Effect of Topical 2% Eucalyptus Extract on 5-FU-Induced Oral Mucositis in Male Golden Hamsters

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Oral mucositis is a common inflammatory complication among patients with cancer. This study evaluated the histopathological, stereological, and antioxidant markers of 2% eucalyptus extract in induced oral mucositis in male golden hamsters. In this animal study, oral mucositis was induced in 30 male golden hamsters by 5-FU (60 mg/kg) on days 0, 5, and 10 of the study. The cheek pouch was scratched with a sterile needle once daily on days 3 and 4. On days 14-17, 2% eucalyptus hydroalcoholic extract gel and *Calendula officinalis* extract gel groups were treated and then compared with a non-treated control group. The histopathological and stereological scores and the pouch content of malondialdehyde, as well as the activities of glutathione and myeloperoxidase in the pouch tissue, were evaluated. Histopathologic scores of oral mucositis were lower in the eucalyptus gel group than those of the calendula and control groups ($p < 0.05$). Also, a lower malondialdehyde level and higher myeloperoxidase and glutathione activities were detected in the eucalyptus group in comparison to the calendula and control groups ($p < 0.001$). The thickness of the mucosa and submucosa increased in the eucalyptus group. The numerical density of the fibroblast and the volume density of the collagen significantly increased in the eucalyptus group. In conclusion, the use of eucalyptus hydroalcoholic extract may be associated with reduced intensity of oral mucositis, diminished concentration of malondialdehyde, increased activity of myeloperoxidase and glutathione, increased volume of mucosa and submucosa, increased fibroblast and collagen in the induced oral mucositis in golden hamsters undergoing 5-FU consumption.

Introduction

Head and neck cancer is the sixth leading cancer by incidence worldwide and eighth by death (1). Surgical resection, radiation, and chemotherapy are the mainstays of treatment for most head and neck cancers and remain the standard of care in most cases. Oral mucositis (OM) is a frequent complication of treatments with radiotherapy or chemotherapy. OM is a painful, erythematous, inflamed, and ulcerated mucosal disease that is covered by a yellow-white fibrin clot pseudomembrane. Pain and loss of taste perception can cause difficulty in swallowing, speaking, and receiving nutrition, which can lead to weakness, low quality of life, and higher treatment costs (2).

After exposure to neoplastic treatments, there is DNA and non-DNA damage in the oral mucosa. Cascade production of free radicals, which cause oxidative stress and reactive oxygen species, is the initial oral mucosal layer and blood vessel injuries. The severity of oral mucositis correlates with the intensity of pro-inflammatory cytokine production, such as tumor necrosis factor (TNF), interleukin-6 (IL-6), and interleukin-1 beta (IL-1 β). Apoptotic changes appear in the form of mucositis in early radiotherapy or chemotherapy (3).

Improvements in the diagnosis and targeted treatment increase the survival rate and convenience of patients (4).

Managing these complications can be done through the following steps: maintaining good oral hygiene, reducing the radiation dose, optimizing pain control with anti-inflammatory drugs, enhancing growth factors, such as palifermin, natural agents, cryotherapy, and low-level laser dose therapy (LLLT) (5,6).

Modern medicine recognizes herbalism derived from natural sources as an alternative medicine, phytotherapy in many diseases and a preventive method against complications that result in chemotherapy-induced OM. *Calendula officinalis* (*C. officinalis*) (7), *Hypericum perforatum* (8), *Pistacia atlantica* and *Zizyphus jujuba* have been used to accelerate the healing of OM (9,10).

Eucalyptus is comprised of more than 100 different natural compounds, such as cineole (eucalyptol), pinene (α , α), limonene (δ , δ), terpineol (α , α), cymene (α), eudesmol, and aromadendrene (11).

The hydroalcoholic extract of eucalyptus contains multiple medicinal properties, such as being antibacterial, antiviral, antifungal, antiinflammatory, analgesic, and antioxidant. The medicinal eucalyptus oil is probably the most powerful antiseptic of its class, especially when it is old as ozone is formed in it on exposure to the air. It has distinct disinfectant action, destroying the lower forms

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of life (12,13).

Considering the effect of multiple properties of eucalyptus and the insufficient data about its efficacy on OM, the present study was conducted to evaluate the healing effects of 2% eucalyptus extract gel on OM induced by the chemotherapeutic agent 5-FU in male golden hamsters based on clinical, histopathologic, stereological, and tissue antioxidant index evaluations.

