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TOPICAL ANTI-INFLAMMATORY EFFECT OF EUCALYPTUS EXTRACT VERSUS JOJOBA GEL ON 5-FLUOROURACIL INDUCED ORAL MUCOSITIS IN HAMSTER

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Abstract

Objectives: The study aimed to evaluate the topical anti-inflammatory effect of eucalyptus extract gel compared with jojoba gel on 5-fluorouracil-induced OM in hamsters. **Materials and Methods:** Sixty male hamsters were divided into two main groups. Group A (n = 15) served as a negative control and did not receive OM induction or treatment. Group B (n = 45) underwent OM induction with two intraperitoneal injections of 5-FU (60 mg/kg) followed by superficial scratching of the left cheek pouch. On day 5, Group B was subdivided into three subgroups (n = 15 each): BI (positive control, untreated), BII (treated daily with JJ gel), and BIII (treated daily with 2% EC extract gel). Five animals per subgroup were euthanized on days 5, 9, and 14. Macroscopic, histological, and immunohistochemical analyses were performed. **Results:** Macroscopic and histological evaluations revealed significant differences in re-epithelization and collagen deposition among groups. Immunohistochemical analysis showed that EC gel treatment significantly decreased interleukin-1 beta (IL-1β) expression and increased transforming growth factor-beta (TGF-β) expression compared with JJ gel. **Conclusions:** Eucalyptus gel demonstrated superior promotion of ulcer re-epithelization and accelerated healing compared with jojoba gel in the 5-fluorouracil induced oral mucositis model.

Keywords: Eucalyptus, Hamster, Jojoba, 5-Fluorouracil, Oral Mucositis.

INTRODUCTION

Cancer is the second most common cause of death for children. Before the age of 20, leukemia is the most frequent childhood cancer. Teenagers are more likely to develop brain and other nervous system tumors (21%), closely followed by lymphoma (19%). The forms and distribution of cancer in adolescents are different from those in children.[1] WHO's estimation indicated that Egypt had 6,803 new cases of childhood cancer among patients aged 0–14, accounting for 20% of the total cases in the region.[2]

Cytotoxic drugs such as 5-fluorouracil (5-FU) are non-selective and can kill both normal and cancerous cells. This lack of selectivity is considered one of the limitations of 5-FU usage that results in serious toxic problems. Acute complications include harm to the most

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vulnerable organs, including the bone marrow, gastrointestinal mucosa, hair follicles and gonads, because of their fast-dividing cells. Thus, some of the early side effects of chemotherapy include myelosuppression, which causes neutropenia, severe infections, nausea, vomiting, diarrhea, hair loss, and decreased fertility.[3]

A common and crippling side effect of chemotherapy is OM. OM has detrimental effects on patient function and quality of life, including malnutrition, reduced saliva production, speech difficulties, and ulceration. About 40% of patients receiving traditional chemotherapy and 60% to 85% of patients receiving high-dose chemotherapy in preparation for a bone marrow transplant are affected by OM.[4]

The mechanism of OM is dynamic, multifaceted, and complex. A cascade of five phases can occur simultaneously and mechanistically that begins with an (i) initiation involving cell injury caused by 5-FU leading to elevation of inflammatory cytokines, free radical-induced cellular and DNA damage with basal and supra-basal epithelial cell death (ii) release of the pro-inflammatory cytokines as interleukin 6 (IL-6), IL-1β and tumor necrosis factor (TNF-α), thus enhancing the injury response through promoting connective tissue and endothelial damage, and stimulating cell death through decreasing the tissue oxygenation, (iii) signaling and amplification lead to further tissue damage due to a decreased ability of epithelial regeneration and proliferation, followed by, (iv) ulceration that is considered as a clinical sign of OM to be visible through the disruption of the mucosa and submucosa integrity, then (v) healing which characterized by epithelial proliferation and tissue differentiation to restore the epithelial integrity. Therefore, each phase may constitute a possible therapeutic target.[5]

Creating a therapeutic alliance with caregivers, dieticians, and nurses is crucial to improving each chemotherapy cycle and preventing the catabolic state. This involves asking for feedback and offering options. The medicinal herbs possess several advantageous qualities, such as antioxidant, anti-inflammatory, antibacterial, antiviral, and antifungal effects. Herbal medicine and medicinal plants have been recognized as secure treatments for conditions associated with cancer treatments or malignancies.[6]

Recently, EC has attracted considerable attention due to its many potential advantages in a variety of biological applications. It is considered a strong suppressor of cytokines. It has demonstrated potent antimicrobial properties against a range of viruses, fungi, and bacteria. They are useful in preventing infections and promoting wound healing. It has active ingredients that can reduce inflammation, which makes them useful for treating inflammatory diseases such as skin problems and respiratory illnesses.[7]

Jojoba (JJ) oil is widely used in traditional medicine to treat conditions affecting the skin and scalp, wounds, sore throats, obesity, and even cancer. It also boosts immunity and promotes hair growth. JJ oil's pharmacological diversity is shown by recent studies, which have shown that it has antibacterial, anti-inflammatory, anti-pyretic, anti-acne, antidiabetic, and antioxidant qualities. Its use in pharmaceuticals has significantly increased, especially in parenteral, topical, and transdermal preparations. It greatly accelerates the closure of wounds in keratinocytes and fibroblasts.[8]

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Up till now, no published articles have been conducted to compare the anti-inflammatory effects of EC and JJ oil on 5-FU induced OM. So, the aim of this study was to evaluate and compare the topical anti-inflammatory effect of EC extract gel versus JJ gel on 5-FU induced OM in hamsters.

METHODS

Study design

The present study aimed to evaluate the anti-inflammatory properties of EC versus JJ gel on 5-FU induced OM on 60 male Syrian golden hamsters weighed (70-80 g). They were housed in a pathogen-free room at the animal house, Faculty of Dentistry, Suez Canal University. The room temperature was maintained at ± 24°C with 12/12 hours light/dark *ad Libitum*. The animals were fed with diet and water. All experimental procedures were performed after the approval of Research Ethics Committee (REC), Faculty of Dentistry, Suez Canal University, number: 461/2022.

