50 | | |
| GT | 5 | 0.00 (0-1) | 9.50 | | |

[1]$X^2$:Kruskal-Wallis test, NG:Negative control group, PG:Positive control group, CLV:Clove hydrosol group, GT: Green tea hydrosol group. (P<0.05).

erosion in the negative control group (Table 6).

Comparison of Fibrosis: In the day 4 groups, minimal fibrosis was observed in only one sample in the green tea group, while no fibrosis was detected in the other groups. However, in the day 8 groups, minimal fibrosis developed in all groups except the negative control group. There were no statistically significant differences between the groups in fibrosis grades on day 4 or day 8 (Table 7).

Comparison of Inflammatory Cell Infiltration: On day 4, minimal inflammatory cell infiltration was detected in all groups except the negative controls, but the difference was not statistically significant. On day 8, the frequency of minimal inflammatory cell infiltration was highest in the positive control group (5 samples), followed by the clove (2 samples) and green tea (2 samples) groups, and was lowest in the negative control group (1 sample), but this difference also did not reach statistical significance (Table 8).

## Discussion

This preclinical in vivo study investigated the effects of locally applied clove and green tea hydrosols in a cyclophosphamide-induced oral mucositis rat model, and the findings are discussed in relation to the literature.

On day 4, the mean TAS value was highest in the negative control group and lowest in the positive control group, but there was no statistically significant difference between the groups. On day 8, the mean TAS value was significantly lower in the positive control group compared to the other groups. In the literature, cyclophosphamide has been reported to increase the production of ROS in the oral mucosa by irreversible DNA strand breaks in cells (Sonis et al., 2004). This is consistent with the lower mean TAS observed in the cyclophosphamide-treated groups in our study. In contrast, mean TAS values were higher in the clove and green tea groups compared to the positive controls. Mouthwash containing green

tea was reported to reduce the overproduction of ROS in patients undergoing stem cell transplantation and neutralizes ulcerative damage in OM (Baharvand et al., 2017; Carulli et al., 2013).

In the formation of OM, increased oxidative stress due to chemotherapy causes an increase in oxidants in the blood (Kaynar, 2010). As expected, the lowest TOS values on both day 4 and day 8 were observed in the negative control groups, followed by the green tea groups. The effect of locally applied clove and green tea hydrosols on serum TAS and TOS values may be a result of the rats' ingestion or sublingual absorption of the hydrosols after oral administration. We consider these findings a positive secondary outcome.

In this study, we evaluated the effects of clove and green tea hydrosols on erosion, fibrosis, and inflammatory cell infiltration in oral mucosal tissue samples collected on day 4 and day 8.

Comparison of erosion; it was expected that the negative control groups would not develop erosion and would have normal mucosa. In the development of OM, chemotherapy and increased ROS levels stimulate apoptosis (Sonis, 2004). Göktuna (2017) reported OM onset at 4.16±2.13 days and resolution at 8.72±2.32 days. The increasing severity of erosion in the positive control groups of our study is consistent with the literature.

Only minimal erosion was observed in almost all of the samples in the clove and green tea groups. This result suggests that clove and green tea hydrosol reduced the severity of erosion in the oral mucosa. There are a limited number of clinical trials testing the use of clove and green tea in OM. However, the cloves and green tea used in these studies were administered in combination with other herbal and nutritional supplements (Carulli et al., 2013; Giacomelli et al., 2015; Kong et al., 2016; Lalla et al., 2020). Studies examining the effect of cloves and green tea on OM reported that mouthwash containing green tea significantly reduced the incidence and severity of OM (Carulli et al., 2013; Giacomelli et al., 2015), while mouthwash containing clove delayed the onset and shortened the duration of OM (Kong et al., 2016). Our study showed that the clove and green tea groups developed less erosion compared to the positive control groups, and that clove application for 8 days slowed the development of erosion.

Comparison of fibrosis; on day 4 of the experiment, only one sample in the green tea group had minimal fibrosis, while the other groups had no fibrosis. However, minimal fibrosis was detected in all groups except the negative controls on day 8. The literature data indicate that proinflammatory cytokines, which increase in the later phases of chemotherapy-induced OM (messenger signal regulation, signal increase), activate the ceramide and caspase pathways (a series of pathways including the nuclear factor kappa beta [NF-κB]-mediated transcription pathway), and that the activation of these pathways leads to the further production of proinflammatory cytokines (tumour necrosis factor-α, interleukin [IL]-1, and IL-6) and apoptosis in the epithelium, endothelium, and connective tissue. It has also been noted that fibronectin degradation occurs during this phase of mucositis (Sonis et al., 2004). In the present study, our observations of fibrosis in one sample on day 4 and increased fibrosis in the cyclophosphamide-treated groups on day 8 are consistent with the literature. On day 8, fibrosis was seen in fewer samples in the clove and green tea groups compared to the positive control group. This result suggests that cloves and green tea reduce fibrosis development by decreasing cytokine release via their anti-inflammatory effect.