Material and Methods

Ethical considerations were confirmed by the Animal Care Committee of Shiraz University of Medical Sciences (NO: 8794115). The experiments were carried out in accordance with the guidelines laid down by the National Institute of Health (NIH) regarding the care and use of animals for experimental procedures.

Animals and Housing

Thirty male golden hamsters (100 to 120 g, 6-8 weeks old) were taken and prepared from the laboratory animal center of Shiraz University of Medical Sciences. Animals were housed in standard cages under equal light and dark periods of 12 h (lights on at 8:00 pm), with an ambient temperature of $22 \pm 2^\circ\text{C}$ and 55% relative humidity. Hamsters were given a 5-day acclimation period with access to normal chow and free water before the experiment.

C. officinalis Extract Gel Formulation

A gel-forming agent was prepared with 2% sodium carboxymethyl cellulose (NaCMC) dispersed in 5% glycerol under continuous stirring with a suitable drive mixer at 500 rpm. Then, adequate amounts of *C. officinalis* extract (0.5% and 10%) were added to sufficient amounts of deionized water. The mixtures were added to the NaCMC with glycerol gradually. The resultant gels were homogenized for 30 min and each formulation was filled in an aluminum tube. They were then sealed, and each formulation was used as required during the 1-month formulation.

Preparation of Eucalyptus Hydroalcoholic Extract

The leaves of the eucalyptus were dried at room temperature and powdered in a grinder. Aqueous ethanol (75%) was added to the powdered materials (500 g) and stirred for 1 h. The mixture was kept at room temperature for 48 h. Following filtration, ethanol was evaporated under reduced pressure at 40°C . The remained water extract was dried at the oven temperature of 50°C . Finally, the 2% eucalyptus hydroalcoholic extract was prepared in a pharmacy laboratory.

Chemotherapy-Induced Oral Mucositis

Thirty adult male golden hamsters were randomly

allocated into 3 equal groups. All animals received three intraperitoneal injections of 5-FU on days 0, 5, and 10 at a dose of 60 mg/kg with insulin needles, following the protocol proposed by Sonis et al. (14). To mimic the clinical effects of the chronic irritation, the cheek pouch mucosa was scratched superficially with the tip of an 18-gauge sterile needle after the area had been anesthetized. Two horizontal linear scratches were made across the everted cheek pouch once daily on days 3 and 4. The treatment started on day 12. The control group did not receive any treatment; the calendula group was treated with a *Calendula officinalis* extract gel; and the eucalyptus group was treated with a 2% eucalyptus extract gel on the cheek pouch mucosa of both sides once a day (morning) and the affected area was completely covered. To make sure that the drug was not swallowed, animals were not allowed to eat or drink for 45 min after the administration of the drug. Five hamsters from each group were randomly selected for euthanasia on days 14 and 17. At the time of euthanasia, they were anesthetized with 100 mg/kg ketamine 10% (Alfasan, Netherlands) and 10 mg/kg xylazine 2% (Alfasan, Netherlands).

Histopathological Study

Both side cheek pouches tissue of hamsters in all groups were harvested on days 14 and 17. Tissues obtained from the right side pouch were proceeded and stained with hematoxylin-eosin and examined by light microscopy ($\times 40$) (15). The specimens were examined histopathologically and received scores of 0 to 3, as described in Lima's method (16):

- Score 0, normal epithelium and connective tissue without vasodilatation; absent or mild inflammatory infiltrate; absence of bleeding, ulcers, and abscesses;
- Score 1, mild vascular hyperemia; areas of re-epithelialization; mild inflammatory infiltrate with a prevalence of mononuclear cells; no hemorrhagic areas, ulcerations or abscesses;
- Score 2, moderate vascular redness; areas of epithelial degeneration; inflammatory infiltration with a prevalence of neutrophils; hemorrhagic areas, edema, and occasional ulcerations; absence of abscesses;
- Score 3, severe hyperemia and vascular vasodilatation; inflammatory infiltration.

Measurement of Malondialdehyde (MDA)

Tissues obtained from the left side pouch were stored under liquid nitrogen until measurement. Tissue malondialdehyde (MDA) assessment was performed via the measurement of thiobarbituric acid reactive substances (TBARS) in PBS tissue homogenate (17) because MDA is one of the end-products of lipid peroxidation (LPO), measured

by estimating the MDA levels (18).