Utoral ampoule (5 ml) contains 250 mg of 5-FU. It was purchased from Hikma Specialize Pharmaceuticals, Egypt. Jogel is composed of 10% JJ oil and 0.5% lidocaine hydrochloride. It was purchased from Sedico, Egypt. EC gel was composed of 2% sodium carboxymethyl cellulose (NaCMC), 5% glycerol, and 2% EC extract. It was manufactured at Nawah Scientific Research Institute, Cairo, Egypt.

Preparation of the EC Gel:

The dried leaves of the EC were powdered in a grinder. Aqueous ethanol (75%) was added to the powdered EC (500 g), stirred for 1 hour, and preserved at room temperature for 48 hours. Then, ethanol was evaporated under reduced pressure at 40 ºC and the remaining water was dried at 50ºC. 2% Na-CMC was dispersed in 5% glycerol to obtain gel formulation under continuous stirring at 500 rpm. 2% of EC extract was added to deionized water then mixed gradually to Na-CMC with glycerol. The resultant gel was homogenized for 30 minutes and filled in an aluminum tube.[9]

Sample size calculation

According to **Daniel, 2009**[10]; **Charan and Biswas, 2013**[11], the sample size for this study revealed 60 samples, five in each group using the following equation:

$$\mathbf{N} = \frac{(Z_\alpha)^2 * (S)^2}{(d)^2}$$

N = Total sample size, Z_α= Standard normal variation (1.96) at P< 0.05, SD = Standard deviation of variable (7.9), D = Absolute error or precision (2)

Oral mucositis induction:

The hamsters received two intraperitoneal injections of 5-FU (60 mg/kg) on day 0 and day 2 with insulin needles. To mimic the clinical effects of the chronic irritation, hamsters were anesthetized with 100 mg/kg ketamine and 10 mg/kg xylazine. one cm^2 area of left

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cheek pouch was scratched superficially with the tip of an 18-gauge sterile needle at an equal force and depth on days 2 and 3.[12]

Animal grouping:

Sixty hamsters were divided into two main groups as follows: Group A (n=15): served as negative control that did not receive OM or any treatment till the end of experiment. Group B (n=45): received OM induction and on day 5, [13] all animals in this group were subdivided into three equal subgroups according to the type of treatment used as follows: Subgroup BI (n=15): acted as positive control. It did not receive any treatment. Subgroup BII (n=15): was treated by topical application of jogel once a day. Subgroup BIII (n=15): was treated by topical application of 2% of EC gel once a day. Hamsters were not allowed to eat or drink for 45 min after the administration of the drugs. Five animals from each group were randomly selected for euthanasia on (days 5, 9 and 14) by using overdose of pentobarbital. After euthanasia, the dead hamsters were disposed of by burning in Animal Aching Unit of Medicine, Suez Canal University.

Macroscopic analysis:

Each animal was weighed throughout the experimental period on days 0, 5, 9 and 14. The scratches were assessed daily from day 3 till day 14, according to the following scoring. Score (0) Healthy cheek pouch without any erosion or vasodilatation. Score (1) Presence of erythema only. (2) Presence of sever erythema and surface erosion. Score (3) Ulcers did not exceed 25% of the cheek pouch surface area. Score (4) Cumulative formation of ulcers of about 50% of the surface area. Score (5) Complete ulceration of the cheek pouch mucosa.[5]

Histopathologic analysis:

The left cheek pouch was fixed in 10% formalin, dehydrated, and embedded in paraffin. Sections of 5 μm thickness were obtained for hematoxylin and eosin (H&E) staining and examined by light microscope. The specimens were examined and received scores of 0 to 3. Score (0) Normal epithelium and connective tissue with no signs of inflammation. Score (1) Mild vascular hyperemia; areas of re-epithelialization; mild inflammatory infiltrate; no hemorrhagic areas, ulcerations, or abscesses. Score (2) Moderate vascular hyperemia; areas of epithelial degeneration; inflammatory infiltration with a prevalence of neutrophils; hemorrhagic areas, edema, and occasional ulceration; absence of abscesses. Score (3) Severe vascular hyperemia and dilatation; redness, edema, areas of necrosis and abscesses, pus formation, ulcers, and marked inflammatory infiltration. Tissue sections were examined by two oral pathologists under light microscopy (100X magnification).[14]

Immunohistochemical (IHC) analysis:

It was performed according to manufacturer's instructions by obtaining 3-5 mm-thick sections from paraffin-embedded tissue blocks that were cut and mounted on a positively charged salinized glass slide. The detection kit DAB HRP cytoplasmic brown detection system; Servicebio; catalog no. GB12113 FOR IL-1-β and BSB 0205 for TGF-β - mouse

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monoclonal antibodies. Tissue sections were examined and analyzed at 400X magnification. The area of the screen was measured by digitizing. The optical density of these sections was assessed quantitatively through using image analyzer computer system (Image J Fuji) at the pathology laboratory, Faculty of Medicine, Suez Canal University.

Statistical analysis:

A descriptive statistical analysis was conducted using the SPSS software for windows version 26.0 (Statistical Package for Social Science, Armonk, NY: IBM Corp). All data were collected in the form of mean ± standard deviation (SD). One-way ANOVA was used to compare between the groups. Duncan's tests were used to evaluate statistical significance. Kruskal Wallis test was used for non-parametric data. The confidence interval was defined as 95% and the value of $p < 0.05$ was considered statistically significant.

