Original Article

Topical Olive Leaf Extract Improves Healing of Oral Mucositis in Golden Hamsters

Najmeh Showraki [1], Maryam Mardani [2], Masoumeh Emamghoreishi [3], Azadeh Andishe-Tadbir [4], Alireza Aram [5], Peiman Mehriar [6], Mahmoud Omidi [7], Masood Sepehrimanesh [8], Omid Koohi-Hosseinabadi [9], Nader Tanideh [3, 5]

[1] Post Graduate Student, Dept. of Oral Medicine, School of Dentistry, Shiraz University of Medical sciences, Shiraz, Iran.
[2] Dept. of Oral Medicine, School of Dentistry, Shiraz University of Medical sciences, Shiraz, Iran.
[3] Dept. of Pharmacology, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran.
[4] Dept. of Oral and Maxillofacial Pathology, Prevention and Treatment of Oral and Dental Diseases Research Center, School of Dentistry, Shiraz University of Medical Sciences, Shiraz, Iran.
[5] Transgenic Technologies Research Center, Shiraz University of Medical Sciences, Shiraz, Iran.
[6] School of Dentistry, University of Southern California, Los Angeles, USA.
[7] Dept. of Pharmacology and Toxicology, School of Pharmacy, Shiraz University of Medical Sciences, Shiraz, Iran.
[8] Gastroenterohepatology Research Center, Shiraz University of Medical Sciences, Shiraz, Iran.
[9] Laboratory Animals Center, Shiraz University of Medical Sciences, Shiraz, Iran.

KEY WORDS
5- fluorouracil;
Anti-inflammatory;
Antioxidant;
Olive Leaf;
Oral Mucositis;

Received: *November 2015;*
Received in revised form: *January 2016;*
Accepted: *April 2016;*

ABSTRACT

Statement of the Problem: Oral mucositis (OM) is a common side effect of anti-cancer drugs and needs significant attention for its prevention.

Purpose: This study aimed to evaluate the healing effects of olive leaf extract on 5-fluorouracil-induced OM in golden hamster.

Materials and Method: OM was induced in 63 male golden hamsters by the combination of 5-fluorouracil injections (days 0, 5 and 10) and the abrasion of the cheek pouch (days 3 and 4). On day 12, hamsters were received topical olive leaf extract ointment, base of ointment, or no treatment (control) for 5 days. Histopathology evaluations, blood examinations, and tissue malondialdehyde level measurement were performed 1, 3 and 5 days after treatments.

Results: Histopathology score and tissue malondialdehyde level were significantly lower in olive leaf extract treated group in comparison with control and base groups (p= 0.000). Significant decreases in white blood cell, hemoglobin, hematocrit , and mean corpuscular volume and an increase in mean corpuscular hemoglobin concentration were observed in olive leaf extract treated group in comparison with control and base groups (p< 0.05).

Conclusion: Our findings demonstrated that daily application of olive leaf extract ointment had healing effect on 5-fluorouracil induced OM in hamsters. Moreover, the beneficial effect of olive leaf extract on OM might be due to its antioxidant and anti-inflammatory properties.

Corresponding Author: Tanideh N., Dept. of Pharmacology, Shiraz University of Medical Sciences, Shiraz, Iran. PO. Box: 71935-1311 Tel & Fax: +98-71-36474263 Email: tanidehn@gmail.com

Cite this article as: Showraki N., Mardani M., Emamghoreishi M., Andishe Tadbir A., Aram A., Mehriar P., Omidi M., Sepehrimanesh M., Koohi-Hosseinabadi O., Tanideh N. Topical Olive Leaf Extract Improves healing of Oral Mucositis in Golden Hamsters. *J Dent Shiraz Univ Med Sci.,* 2016 December; 17(4): 334-342.