Myeloperoxidase (MPO) Assay

The MPO activity can be measured in the tissues by using hydrogen peroxide and o-dianisidine dihydrochloride as substrates (19). Briefly, the cheek pouch tissues which were collected on days 14 and 17 were weighed and homogenized in a solution, containing 0.5% (w/v) hexadecyltrimethylammonium bromide dissolved in 10 mmol/L potassium phosphate buffer (pH 7.4), and centrifuged for 30 min at 20,000 g at 4 °C. An aliquot of supernatant was then removed and added to a reaction mixture, containing 1.6 mmol/L tetramethylbenzidine and 0.1 mmol/L hydrogen peroxide (H₂O₂). The rate of change in the absorbance was measured spectrophotometrically at 650 nm. MPO activity was defined as the quantity of enzyme required to degrade 1 mmol of H₂O₂ at 37 °C and expressed in U/g wet tissue.

Tissue Glutathione (GSH) Measurements

The assay mixture consisted of 2.0 ml of 75 mmol phosphate buffer (pH 7.0), 50 µL of 60 mmol GSH, 0.1 mL of 30 U/ml GSH reductase, 0.1 ml of 15 mmol disodium salt of EDTA, 0.1 ml of 3 mmol reduced nicotinamide adenine dinucleotide phosphate reduced form (NADPH), and supernatant to a final volume of 3.0 mL. The reaction was initiated by adding 0.1 mL of 7.5 mmol H₂O₂. The rate of change in the absorbance due to the conversion of NADPH to nicotinamide adenine dinucleotide phosphate (NADP) was recorded spectrophotometrically at 340 nm for 3 min. GSH activity was expressed as micromoles of NADPH oxidized to NADP min⁻¹ mg⁻¹ protein using the extinction coefficient (6.22 mM⁻¹ cm⁻¹) for NADPH 24.

Stereological Study

The oral mucosa was sectioned axially and ten 4-µm-thick and ten 26-µm-thick sections per animal were sampled

in a systematic uniform random pattern. The sections were stained with both Heidenhain's AZAN trichrome stain and hematoxylin-eosin. The microscopic analyses of the oral mucosa were performed using a video-microscopy system made up of a microscope (E-200; Nikon™; Tokyo, Japan), a video camera, and a flat monitor. Four µm sections were used to estimate the volume density of the oral epithelium, connective tissue, and collagen bundles using the point-counting method. Briefly, a point grid was overlaid on the monitor and the subsequent formula was used to estimate the volume density (V_V) of the favored structure:

$$V_V = \Sigma P(\text{structure}) / \Sigma P(\text{reference})$$

where $\Sigma P(\text{structure})$ and $\Sigma P(\text{reference})$ are the total points hitting the favored structure and reference tissue.

To estimate the mean height of the layers (epithelium, whole mucosa), the isotropic lines were imposed on the images of the 4-µm-thick sections of the mucosa. The thickness was measured vertically at the meeting points of the lines and mucosa.

The numerical density (N_v: number of the cells per unit volume of the connective tissue) of the fibroblasts was approximated by employing the 26-µm-thick sections. Also, the "optical disector" method (Fig. 1) and the following formula were applied:

$$N_v(\text{cells / connectivetissue}) = \frac{\Sigma Q^-}{\Sigma P \times \left(\frac{a}{f}\right) \times h} \times \frac{t}{BA}$$

where "ΣQ⁻" is the number of the fibroblast nuclei coming into focus during scanning "h"; "ΣP" is the total count of the unbiased counting frame in all fields; "a/f" is the frame area; "h" is the height of the disector; "t" is the mean section thickness; and "BA" is the block advance of the microtome set at 26 µm. "t" and z-axis movement (h) of the microscope stage were measured using a microcator

Figure 1. A comparison between three different groups in two days of sampling. A: Histopathological score on the 14th day; B: Histopathological score on the 17th day.

(MT 25, Heidenhain, Traunreut, Germany).