RESULTS

Macroscopic findings

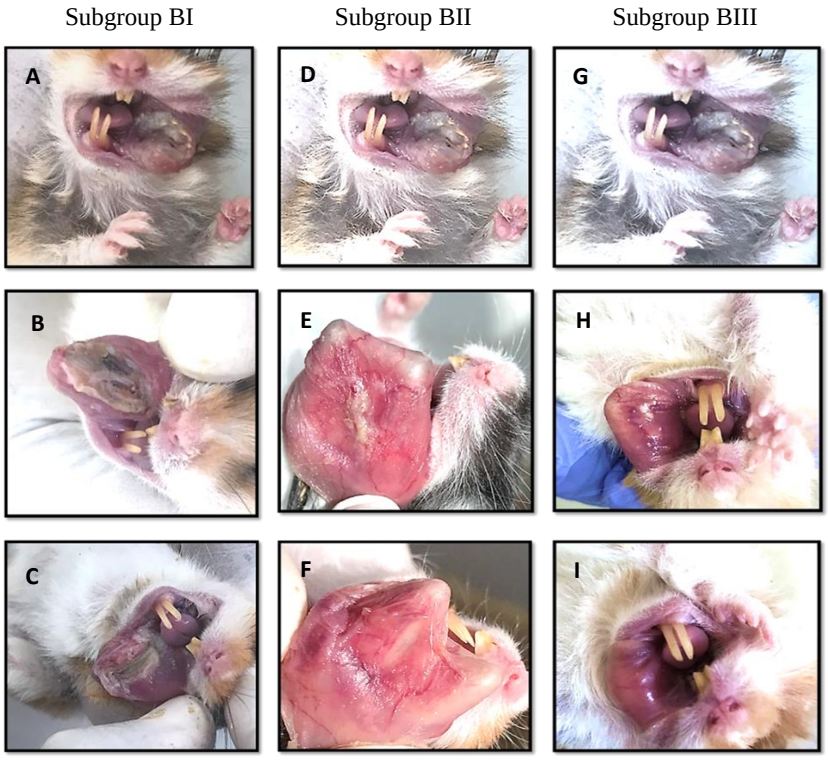

Figure 1: The clinical scoring of oral mucositis induced by 5-FU in hamsters on days 5,9,14 respectively in different subgroups. (A) Score 4; (B) Score 5; (C) Score 3; (D) Score 4; (E) Score 2; (F) Score 1; (G) Score 4; (H) Score 2; (I) Score 0

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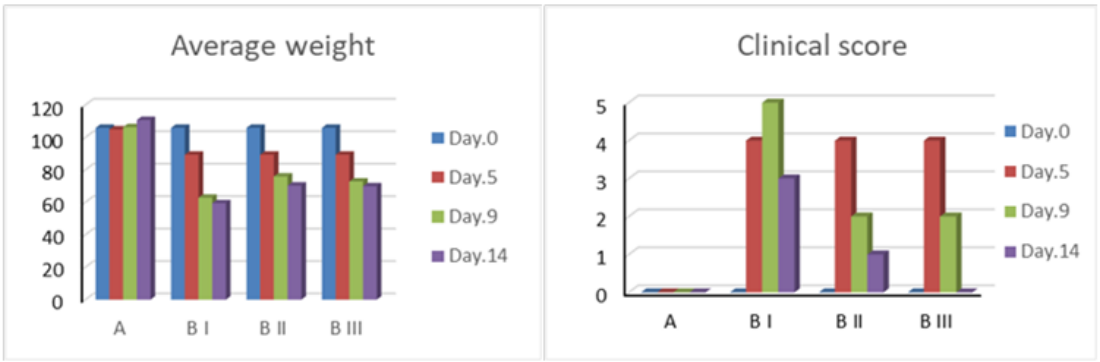
In group A, the hamsters appeared healthy and active. Their average weight did not change from day 0 (105.9 ± 8.1) till the end of the experiment (110.7 ± 7.9). In subgroups BI, BII, BIII, most hamsters appeared debilitated and exhausted and some developed diarrhea. On day 14, the average weight was significantly decreased (59.4 ± 3.7), (70.3 ± 4.9), (69.9 ± 6.1) respectively in comparison to the same group on day zero (105.9 ± 8.1). The average clinical score in different subgroups with multiple intervals in the experiment **(Figures 1 and 2).**



A **B**

Figure 2: (A) The mean weight of hamsters, (B) The mean clinical score of oral mucositis in different groups throughout the experiment

Histopathological and immunohistochemical analysis (Figures 3, 4 and 5):

Group A revealed that normal hamster buccal pouch with thin keratinized stratified squamous epithelium. The underlying submucosa consisted of a thin layer of dense collagenous fibrous tissue, a layer of longitudinal stratified muscle fibers and loose areolar connective tissue (CT) (score 0) **(Figure 3A)**. The IHC revealed weak cytoplasmic reaction to IL-1β (16.9 ± 2.2a) limited to basal layer of the epithelium **(Figure 3B)**. Regarding TGF-β immune reactivity, there was strong cytoplasmic expression (162.7± 3.4) within the epithelium and the underlying lamina propria (**Figure 3C).**

Group B (**Day 5):** At the wound area, there was a complete loss of the epithelial surface with protruded coagulative necrosis mixed with a prevalence of neutrophils and macrophages. The underlying CT revealed intense inflammatory infiltration and severe vascular hyperemia. The same histological score (score 3) was observed in different subgroups B I , B I I and B I I I (**Figure 3D)**. The IHC showed strong cytoplasmic expression to IL-1β (85.7± 1.4) within the lamina propria while TGF-β expression was markedly decreased (82.6 ± 2.5) in comparison with group A **Figure 3 (E and F)**.

Subgroup B I (**Day 9):** The borders of the epithelial lining appeared edematous with infiltration of inflammatory cells. The underlying CT showed severe vascular hyperemia with dilatation of blood vessels. There was marked infiltration of acute inflammatory cells, mainly macrophages and polymorphonuclear leucocytes. The histological score was score 3 (**Figure 3G)**. The IHC results showed strong cytoplasmic expression to IL-1β

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(98.3 ± 2.9) while TGF-β showed moderate expression (95.8 ± 1.8) with the positive control group **Figure 3 (H and I).**

Day 14: Areas of re-epithelialization began to appear at the periphery of the lesion. There was intense to moderate inflammatory infiltration with moderate vascular hyperemia. The histological score began to decline into score 2 (**Figure 3J)**. After the analysis of IHC photomicrographs, IL-1β showed moderate cytoplasmic expression (70.2 ± 4.1) while TGF-β showed moderate expression (100.7 ± 4.4) **Figure 3 (K and L).**