Introduction

Mucositis is a common, serious mucosal inflammatory reaction and clinical problems caused by the cytotoxic effects of cancer chemotherapeutics. [1-3] This condition may involve the entire gastrointestinal tract causing mouth and throat pain, ulcerations and diarrhea. The exact pathogenesis of mucositis is unclear. However, reactive oxygen species generated by cytotoxic agents used in cancer chemotherapy may activate the processes of oxidative stress leading to mucosal atrophy. This in turn may make the oral mucosa more susceptible to trauma and ulcerations. [2] Approximately 40% of pa-

tients receiving standard cancer chemotherapy regimens developed mucositis. [1-3]

Depending on the severity of injury and discomfort, symptoms range from moderate burning sensation of the mouth to sever pain associated with ulcerations. This can be a limiting factor for cancer chemotherapy leading to poor treatment outcomes and increased financial burden. Due to these issues, controlling mucositis has become an important concern in care of cancer patients. [2, 4] There is no effective treatment available for oral mucositis (OM). Various agents, which are currently used for managing OM, are only palliative or infection controller including anti-microbial agents, magnesium hydroxide, hydroxypropyl cellulose, local anesthetics, corticosteroids, pentoxyfyline, supplementary amino acids, vitamins, growth factors, antioxidants, cryotherapy , and low-level laser therapy. [5-6] Therefore, developing new treatments for OM with greater efficacy and fewer side effects is highly warranted.

In recent years, ethnomedicine has appealed the attentions of many researchers as a potential source of discovering new remedies. Some herbal medicines such as chamomile, Aloe Vera, *Calendula officinalis*, *Carum carvi*, and *Zizyphus jujube* have been evaluated for treating OM. [7-12] Since oxidative stress processes and inflammatory reactions have been indicated in the pathophysiology of OM, herbs with strong antioxidant and/or anti-inflammatory properties such as olive can be a potential candidate for treating OM.

Different parts of olive tree such as fruits, leaves and seeds in the forms of extracts, herbal tea and powder are commonly used as remedies in European and Mediterranean countries and contain many potential bioactive compounds. [13] The main chemical constituents of olive which have pharmacological effects are polyphenol compounds particularly oleuropeine and hydroxytyrosol. Polyphenols are more abundant in leaf extracts than fruits of olive. In addition, olive leaves are inexpensive crude material with high beneficial properties. [14-15] Furthermore, earlier studies have indicated cardio protective, hypotensive, hypoglycemic and antioxidative activities of olive leaf extract. [16-19] On the other hand, mucosal protective effects of olive leaf extract have been described in some studies. [20-21] Therefore, the aim of this study was to assess the healing effect of topical application of olive leaf extract ointment on OM induced by the chemotherapeutic agent 5-fluouracil (5-FU) in golden hamsters based on clinical, histopathology, and serum biochemical evaluations.

Materials and Method

Animals

Sixty three male golden hamsters, weighing 110±10 g and eight weeks old, were purchased from Laboratory Animals Center of Shiraz University of Medical Sciences, Shiraz, Iran. Hamsters were randomly divided into three equal groups and were maintained under standard conditions (20±2 ^{o}C, 50% relative humidity and 12/12 hr light/dark cycles) with access to a standard diet and water *ad libitum*. Every procedure was designed to minimize discomfort to the animals and to use the fewest animals needed for statistical analysis. Also, all experimental protocols were approved by the Animal Care and Use Committee at the Shiraz University of Medical Sciences and were performed in accordance with the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals.

5-FU induced OM

OM was induced according to Sonis *et al.* procedure. [22] Briefly, hamsters were injected intraperitoneally 5-FU (60 mg/kg, IP) on days 0, 5 and 10. Three superficial vertical linear scratches were made on cheek pouch mucosa on days 3 and 4 with a tip of an 18-gauge sterile needle. On 12th day, the peak of inflammation and redness of mucosa, hamsters received daily topical application of olive leaf ointment (Zaitonex, containing 0.45% oleuropeine) or base of ointment for 5 days. One group received no treatment assigned as the control group. The cheek pouch mucosa of both sides of the affected area was completely covered once a day in the morning. Animals were not allowed to eat or drink for one hour after the application of treatments in order to make sure that the coverage was not swallowed.

Histopathologic studies

Seven hamsters from each group were randomly sacrificed on days 13, 15, and 17. At the time of sacrifice, they were anesthetized with an overdose of pentobarbital. Excisional biopsies of the cheek pouch mucosa of both sides were obtained. The gross macroscopic changes were evaluated by an expert veterinarian and related lesions were reported. Tissues of right side cheek pouch were placed in 10 % buffered formalin, prepared

Figure 1: Evaluation of the healing effect of olive leaf extract ointment by clinical scoring of oral mucositis (arrows) induced by 5-fluorouracil in hamsters on day 15. A, Score 0; B, score 1; C, score 2; D, score 3 based on related histopathological scores

for histopathologic examination and stained with hematoxylin and eosin. The specimens were histopathologically scored based on the study of Lima *et al.* [6] as follows:

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

Blood analysis

Heart blood was taken by cardiocentesis and used for complete blood count (CBC) test including white blood cell (WBC), red blood cell (RBC), and platelet counts plus hematocrite (HCT), hemoglobin, mean of corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC).