Statistical Analysis

All statistical analyses were carried out using SPSS® statistical software, version 20.0 (SPSS Inc., Chicago, IL, USA) for Windows®. Quantitative and qualitative variables are described as the mean $\pm$ standard deviation (SD). One-sample Kolmogorov-Smirnov test was used to test the normality of distribution of the collected data. An independent-samples Kruskal-Wallis Test was used to assess significant differences between the study groups with regard to the MDA level, GSH and MPO activities, and the pathologic score. Additionally, the independent-samples T-Test was used to compare the treated group on different days. Differences were considered significant when $p < 0.05$.

Results

Histopathological Assessment

Histopathological changes according to the determined scores showed that the peak inflammation of OM happened on the 12th day (based on clinical evaluation), and the treatment was initiated on this day. There was a significant histopathological difference between the treated group and the control group. No difference was observed between the control group and the gel-based group on different days. Over the whole treatment period, the histopathological scores in the treated group were lower than those in the gel-based and control groups, indicating less severe inflammation in the eucalyptus-treated group (Fig. 1).

Epithelialization and wound healing were detected in the eucalyptus-treated group whereas extensive infiltration of inflammatory cells, hemorrhage, and ulcers were observed in the control and gel-based groups. On days 14 and 17, the histopathological scores for the treated group were lower than those in the gel-based group

($p=0.005$, $p=0.016$, respectively). Also on days 14 and 17, the histopathological scores for the treated group were lower than those of the control group ($p=0.005$, $p=0.002$, respectively).

Tissue MDA Level Assessment

The measurement of tissue MDA concentrations showed no differences in the MDA concentrations between the gel-based and control groups on different days. The MDA concentration in the control and gel-based groups was higher than that in the treated group on the 14th and 17th day ($p=0.002$ in both days), indicating more oxidative stress in the gel-based and control groups. On days 14 and 17, the MDA concentration for the treated group was lower than that of the gel-based group ($p=0.009$ and $p<0.001$, respectively). Nevertheless, the MDA concentration in the Eucalyptus-treated group on the 14th and 17th day was significantly lower than that of the control group ($p=0.001$ and $p=0.009$, respectively). The difference between the treated groups was significant ($p=0.002$). Tissue MDA concentrations on the 14th day (5.05 ± 0.71) were higher than those on the 17th day (2.06 ± 0.27) (Fig. 2).

Tissue MPO Level Assessment

Tissue MPO activity in the study groups showed significant differences in the MPO activity between all three groups on the 14th and 17th day ($p=0.008$, $p=0.005$, respectively). On the 14th day, there was a significant difference between the treated group, on one hand, and the control ($p=0.004$) and gel-based ($p=0.016$) groups, on the other hand. On the 17th day, significant differences were detected between the treated group, on the one hand, and the control ($p=0.001$) and gel-based ($p=0.033$) groups, on the other hand. The difference between the Eucalyptus-treated groups was significant ($p<0.001$). The

Figure 2. A comparison of malondialdehyde (MDA) concentrations between three different groups in two days of sampling. A: MDA concentration on the 14th day; B: MDA concentration on the 17th day.

tissue MPO activity on the 14th day (126 ± 5.91) was lower than that on the 17th day (224.6 ± 8.61).

Tissue GSH Level Assessment

The difference between the GSH level in all the study groups was significant ($p=0.005$ and $p=0.002$, respectively). GSH had more activity in the treated group than in the control ($p=0.001$) and gel-based ($p=0.034$) groups on the 14th day. Respectively, on the 17th day, significant differences were detected between the treated group, on the one hand, and the control ($p<0.001$) and gel-based ($p=0.009$) groups, on the other hand. There were no differences between the tissue GSH level in the control and gel-based groups on the 14th and 17th day. The difference between the treated groups was significant ($p<0.001$). Tissue GSH level on the 14th day (1.12 ± 0.04) was lower than that on the 17th day (1.76 ± 0.08) (Fig. 3).

Stereology Assessment

The mean of the epithelium thickness showed a 50% increase in the eucalyptus 14-day group in comparison to that of the control and gel-based groups. The epithelium thickness indicated a 61% increase in the eucalyptus 17-day group in comparison to that of other groups ($p<0.01$, Fig. 4A, Fig. 5).

The total thickness of the mucosa and submucosa showed a 60% increase on average in the thickness of both layers in the Eucalyptus-treated (14 or 17 days) hamsters in comparison to that of the animals in the control and gel-based groups ($p<0.01$, Fig. 4B).