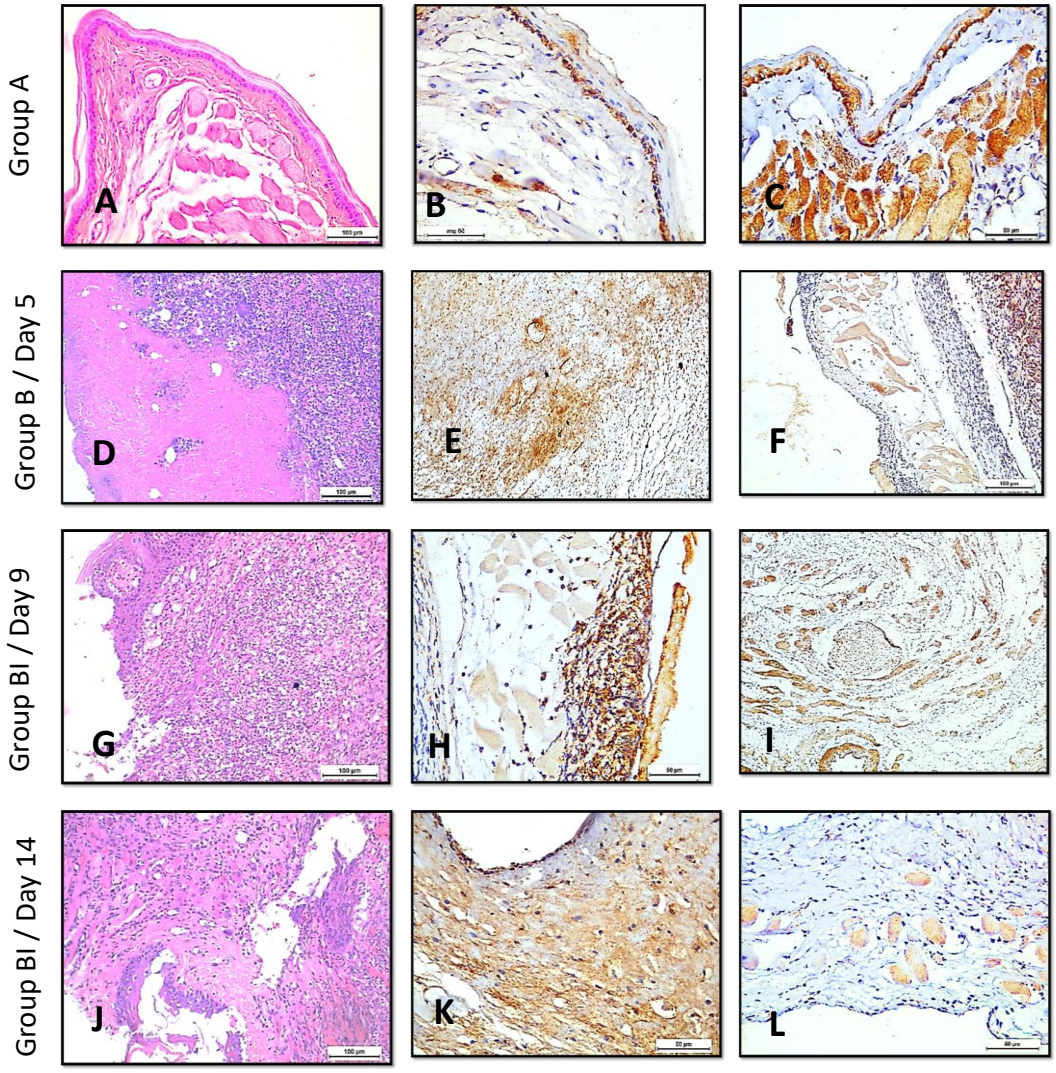

Figure 3: Photomicrographs showing the histological scoring in different groups (A) Score 0; (D) Score 3; (G) Score 3; (J) Score 2. The immunohistochemical cytoplasmic expression of IL-1β (B) Weak; (E & H) Strong; (K) Moderate; and the immunohistochemical cytoplasmic reaction to TGF-β (C) Strong; (F) Weak; (I & L) Moderate

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Subgroup B Ⅱ (**Day 9):** Lateral migration of the epithelium extended from the wound periphery. Moderate inflammatory infiltration with a prevalence of neutrophils with moderate vascular hyperemia. The histological score decreased to score 2 (**Figure 4A)**. The IHC results showed moderate cytoplasmic expression to IL-1β (70.2± 2.1) while TGF-β showed moderate expression (90.4±3.6) **Figure 4 (B and C).**