Comparison of inflammatory cell infiltration; no inflammatory cell infiltration was observed in the negative control groups. As mentioned above, the increased production of proinflammatory cytokines (TNF-α, IL-1 and IL-6) by the chemotherapy-activated transcription factor NF-κB causes macrophage activation. This macrophage activation leads to tissue damage and the production of more TNF-α, which increases inflammation (Sonis et al., 2004). In this study, minimal inflammatory cell infiltration was observed in more samples in the positive control groups. The clove and green tea groups had a similar lower rate of minimal inflammatory cell infiltration. This result demonstrates the anti-inflammatory effect of clove and green tea. Clove has been reported to inhibit cyclooxygenase-2 expression and NF-κB activation in macrophages, thereby preventing inflammatory cytokine expression and exerting an anti-inflammatory effect (Magalhães et al., 2010). In a study by Almeida et al. (2014) examining the anti-inflammatory activity of green tea on small intestinal mucositis associated with methotrexate, it was reported that green tea showed significant anti-inflammatory effects. A study investigating the efficacy of green tea in preventing nicotine-induced inflammatory and epithelial changes in the buccal mucosa of albino rats showed that green tea significantly prevented inflammatory cell infiltration in response to nicotine-induced damage (Shahbaz et al., 2017).

## Conclusion

In a rat cyclophosphamide-induced OM model, locally applied clove and green tea hydrosols both had a preventive effect but there was no significant difference between the preventive effects of the clove and green tea hydrosols. Based on these results, we recommend performing clinical studies in patients at high risk of developing OM to corroborate our data and compare clove and green tea hydrosols with proven interventions.

## Acknowledgements

I thank Prof. Dr. Serap Birincioğlu for tissue examinations. This work was supported by Aydın Adnan Menderes University Scientific Research Projects Unit (HF-22002).

This article is summarized from the first author's master's thesis. This manuscript was presented as a verbal presentation at the 1st International Association of Oncology Nursing Congress.



## Authors Contributions

ETK, SBÖ and SGE contributed to the project idea, design and execution of the study. ETK and SGE contributed to the acquisition of data. ETK drafted and wrote the manuscript. SBÖ reviewed the manuscript critically. All authors have read and approved the finalized manuscript.

## Conflict of Interest

The authors declare no conflicts of interest.

## References

Almeida, S.B.D., Monteiro, M.C.S.A., Lima, A.V.P.D., Menezes, D.B.D., & Monteiro, S.M.N. (2014). Protective effect of Camellia sinensis on methotrexate-induced small intestinal mucositis in mice. *Food and Nutrition Sciences*, 5, 443-448. https://doi.org/10.4236/fns.2014.55052

Al-Refai, A.S., Ali, A.B.H., & Kamal, K.A. (2014). Immunohistochemical Study of the Effect of Green Tea Extract on Methotrexate-Induced Oral Mucositis in Albino Rats. *Journal of Cytology & Histology*, 5(3), 1-7. https://doi.org/10.4172/2157-7099.1000227

Al-Refai, A.S. (2017). Chemoprotective effect of ascorbic acid on cyclophosphomide induced oral toxicity. *Zanco Journal of Medical Sciences,* 21(1), 1540-1551. https://doi.org/10.15218/zjms.2017.002

Aykaç, G., Uzun, M.B., & Özçelikay, G. (2014). Tea in Social Aspect "Camellia Sinensis". *Lokman Hekim Journal,* 4(1), 1-5

Babic, A., & Murray, J. (2019). Role of Nursing in HSCT. E. Carreras, C. Dufour, M. Mohty, & N. Kröger (Eds). In: The EBMT Handbook Hematopoietic Stem Cell Transplantation and Cellular Therapies, Switzerland, Springer Nature, 237-243. https://doi.org/10.1007/978-3-030-02278-5

Baharvand, M., Jafari, S., & Mortazavi, H. (2017). Herbs in Oral Mucositis. *Journal of Clinical and Diagnostic Research,* 11(3), 5-11. https://doi.org/10.7860/JCDR/2017/21703.9467