The mean volume density of the collagen showed an increase of 35% on average in the eucalyptus (14 or 17 days) group in comparison to that of the control and gel-based groups ($p<0.01$, Fig. 4C).

The numerical density of the fibroblast significantly increased by 88% on average in the Eucalyptus (14 or 17 days) group in comparison to that of the control and gel-

based groups ($p<0.01$) (Figs. 4D).

Discussion

This study was conducted to evaluate the histopathological, stereological, and antioxidant markers of 2% eucalyptus on induced oral mucositis in male golden hamsters. A large and increasing number of patients are consuming medical herbs without accurate information about their safety and efficacy and seek the advice of their physicians regarding their use. The findings of this study provide physicians with some convincing data which encourage them to help their patients make wise decisions about the use of the medical herbs. NSAIDs are commonly used for inflammatory diseases.

Some herbal medicines with no side effects are used for the treatment of OM in animal models. Tanideh et al. (7) showed that the accelerated healing effect, antitoxicity, and antimicrobial properties of *C. officinalis* may make it an appropriate drug for the treatment of OM. Showraki et al. (20) demonstrated that daily application of olive leaf extract ointment had a healing effect on 5-fluorouracil induced OM in hamsters. Moreover, the reported beneficial effects of olive leaf extract on OM might be due to its antioxidant and anti-inflammatory properties. Mardani et al. (21), evaluated the effects of *Carum Carvi L* hydroalcoholic extract on oral mucositis and showed that the use of CHE in topical form may be associated with reduced intensity of OM. This may be due to its appropriate antibacterial activity and the terpinene contents in it. Koohi-Hosseiniabadi et al. (22) also investigated the healing effects of *Elaeagnus angustifolia* in 5-fluorouracil (5-FU)-induced OM in golden hamsters. They showed that the hydroalcoholic extract of *E. angustifolia* can be used as an appropriate drug for the treatment of oral mucositis based on the extract healing stimulatory and anti-inflammatory properties. Eucalyptus has multiple medicinal properties, such as being antibacterial, antiviral, antifungal, anti-inflammatory, analgesic, and antioxidant.

Figure 3. A comparison of glutathione (GSH) activity between three different groups in two days of sampling. A: GSH activity on the 14th day; B: GSH activity on the 17th day.

Eucalyptus can reduce the inflammation process due to its main components, including eucalyptol, thymol, gallic acid, caffeic acid, quercetin, and rutin (12). Sodium carboxymethyl cellulose (NaCMC) is a bioadhesive polymer used for delivery of drugs to the oral cavity, including eucalyptus extract (13).

Darshan et al. (23) demonstrated that the presence of tannins (gallic acid) had astringent action and disinfectant and anti-inflammatory effects. Eucalyptus has been used as a traditional medicine for the treatment of different diseases. The consumption of eucalyptol as medicine is safe and it can be taken orally up to 12 weeks. It is able to eliminate

the microorganism and fungi. The main components which give it such properties are cineole, eucalyptol, tannin, linalool, chlorogenic acid, thymol, limonene, and pinene. The monoterpenoid components present in the essential oils from Eucalyptus are commercially available for the treatment of flu, respiratory infections, and inflammations disorders (24).

Our findings derived from the histopathologic assessment showed that the 2% eucalyptus had lower microscopic scores compared to other groups on the 14th and 17th day. The reduction in the histopathologic scores indicates the healing process of this therapeutic agent. The

Figure 4. The plots of the quantitative parameters of the oral mucosa in the chemotherapy-exposed hamsters which received no treatment (control), gel-based agent, and eucalyptus extract gel for 2 or 5 days. A: Thickness of Epithelium (μm); B: Thickness of mucosa and submucosa (μm); C: Volume Density of Collagen (%); D: Numerical density of fibroblast ($\times 1000/\text{mm}^3$). The significant differences between the gel-based and the eucalyptus group are shown on the plots.

overall histopathologic changes were expected as some previous studies had reported the presence of quercetin in *calendula officinalis*. Our findings are in agreement with those of Hukkeri et al. (25), who investigated the wound healing property of *Eucalyptus globulus* leaf extract.

Oxidative stress from free radicals is involved in the pathophysiology of many inflammatory diseases. Our data showed that eucalyptus as a protective rule of antioxidative enzymes included MDA, MPO, and GSH in the pathogenesis of OM. In this study, we observed that the eucalyptus group could decrease MDA concentration and elevate MPO and

GSH activity compared to other groups.