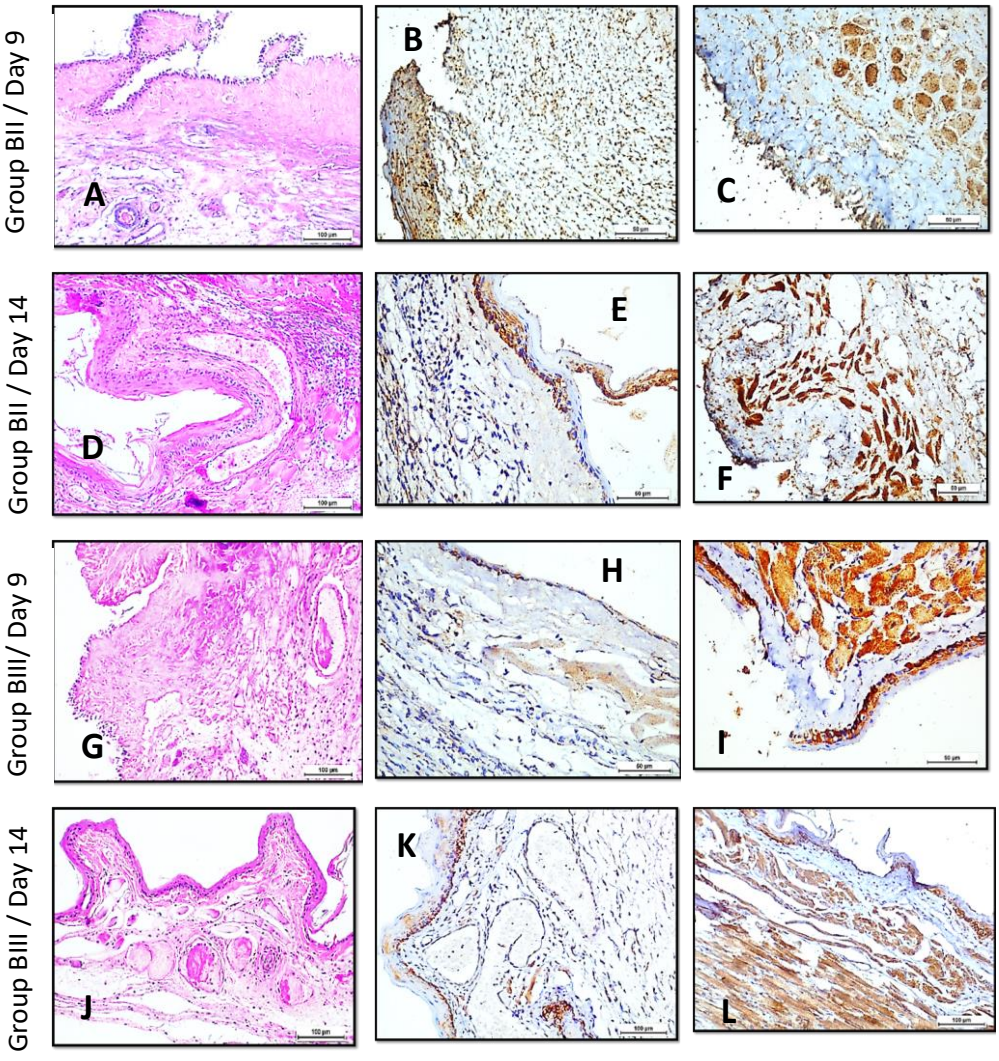

Figure 4: Photomicrographs showing the histological scoring in different groups (A) Score 2; (D) Score 2; (G) Score 2; (J) Score 1. The immunohistochemical cytoplasmic expression of IL-1β (B) Moderate; (E) Weak to moderate; (H) Moderate; (K) Weak; and the immunohistochemical cytoplasmic reaction to TGF-β (C) Moderate; (F & I & L) Strong

(**Day 14):** The epithelial thickness increased but the lamina propria still filled with moderately dilated blood vessels which were engorged with red blood cells (RBCS) and moderate inflammatory infiltration, so the histological score remains 2 (**Figure 4D)**.

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The IHC results showed weak to moderate cytoplasmic expression to IL-1β (38.6 ± 3.3) while TGF-β showed marked increase in its expression (120.8±2.1) **Figure 4 (E and F).**

Subgroup B Ⅰ Ⅰ Ⅰ (Day 9): Re-epithelization started to extend all over the wound surface. The underlying lamina propria showed well organized vascularized CT Moderate inflammatory infiltration with a prevalence of neutrophils with moderate vascular hyperemia. The histological score decreased to score 2 (**Figure 4G).** The IHC results showed moderate cytoplasmic expression to IL-1β (45.2± 3.6) while TGF-β showed strong expression (130.7±1.9) **Figure 4 (H and I).**

(Day 14): Complete coverage of ulcerated areas with normal epithelium was noticed in some sections. Mild inflammatory infiltration was noticed. The subepithelial lamina propria showed well organized granulation tissue with abundant mature collagen bundles. Some of the newly formed blood vessels appeared engorged with RBCs. The histological score became score 1 (**Figure 4J).** The IHC results showed mild cytoplasmic expression to IL-1β (28.7 ± 1.4) while TGF-β showed strong expression (170.1±3.7) **Figure 4 (K and L).**

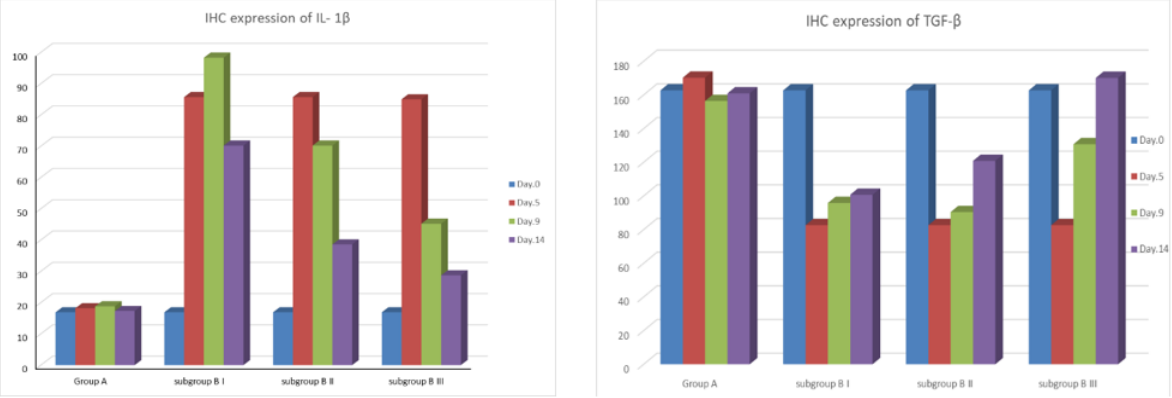

Figure 5: The average level of IL-1β and TGF-β in different groups throughout the experimental intervals

DISCUSSION

Chemotherapy -induced OM is a common side effect of 5- FU which has an anti-tumor effect. This study was conducted to evaluate the daily topical anti- inflammatory effect of jojoba versus eucalyptus gels, to contribute in efforts to find the appropriate treatment to OM. Topical therapy is widely used as a supportive approach in wounds management. So, the use of natural products in the management of ulcer healing has been proposed.[15]

Preclinical investigations that serve as a translational pathway for drug discovery heavily rely on animal models, which are an extension of in vitro studies. **Sonis et al., 1990** [12] developed the use of the experimental model “hamster buccal pouch” in the induction protocol of OM. Numerous studies on the pathophysiology and inflammatory processes of OM have been conducted using hamsters. Its allure stems from the similarities between the hamster and human oral cavities, including blood cell count, bacterial flora, and OM

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onset. This model reproduced histopathological changes of chemotherapy-induced OM in humans, such as stimulation of proinflammatory cytokines in addition to induction of apoptosis. [16]

There was wide variation in doses and injection regimes used to induce OM between multiple studies. The current study was online with **Ogihara et al., 2023**[17], who reported the use of twice dose of 60m/kg of 5-FU. In contrast to our study, **Zhang et al., 2018**[18] injected five dosages of 50 to 100 mg/kg intraperitoneal per day of 5-FU to induce OM. There was an increase in the severity of OM and mortality rate. **Bertolini et al., 2017**[19] suggested that intraperitoneal injection of 5-FU could induce intestinal mucositis reproducibly but did not affect the oral mucosa significantly. Therefore, local mechanical trauma or chemical injury has been incorporated into OM models, which may be due to the highly keratinized nature of the oral epithelium, making it less susceptible to mucosal breakage.