Measurement of tissue malonedialdehyde (MDA) level

Tissue from the left side was kept in a cryotube under liquid nitrogen for subsequent measurement of MDA level as an index of lipid peroxidation. Briefly, 200 mg of tissue was homogenized with phosphate buffer solution in the ratio of 1w: 5v. The homogenized tissue (400 µL) was then mixed with 800 µL of trichloroacetic acid (TBA) and centrifuged (3000 rpm, 4° C, 30 min). Then 600 µL of the supernatant was mixed with 150 µL of 1% TBA and incubated in water bath for 15 min. After cooling down, 6 ml of *n*-butanol was added and the mixture was centrifuged (3000 rpm, 4 °C, 10 min). Subsequently the absorption of butane phase was measured by spectrophotometer at 532 nm. [14] Tetraetoxy propane was used as control. MDA tissue concentration was calculated with following equation:

$$MDA\ concentration\left(\frac{nmol}{ml}\right) = [(A_T - A_{NC}) + 0.0606]/0.0537$$

In which, A_T is absorbance of the test sample and A_{NC} is absorbance of the negative control at 532 nm.

Statistical analysis

The data were presented as mean and standard error of mean. Normal distributions of the data were assessed by Kolmogorov-Smirnov test. Two-way ANOVA and *post hoc* test of Tukey were used for comparison of mean differences in tissue MDA level and blood factors between three experimental groups on different days (days 13, 15, and 17) . Mann-Whitney U test with Bonferroni correction was used for comparing histopathology scores between different groups on different days. SPSS 16.5 software was used to analyze the data and $p< 0.05$ was considered statistically significant. Illustrations were made by GraphPad Prism 5.

Results

Histopathology analysis

The macroscopic scoring images of OM in the hamster's cheek pouches on day 15 are shown in Figure 1. Score 1 of OM (A) in group that received topical OLE ointment and severe OM (score 3, D) in control group are demonstrated. Figure 2 shows the histopathology scores of lesion in cheek pouches of 3experimental groups. Epithelialization and wound healing were detected in the OLE ointment group, whereas extensive infiltration of inflammatory cells, hemorrhage, and ulcer were observed in the control and gel base groups (Figure 2).

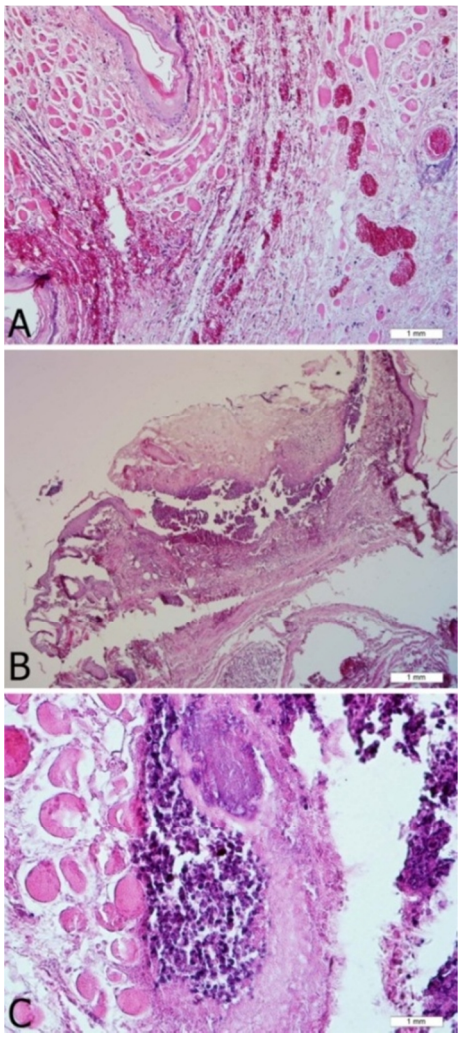

Figure 2: Evaluation of the healing effect of olive leaf extract (OLE) ointment by histopathology scoring of the oral mucositis induced by 5-fluorouracil on day 15. **A:** Score 1 from the group treated with OLE ointment showing discrete vascular ingurgitation, discrete inflammatory infiltration with mononuclear prevalence (H&E ×200). **B:** Score 3from the control group showing inflammatory infiltration with neutrophil prevalence, presence of hemorrhagic areas, edema and eventual ulceration and abscess formation (H&E ×40). **C:** Score3, higher magnification of previous picture show abscess formation (H&E ×400).