The stereological results of our study indicated that the hydroalcoholic extract of eucalyptus could promote the epithelium and connective tissue regeneration in chemotherapy-induced OM groups compared to other groups. These findings were in agreement with those obtained in the previous studies, demonstrating that eucalyptus citriodora was able to reduce the epithelialization period (15).

Based on our findings, the numeric density of the fibroblast increased in the Eucalyptus-treated group in

Figure 5. The micrograph of the thickness of the epithelium and connective tissue of the mucosa of different groups. A: Control group on the 14th day; B: Control group on the 17th day; C: Gel-based group on the 14th day; D: Gel-based group on the 17th day; E: Eucalyptus-treated group on the 14th day; F: Eucalyptus-treated group on the 17th day.

comparison to that of the control and gel-based groups. These findings are in line with those of the study conducted to investigate the wound healing potential of leaves of eucalyptus citriodora in rats, the results of which showed that Tannins could promote wound healing through several cellular mechanisms, including scavenging of free radicals and reactive oxygen species, promoting the contraction of the wound, and increasing the formation of capillary vessels and fibroblasts (26). The re-epithelialization of wound involves the migration of keratinocytes from the edges of the wound. The role of fibroblasts in the re-epithelialization process and the reconstitution of the dermal-epidermal junction has been documented (27).

Collagen is the main structural protein in various connective tissues in animals. The fibroblast is the most common cell that creates collagen. Since eucalyptus had

a promotive effect on fibroblast, an increase in collagen, as its product, is expected. The results of the current study confirmed an increase of 35% in the treated groups compared to other groups. Previous studies have claimed that the synthesis of collagen increases in the eucalyptus-treated groups (15).

Compared with the control and gel-based groups, the Eucalyptus group increased re-epithelialization. These data indicate that Eucalyptus can accelerate wound healing in OM through anti-inflammatory pathways (28). This finding and the established safety profile of Eucalyptus provide a rationale for studying the topical application of this agent in a clinical setting.

Based on the results of this study, eucalyptus might be effective in OM treatment. However, further studies must focus on its active anti-inflammatory and antioxidative

Figure 6. The micrograph of the fibroblast population in the oral mucosa of different groups. A: Control group on the 14th day; B: Control group on the 17th day; C: Gel-based group on the 14th day; D: Gel-based group on the 17th day; E: Eucalyptus-treated group on the 14th day; F: Eucalyptus-treated group on the 17th day.

components in different diseases and clinical trials.

Resumo

A mucosite oral é uma complicação inflamatória comum em pacientes com câncer. Este estudo avaliou os marcadores histopatológicos, estereológicos e antioxidantes de Eucalyptus 2% na mucosite oral induzida em hamsters dourados machos. Neste estudo em animais, a mucosite oral foi induzida em 30 hamsters golden masculinos por 5-FU (60 mg / kg) nos dias 0, 5 e 10 do estudo. A bolsa da bochecha foi arranhada com uma agulha estéril uma vez ao dia nos dias 3 e 4. Nos dias 14 a 17, os grupos de gel de eucalipto a 2% e curativos à base de gel foram tratados e comparados com um grupo controle. Foram avaliados os escores histopatológicos e estereológicos e o conteúdo de malondialdeído na bolsa, bem como as atividades de glutatona e mieloperoxidase no tecido da bolsa. Os escores histopatológicos de mucosite foram menores no grupo de gel de eucalipto a 2% do que os do gel e do grupo controle ($p < 0,05$). Além disso, um nível mais baixo de malondialdeído e maiores atividades de mieloperoxidase e glutatona foram detectadas no grupo tratado com eucalipto em comparação aos grupos à base de gel e controle ($p < 0,001$). A espessura da mucosa e submucosa aumentou no grupo Eucalyptus. A densidade numérica do fibroblasto e a densidade do volume do colágeno aumentaram significativamente nos grupos tratados com eucalipto. Em conclusão, o uso do extrato hidroalcoólico de Eucalyptus pode estar associado a menor intensidade de mucosite oral, diminuição da concentração de malondialdeído, aumento da atividade de mieloperoxidase e glutatona, aumento do volume de mucosa e submucosa, aumento de fibroblastos e colágeno na mucosite oral induzida em hamsters dourados em consumo de 5 UF.

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