The macroscopic findings of the present study were consistent with authors who used 5-FU as a chemotherapeutic agent in the induction protocol of OM. [20] Greater weight loss is considered as an indicator of discomfort and pain while eating. It was expected in the positive control group, which received 5-FU only. However, weight loss that occurred in the EC and JJ groups was not expected as there was an improvement in OM. Possible explanations for these findings may be attributed to the medication effects on taste buds and / or intestinal mucosa which is associated with difficulty in food absorption. Also, the handling of animals for topical application of the medicaments generated stresses that were reflected in food intake.[21]

In the present study, the histopathological findings in the positive control group were agreed with **Sonis et al.**, **2004**[22] who proposed a five-stage model of the pathogenesis of OM induced by chemotherapeutic agents, which include initiation, Primary damage response, signal amplification, ulceration, and healing stage. Consistent with our results, **Lee et al., 2016**[23] investigated that 5-FU/scratching group exhibited severe ulceration, fibrosis, and festering wounds. Also, **Ogihara et al., 2023**[17] reported that the area of OM rapidly expanded and gradually increased over 5–7 days then gradually decreased within 12 days after the injection.

In the current study, there was a significant improvement in the histopathological findings of the treated groups when compared to the positive control. Different criteria in the histological scoring system confirmed the improvement within different grades such as acute inflammatory cells infiltration, vascular hyperemia and areas of re-epithelialization. The wound repair is characterized by three basic phases. The inflammatory and hemostasis phase is characterized by sealing of the wound by neutrophils, macrophages and lymphocytes infiltration. The proliferative phase shows epithelial proliferation, angiogenesis, collagen synthesis and extracellular matrix formation. In the maturation and remodeling phase, it starts when enough collagen is present that strengthens the wound closure then myofibroblast undergoes apoptosis and matrix production ceases. [24]

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Koohi et al., 2015[25] discovered that 20% JJ gel was useful in treating 5-FU-induced OM in golden hamsters, which is in line with our findings. Histopathological scores were lower with less inflammatory infiltration along with the epithelization and wound healing. Our results were consistent with those of **Ayoub et al., 2021**[13] who applied joggle (JJ gel) to the ulcer site for 14 days. The JJ group exhibited significant healing of the ulcerative areas with congested blood vessels and protection against acetic acid. In comparison to the ulcer group, there was a greater degree of collagen deposition and a lower level of inflammatory markers. Moreover, **Wadkar and Pinjari, 2023**[26] stated that JJ oil may be helpful in treating wounds in therapeutic conditions. JJ oil is better characterized as liquid wax than oil because of its many bioactive properties; it is primarily made up of wax esters, sterols, and vitamins, with very small amounts of free fatty acids, alcohols, and hydrocarbons

In current study, EC extract was dissolved in ethanol according to **Mumtaz et al., 2022**[27] who dried EC leaves at 10°C, 30°C, 50°C, and 100°C before dissolving them in ethanol, methanol, and acetone to create a total of 12 extracts, and found that ethanol-based EC extract showed noticeably higher cell proliferation than extracts based on methanol and acetone. EC has been used as a traditional medicine for the treatment of different diseases. EC was selected for the current investigation as a potent cytokine suppressor that it treats inflammation in steroid-sensitive illnesses in the long run.[28]

According to **Tanideh et al., 2020,**[9] the current findings demonstrated that EC could shorten the epithelization period and encourage the regeneration of epithelium and connective tissue by stimulating the production of collagen and fibroblasts. Whereas the fibroblast is the most common cell that creates collagen that is considered the main structural protein in various connective tissues in animals.

In agreement with our findings, **Qiujian et al., 2021**[29] discovered that ellagic acid, a polyphenol compound derived from EC leaves, protects mice against acute gastric ulcers. Mice's stomach ulcers significantly improved after receiving different doses of EC. Also, **Mohammed et al., 2022**[30] found that topical application of 5% EC ointment reduced inflammatory cells and edema in the wound area when used to treat a third-degree skin burn in a rat model. Pro-inflammatory markers were significantly reduced in the injured tissues, while antioxidant levels were increased.

The positive control group in the current study exhibited strong cytoplasmic expression of IL-1β according to IHC analysis. **De Araújo et al., 2015** [31] assessed the impact of azilsartan (AZT), an angiotensin II receptor antagonist, on 5-FU induced OM in *Syrian* golden hamsters, which is in line with our findings. AZT oral treatment improved the state of OM by significantly lowering IL-1β levels.

The expression analysis of IL-1β revealed a significant change following the topical application of EC treatment. **Brizeno et al., 2016** [32] reported that leukocyte adhesion molecules might contribute to an increase in vascular permeability through direct endothelial damage. This information helped to explain our findings, which showed that the treated groups' connective tissue exhibited a markedly lower level of IL-1β expression.

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Vascular permeability and the degrees of inflammatory infiltration were found to be strongly correlated. **Bayramoglu et al., 2022** [33]examined the impact of taxifolin on rats with methotrexate-induced oxidative and inflammatory OM. They found that the methotrexate group had higher levels of IL-1β, conversely the taxifolin-treated group revealed lower expression of IL-1β.

In our study, TGF-β was a good indicator to evaluate the healing effects of JJ and EC. In the treated groups, their expression level was markedly elevated while it declined in the positive control group. In line with our results, **Bayer et al., 2017** [34] compared the effect of laser and ozone in treatment of OM and found that laser was more effective but the difference in TGF-β was little between the experimental groups.