There were no differences in histopathologic scores between the gel base and control groups on different days (p= 0.78) (Figure 3). During the whole treatment period, histopathology scores were lower in the OLE ointment group than in the gel base and control groups (p= 0.037) Also, the histopathologic scores decreased during the experimental period in OLE ointment treated group (Figure 3).

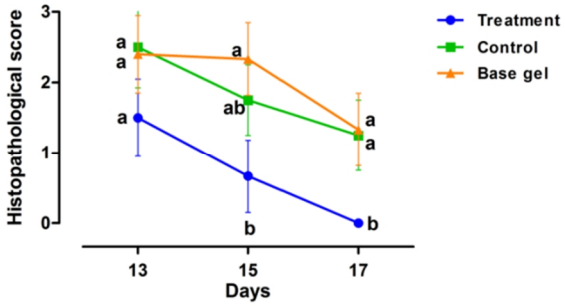

Figure 3: Changes in histopathology scores of oral mucositis induced by 5-fluorouracil in hamsters for evaluation of the therapeutic effect of olive leaf extract ointment. During the whole treatment period, histopathology scores were lower in the treatment group than in the gel base and control groups. Different superscript letters in each day indicate significant difference (p< 0.05).

Blood factors analyses

WBC and RBC counts of studied groups were shown in Figure 4A and B. On day 17, WBC count was higher in the base gel group in comparison to control and OLE ointment groups (p= 0.021). Also, WBC count was higher in control group in comparison to the OLE ointment group (p= 0.011). There were no significant differences in RBC counts between the three studied groups during the study period (p= 0.68). Figure 4C and 4D shows hemoglobin and HCT levels for the three studied groups. On day 13, hemoglobin and hematocrit levels of the OLE ointment group was less than those of other two groups, but these levels increased in OLE ointment group (p= 0.012) and decreased in base gel and control groups during the study period (p= 0.026).

Figure 4E and F show the MCV and MCH changes in three groups during the study. On day 15, MCV of the control group was more than that of the treatment and base gel groups (p= 0.91). On day 17, MCV of the control and base gel groups was higher than that of treatment group (p= 0.78). Considering the MCH level, hamsters in base gel group had higher MCH than that of control group on day 13 (p= 0.033). There were no significant differences on the other days between other gro-