Baysal, E., & Sarı, D. (2016). Current approaches in oral mucositis prevention, care and treatment in hematopoietic stem cell transplantation: Literature review. *Journal of Human Sciences,* 13(3), 5721-5739. https://doi.org/10.14687/jhs.v13i3.4036

Carulli, G., Rocco, M., Panichi, A., Chios, C.F., Ciurli, E., Mannucci, C., et al. (2013). Treatment of Oral Mucositis in Hematologic Patients Undergoing Autologous or Allogeneic Transplantation of Peripheral Blood Stem Cells: A Prospective, Randomized Study With a Mouthwash Containing Camelia Sinensis Leaf Extract. *Hematology Reports,* 5(1), e6. https://doi.org/10.4081/hr.2013.e6

Cidon, E.U. (2018). Chemotherapy induced oral mucositis: prevention is possible. *Chinese Clinical Oncology,* 7(1), 6. https://doi.org/10.21037/cco.2017.10.01

Çıtlak, K., & Kapucu, S. (2015). Current Approaches to Oral Mucositis Prevention and Treatment In Patients Receiving Chemotherapy: Evidence-Based Practices, *Journal of Hacettepe University Faculty of Nursing,* 70-77

Danthu, P., Simanjuntak, R., Fawbush, F., Tsy, J.L., Razaβimamonjison, G., Abdillahi, M.M., et al. (2020). The clove tree and its products (clove bud, clove oil, eugenol): prosperous today but what of tomorrow's restrictions?. *World,* 61(58,172), 52-915. https://doi.org/10.17660/th2020/75.5.5

Erel, O. (2005). A new automated colorimetric method for measuring total oxidant status. *Clinical Biochemistry,* 38, 1103-1111. https://doi.org/10.1016/j.clinbiochem.2005.08.008

Erel, O. (2004). A novel automated direct measurement method for total antioxidant capacity using a new generation, more stable ABTS radical cation. *Clinical Biochemistry,* 37, 277-285. https://doi.org/10.1016/j.clinbiochem.2003.11.015

Ghorbani, A., Sadrzadeh, A., Habibi, E., Dadgar, K., Akbari, J., Moosazadeh, M., et al. (2018). Efficacy of Camellia sinensis extract against Candida species in patients with denture stomatitis. *Current Medical Mycology,* 4(3), 15. https://doi.org/10.18502/cmm.4.3.174

Giacomelli, I., Scartoni, D., Fiammetta, M., Baki, M., Zei, G., Muntoni, C., et al. (2015). Oral lapacho-based medication: an easy, safe, and feasible support to prevent and/or reduce oral mucositis during radiotherapy for head and neck cancer. *Nutrition and cancer,* 67(8), 1249-1254. https://doi.org/10.1080/01635581.2015.1082114

Göktuna, G. (2017). Investigation of the frequency and risk factors of oral mucositis development in patients receiving chemotherapy. [Master's Thesis Izmir Dokuz Eylul University]

Hepokur, C. Investigation of Cytotoxic Effects of Eugenia Caryophyllus (Clove). (2018). *Cumhuriyet Dental Journal,* 21(3), 173-177. https://doi.org/10.7126/cumudj.444426

İzgü, N. (2017). Complementary Therapies in the Management of Induced Oral Mucositis during Cancer Treatment, *Journal of Education and Research in Nursing,* 14(4), 304-310. https://doi.org/10.5222/HEAD.2017.304

Kaynar, L. (2010). The Effect of Royal Jelly on Methotrexate-Induced Mucositis and Oxidative Stress in Rats. [Subspecialty Thesis, Erciyes University] https://tez.yok.gov.tr/UlusalTezMerkezi/tezDetay.jsp?id=y7foSS3jHkgqRXXOTNubaw&no=iTRHHnx5yeWIJnYeJn9T2w

Kong, M., Hwang, D.S., Yoon, S.W., Kim, J. (2016). The effect of clove-based herbal mouthwash on radiation-induced oral mucositis in patients with head and neck cancer: A single-blind randomized preliminary study. *Oncotargets and Therapy,* 9, 4533-4538

Lalla, R.V., Solé, S., Becerra, S., Carvajal, C., Bettoli, P., Letelier, H., et al. (2020). Efficacy and safety of Dentoxol® in the prevention of radiation-induced oral mucositis in head and neck cancer patients (ESDOM): a randomized, multicenter, double-blind, placebo-controlled, phase II trial. *Supportive Care in Cancer,* 28, 5871-5879. https://doi.org/10.1007/s00520-020-05358-4