Other growth factors like fibroblast growth factors and platelets derived growth factor were more effective in the healing assessment. **Park et al., 2018,** [35] which used EC extract to treat UV-induced damage on human dermal fibroblasts and discovered that when the healing effect started to increase, the expression of TGF-β and procollagen type Ⅰ was elevated. **Tütüncüoğlu et al., 2022**[36] showed how using an oral irrigator to brush teeth can improve peri-implant mucositis. TGF-β levels in the surrounding tissues increased and IL-1β expression decreased in tandem with the reduction in inflammation.

The clinical, histological and immunohistochemical data gathered from the present study after 5, 9 and 14 days of ulcer induction, revealed differences in the rate of epithelization and the healing process between the different groups. However, the eucalyptus treated group showed significant enhancement of ulcer re-epithelization with accelerated healing time.

CONCLUSIONS

Oral mucositis remains a dose-limiting complication of chemotherapy, and its management presents a continuing challenge. The hamster model using intraperitoneal administration of 5-fluorouracil (60 mg/kg) provides a reliable in vivo system for investigating oral mucositis. In this study, daily topical application of Eucalyptus gel produced a significantly greater healing effect than jojoba gel, without evidence of toxicity. The beneficial effect of Eucalyptus was associated with reduced interlukin1-beta expression and increased transforming growth factor beta expression, indicating its potential as a therapeutic agent for oral mucositis.

Limitations

1) Obtaining hamsters within the inclusion criteria having ideal weight from (70-80) gm took time.
2) Mortality of hamsters as a result to secondary infections and side effects of fluorouracil.
3) The markers that were used in this study (IL1-beta and TGF-beta) couldn't detect different pathways that affect the re- epithelization, stimulation of cell proliferation and collagen formation.

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Author's Contributions

SMM and MNE conceived the idea and designed the study. MAO wrote the main manuscript text. MNE prepared the figures. All listed authors participated in experiments and data collection. All authors have read and approved the final manuscript.

Data availability

The datasets generated and analyzed during the current study are available from the corresponding author on reasonable request.

References

1) Siegel RL, Miller KD, Wagle NS, Jemal A. Cancer statistics, 2023. CA: a cancer journal for clinicians. 2023 Jan 1;73(1).

2) Soliman R, Bolous N, Heneghan C, Oke J, Boylan AM, Eweida W, Abouelnaga S, Elhaddad A. An overview of childhood cancer care and outcomes in Egypt: a narrative review. E. cancer medical science. 2024 Feb 28; 18: 1676.

3) Anderson PM, Lalla RV. Glutamine for amelioration of radiation and chemotherapy associated mucositis during cancer therapy. Nutrients. 2020 Jun 4;12(6):1675.

4) Andriakopoulou CS, Yapijakis C, Koutelekos I, Perdikaris P. Prevention and treatment of oral mucositis in pediatric patients: Systematic review and meta-analysis of randomized controlled trials. in vivo. 2024 May 1;38(3):1016-29.

5) Sonis ST, Peterson RL, Edwards LJ, Lucey CA, Wang L, Mason L, Login G, Ymamkawa M, Moses G, Bouchard P, Hayes LL. Defining mechanisms of action of interleukin-11 on the progression of radiation-induced oral mucositis in hamsters. Oral oncology. 2000 Jul 1;36(4):373-81.

6) Safarzadeh S, Shirban F, Bagherniya M, Sathyapalan T, Sahebkar A. The effects of herbal medicines on cancer therapy-induced oral mucositis: A literature review. Phytotherapy Research. 2022 Jan;36(1):243-65.

7) Shiekh RA, Atwa AM, Elgindy AM, Mustafa AM, Senna MM, Alkabbani MA, Ibrahim KM. Therapeutic applications of eucalyptus essential oils. Inflammopharmacology. 2025 Jan;33(1):163-82.

8) Chakrabarty S, Jigdrel K, Mukherjee P, Paul T, Drakpa D, Gupta J. Bioactivities of Jojoba Oil Beyond Skincare. Journal of medicinal food. 2024 Jul 1;27(7):579-88.

9) Tanideh N, Badie A, Habibagahi R, Koohi-Hosseinabadi O, Haghnegahdar S, Andisheh-Tadbir A. Effect of topical 2% eucalyptus extract on 5-fu-induced oral mucositis in male golden hamsters. Brazilian Dental Journal. 2020 Jul 13;31(3):310-8.

10) Daniel WW. BIOSTATISTICS A Foundation for Analysis in the Health Sciences 9TH ED. Wiley; 2009.

11) Charan J, Biswas T. How to calculate sample size for different study designs in medical research? Indian journal of psychological medicine. 2013 Apr;35(2):121-6.

12) Sonis ST, Tracey C, Shklar G, Jenson J, Florine D. An animal model for mucositis induced by cancer chemotherapy. Oral surgery, oral medicine, oral pathology. 1990 Apr 1;69(4):437-43.

13) Ayoub N, Badr N, Al-Ghamdi SS, Alsanosi S, Alzahrani AR, Abdel-Naim AB, Nematallah KA, Swilam N. HPLC/MSn profiling and healing activity of a muco-adhesive formula of salvadora persica against acetic acid-induced oral ulcer in rats. Nutrients. 2021 Dec 22;14(1):28.

14) Lima V, Brito GA, Cunha FD, Rebouças CG, Falcão BA, Augusto RF, Souza ML, Leitão BT, Ribeiro RA. Effects of the tumour necrosis factor-α inhibitors pentoxifylline and thalidomide in short-term experimental oral mucositis in hamsters. European journal of oral sciences. 2005 Jun;113(3):210-7.

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15) Sallehuddin N, Nordin A, Bt Hj Idrus R, Fauzi MB. Nigella sativa and its active compound, thymoquinone, accelerate wound healing in an in vivo animal model: a comprehensive review. International journal of environmental research and public health. 2020 Jun;17(11):4160.