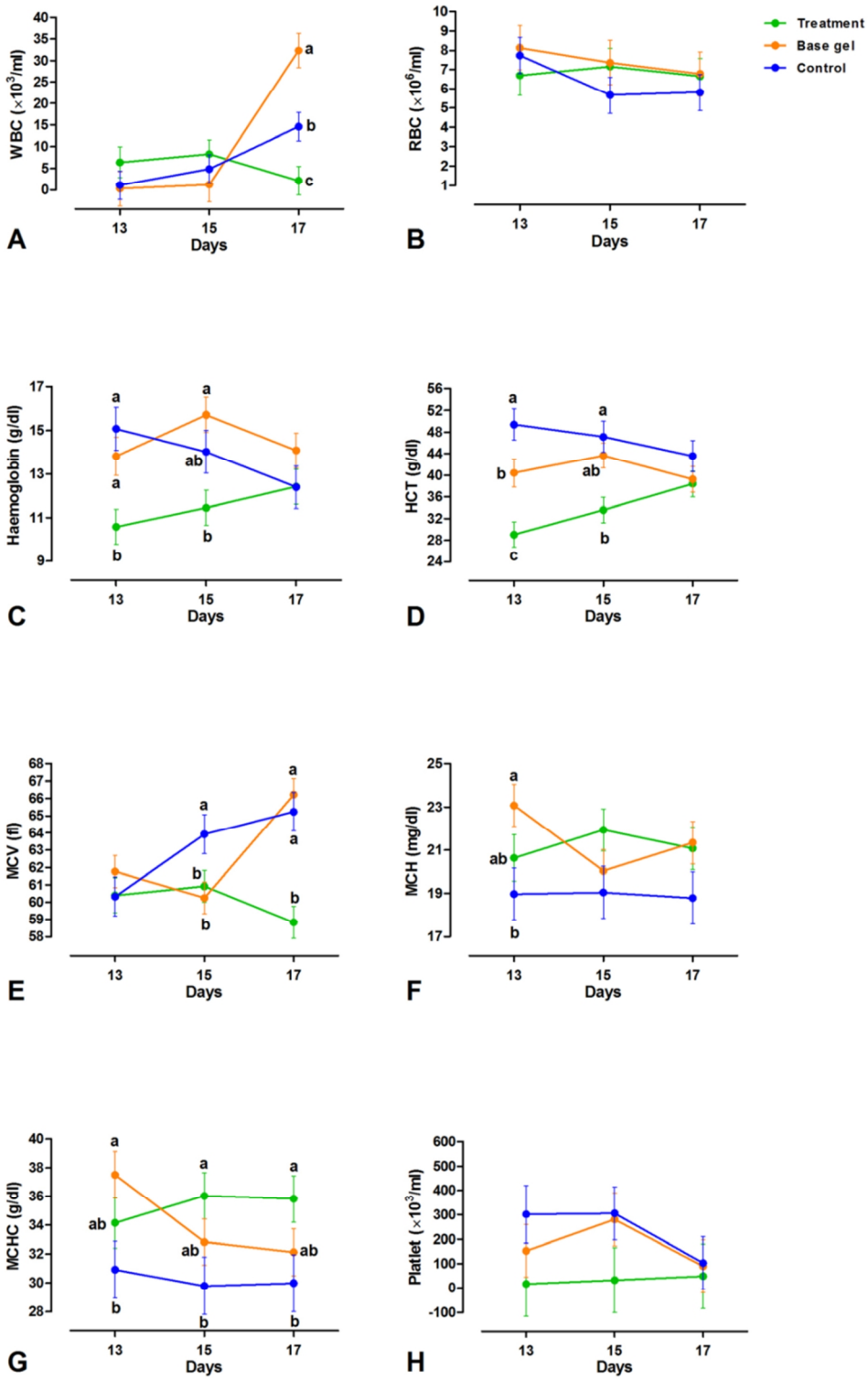

Figure 4: Effects of olive leaf extract ointment on blood parameter level changes in oral mucositis induced by 5-fluorouracil in hamsters. Data presented as mean±SEM (n=21/group) for (A) White blood cell count; (B) red blood cell count; (C) haemoglobin; (D) haematocrit; (E) mean corpuscular volume; (F) mean corpuscular haemoglobin; (G) mean corpuscular haemoglobin concentration; (H) platelet count. Different superscript letters in each day indicate significant difference ($p< 0.05$).

ups ($p= 0.82$). MCHC differences between three groups were shown in Figure 4G. On day 13, MCHC was higher in base gel group than in control group ($p= 0.017$). On days 15 and 17, MCHC was lower in control than in gel base group ($p= 0.013$). Platelet counts for studied groups were shown in Figure 4H. There was no significant differences in platelet counts between three studied groups during the study period ($p= 0.64$).

Tissue MDA level analysis

Tissue MDA levels of studied groups on different days

were shown in Figure 5.

There was no difference in MDA concentrations between the gel base and control groups on different days (p= 0.56). MDA concentration was higher in the control and gel base groups than in the treatment group on all days (p= 0.000). Tissue MDA concentration rapidly decreased on day 15 in the treatment group (p= 0.031).