Magalhães, C.B., Riva, D.R., DePaula, L.J., Brando-Lima, A., Koatz, V.L.G., Leal-Cardoso J.H., et al. (2010). *In vivo* anti-inflammatory action of eugenol on lipopolysaccharide-induced lung injury. *Journal of Applied Physiology,* 108(4), 845-851. https://doi.org/10.1152/japplphysiol.00560.2009

Milind, P., Deepa, K. (2011). Clove: A Champion Spice. *International Journal of Research in Ayurveda & Pharmacy,* 2(1), 47-54

Mutluay Yayla, E., İzgü, N., Özdemir, L., Aslan Erdem, S., Kartal, M. (2016). Sage tea-thyme-peppermint hydrosol oral rinse reduces chemotherapy-induced oral mucositis: A randomized controlled pilot study. *Complementary Therapies in Medicine*, 27, 58-64. https://doi.org/10.1016/j.ctim.2016.05.010

Mutluay Yayla, E. (2017). Evidence-Based Practices for Mucositis. *Journal of Education and Research in Nursing*, 14(3), 223-227. https://doi.org/10.5222/HEAD.2017.223

Negrin, R. S., & Treister, N. S. (2024). Oral toxicity associated with systemic anticancer therapy. UpToDate, 17 (9). https://www.uptodate.com/contents/oral-toxicity-associated-with-systemic-anticancer-therapy

Oğuz, Y., Diker, N., Gülsever, S., Yılmaz Akçay, E. (2023). Effects of Hyaluronic Acid Gel Application on Incisional Oral Mucosal Wound Healing: An Experimental Study, *Turkiye Klinikleri Journal of Dental Sciences,* 29(2), 225-33

Oronsky, B., Goyal, S., Kim, M.M., Cabrales, P., Lybeck, M., Caroen, S., et al. (2018). 11A Review of Clinical Radioprotection and Chemoprotection for Oral Mucositis, *Translational Oncology,* (3), 771-778. https://doi.org/10.1016/j.tranon.2018.03.014

Özsoy, S., Yıldırım, J.G. (2012). Animal Research in Nursing. *Journal of Research and Development in Nursing,* 1, 56–69

Razmara, F., Khayamzadeh, M. (2019). An Investigation into the Prevalence and Treatment of Oral Mucositis After Cancer Treatment. *International Journal of Cancer Management,* 12(11), e88405. https://doi.org/10.5812/ijcm.88405

Rodríguez, O., Sánchez, R., Verde, M., Núñez, M., Ríos, R., Chávez, A. (2014). Obtaining the essential oil of Syzygium aromaticum, identification of eugenol and its effect on Streptococcus mutans. *Journal of Oral Research*, 3(4), 218-224

Saleh, A.O., Khleel, A.K., Almahana, J. (2019). Simulation Assessment of the Protective Role of Vitamin C and E against Cytotoxic Effects of Cyclophosphamide in Rats Tongue Mucosa: Histological and Immunohistochemical Study. *Journal of Physics: Conference*

*Series,* 1279(1), 012014. https://doi.org/10.1088/1742-6596/1279/1/012014

Santosa, D., Pangarsa, E.A., Setiawan, B., Naibaho, R.M., Rizky, D., Dharmana, E., et al. (2021). Establishing the hematopoietic stem cell transplant (HSCT) in a developing country; the journey of HSCT in Semarang, Indonesia. *Bone Marrow Transplantation,* 56(1), 270-273. https://doi.org/10.1038/s41409-020-0973-7

Shahbaz, M., Zaheer, N., Sagheer, A., Arshad, A.I., Zaheer, U., Alam, M.K. (2017). Role of Green Tea Extract (Camellia sinensis) in Prevention of Nicotine-Induced Inflammatory and Epithelial Changes in Buccal Mucosa of Albino Rats. *International Medical Journal,* 24(2), 230

Sonis, S.T., Elting, L.S., Keefe, D., Peterson, D.E., Schubert, M., Hauer Jensen, M., et al. Perspectives on cancer therapy-induced mucosal injury: pathogenesis, measurement, epidemiology, and consequences for patients. (2004). *Cancer: Interdisciplinary International Journal of the American Cancer Society,* 100(S9). https://doi.org/10.1002/cncr.20162

Sonis, S.T. (2004). The pathobiology of mucositis. *Nature Reviews Cancer,* 4(4), 277-284

Trevenzoli, D.A.M., Cock, N.R.O.S., De Souza, L.N.G., Vaz, S.L.D.A., Leite, M.F., De Barros, L,A,P., et al. (2018). Green Tea: a Potential Radioprotective Agent for the Oral Mucosa. *Oral surgery, oral medicine, oral pathology and oral radiology*, 126(3), 175-176