16) Jordan CT, Bradford EM, Cheek DC, Kudrimoti M, Miller CS, Smith MH, Hilt JZ, Dziubla TD. Radiation-induced oral mucositis hamster model using a linear accelerator enhances clinical relevance of preclinical studies for treatment strategy investigation. Animal Models and Experimental Medicine. 2021 Mar;4(1):47-53.

17) Ogihara T, Kagawa M, Yamanaka R, Imai S, Itohara K, Hira D, Nakagawa S, Yonezawa A, Ito M, Nakagawa T, Terada T. Preparation and pharmaceutical properties of Hangeshashinto oral ointment and its safety and efficacy in Syrian hamsters with 5-fluorouracil-induced oral mucositis. Journal of Natural Medicines. 2023 Jan;77(1):53-63.

18) Zhang S, Liu Y, Xiang D, Yang J, Liu D, Ren X, Zhang C. Assessment of dose-response relationship of 5-fluorouracil to murine intestinal injury. Biomedicine & Pharmacotherapy. 2018 Oct 1; 106:910-6.

19) Bertolini M, Sobue T, Thompson A, Dongari-Bagtzoglou A. Chemotherapy induces oral mucositis in mice without additional noxious stimuli. Translational Oncology. 2017 Aug 1;10(4):612-20.

20) Mitsuhashi H, Suemaru K, Li B, Cui R, Araki H. Evaluation of topical external medicine for 5-fluorouracil-induced oral mucositis in hamsters. European journal of pharmacology. 2006 Dec 3;551(1-3):152-5.

21) Pavesi VC, Lopez TC, Martins MA, Sant'Ana Filho M, Bussadori SK, Fernandes KP, Mesquita-Ferrari RA, Martins MD. Healing action of topical chamomile on 5-fluouracil induced oral mucositis in hamster. Supportive Care in Cancer. 2011 May;19(5):639-46.

22) Sonis ST. The pathobiology of mucositis. Nature Reviews Cancer. 2004 Apr 1;4(4):277-84.

23) Lee HR, Yoo N, Kim JH, Sohn KY, Kim HJ, Kim MH, Han MY, Yoon SY, Kim JW. The therapeutic effect of PLAG against oral mucositis in hamster and mouse model. Frontiers in Oncology. 2016 Oct 17; 6:209.

24) Politis C, Schoenaers J, Jacobs R, Agbaje JO. Wound healing problems in the mouth. Frontiers in physiology. 2016 Nov 2; 7:507.

25) Koohi-Hosseinabadi O, Andisheh-Tadbir A, Bahadori P, Sepehrimanesh M, Mardani M, Tanideh N. Comparison of the therapeutic effects of the dietary and topical forms of Zizyphus jujuba extract on oral mucositis induced by 5-fluorouracil: A golden hamster model. Journal of clinical and experimental dentistry. 2015 Apr 1;7(2): e304.

26) Wadkar HS, Pinjari RM. The Therapeutic Wound Healing activities of Various Medicinal Plants. International Journal of Rural Development, Environment and Health Research. 2023 Nov 3; 7(5), pp:71-90

27) Mumtaz R, Zubair M, Khan MA, Muzammil S, Siddique MH. Extracts of Eucalyptus alba Promote Diabetic Wound Healing by Inhibiting α-Glucosidase and Stimulating Cell Proliferation. Evidence-Based Complementary and Alternative Medicine. 2022;2022(1):4953105.

28) Dhakad AK, Pandey VV, Beg S, Rawat JM, Singh A. Biological, medicinal and toxicological significance of Eucalyptus leaf essential oil: a review. Journal of the Science of Food and Agriculture. 2018 Feb;98(3):833-48.

29) Qiujian Y, Zongcai F, Liping H, Jingwei H, Zhongliu Z, Fang L. Ellagic acid (EA), a tannin was isolated from Eucalyptus citriodora leaves and its anti-inflammatory activity. Medicinal Chemistry Research. 2021 Dec;30(12):2277-88.

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30) Mohammed HA, Mohammed SA, Khan O, Ali HM. Topical eucalyptol ointment accelerates wound healing and exerts antioxidant and anti-inflammatory effects in rats' skin burn model. Journal of Oleo Science. 2022; 71(12):1777-88.

31) de Araújo AA, Varela H, de Medeiros CA, de Castro Brito GA, de Lima KC, de Moura LM, de Araújo RF. Azilsartan reduced TNF-α and IL-1β levels, increased IL-10 levels and upregulated VEGF, FGF, KGF, and TGF-α in an oral mucositis model. PloS one. 2015 Feb 17; 10(2): e0116799.

32) Brizeno LA, Assreuy AM, Alves AP, Sousa FB, Silva PG, de Sousa SC, Lascane NA, Evangelista JS, Mota MR. Delayed healing of oral mucosa in a diabetic rat model: Implication of TNF-α, IL-1β and FGF-2. Life sciences. 2016 Jun 15; 155:36-47.

33) Bayramoglu Z, Mokhtare B, Mendil AS, Coban TA, Mammadov R, Bulut S, Suleyman Z, Suleyman H. Effect of taxifolin on methotrexate-induced oxidative and inflammatory oral mucositis in rats: biochemical and histopathological evaluation. Journal of Applied Oral Science. 2022 Sep 19; 30: e20220115.

34) Bayer S, Kazancioglu HO, Acar AH, Demirtas N, Kandas NO. Comparison of laser and ozone treatments on oral mucositis in an experimental model. Lasers in medical science. 2017 Apr; 32(3):673-7.

35) Park B, Hwang E, Seo SA, Cho JG, Yang JE, Yi TH. Eucalyptus globulus extract protects against UVB-induced photoaging by enhancing collagen synthesis via regulation of TGF-β/Smad signals and attenuation of AP-1. Archives of biochemistry and biophysics. 2018 Jan 1; 637:31-9.

36) Tütüncüoğlu S, Cetinkaya BO, Pamuk F, Avci B, Keles GC, Kurt-Bayrakdar S, Lütfioğlu M. Clinical and biochemical evaluation of oral irrigation in patients with peri-implant mucositis: a randomized clinical trial. Clinical oral investigations. 2022 Jan; 26(1):659-71.