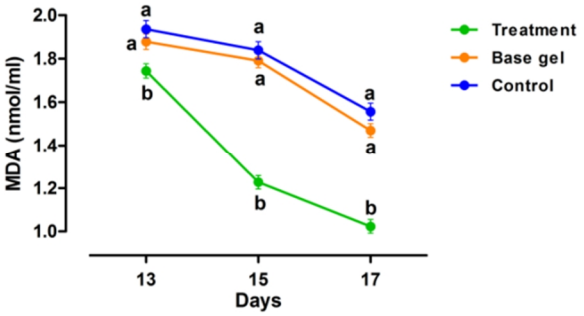

Figure 5: Tissue malondialdehyde (MDA) concentration changes in oral mucositis induced by 5-fluorouracil in hamsters for evaluation of the antioxidant effect of olive leaf extract ointment (mean and SEM). MDA concentration was higher in the control and gel base groups than in the treatment group on all days. Tissue MDA concentration rapidly decreased on day 15 in the treatment group. Different superscript letters in each day indicate significant difference (p< 0.05).

Discussion

This study evaluated and compared the healing effects of OLE on the 5-FU induced OM in golden hamsters. Our findings indicated that the daily application of OLE ointment was effective in the treatment of OM. These beneficial effects may be through its antioxidant and anti-inflammatory activities. In the present study, OLE decreased histopathologic scores of OM. The reduction in histopathologic scores was started one day after beginning the OLE treatment and progressively continued until the end of treatment. This suggests that OLE might have decreased inflammatory responses that peaked 12 days following 5-FU-induced mucositis. In addition, it might reflect the healing properties of OLE as reported previously. [23-25] Increased collagen concentration and fibers' stabilization have been attributed to the wound healing effect of OLE. [25] Furthermore, olive extract have antimicrobial activity [26-28] which can be other effective mechanism by which OLE may exert its wound healing effect. OM ulcers are commonly infected with bacteria and fungi delaying the healing of ulcers with the damaging effects of their byproducts. The antimicrobial properties of OLE by reducing the impact of microorganisms may have beneficial effect for OM ulcers.

Blood evaluations may be beneficial in assessing the level of immunosuppression and inflammation and can be attributed to the overall healing process of mucositis. A high RBC count was observed on day 13 in all studied groups. This can be attributed to high inflammation and less eating and drinking of animals leading to dehydration and haemoconcentration. A decline to a normal RBC count during treatment period was observed in OLE-treated group. In addition, hematocrit which is dependent on the RBC count was higher during treatment period in control than OLE group. These findings indicate superior healing process and less inflammation in OLE than control group. Bone marrow suppression caused by chemotherapeutic agents and the inflammatory disease itself can lead to anemia. Anemia will cause a drop in hemoglobin followed by increased water flow into cells leading to a decrease in MCHC. In the present study, MCHC was lower in the control than OLE group which is suggestive of more inflammation in control group. The influx of water into RBCs and increased erythropoietin level due to bone marrow suppression causes macrocyte production increasing the MCV level. A declined MCV level in OLE group and an increased MCV level in the control group were observed during treatment period in this study. This indicates a decrease in inflammatory response by OLE. During inflammation the platelet count increases and declines after inflammation subsides. However, the platelet count was low during the whole treatment period in the OLE treatment group. This may be due to an inhibitory effect of OLE on platelet as reported in numerous studies. [19]

In the current study, a reduction in tissue MDA levels was observed in the group treated with OLE ointment. As MDA level is an index of lipid peroxidation, the reduction in MDA level seen two days after treatment in OLE group suggests that OLE prevents oxidative processes in oral tissue leading to mucosal protection. This is in agreement with previous studies shown the mucosal protective effect of OLE. [20, 29-30] The MDA reduction in oral mucosa in OLE treated group reflects antioxidant activity of OLE. The antioxidant property of OLE has been shown in previous studies and can be related to its various phytochemicals,

mainly oleuropein or flavonoids. [26, 31-32] This study demonstrated that OLE is effective in treating mucositis in hamster. This finding is in agreement with a study that reported the mouthwash of olive leaf extract has been useful in decreasing the incidence and severity of OM caused by 5-FU in rats. However, in the previous study blood examinations and tissue MDA measurement were not assessed to compare our results with.

The mechanism by which OLE exerts its therapeutic effect in OM is not clear. However, several mechanisms can be proposed for OLE healing effect in OM. Pro-inflammatory cytokines such as IL1β and TNFα have shown to play an important role in the pathophysiology of OM in developing the lesions and mediating injury. The anti-inflammatory properties of OLE have been demonstrated to be due to its ability to reduce the expression of pro-inflammatory cytokines. [29] We did not evaluate the effect of OLE on cytokines; however, the indirect measures of inflammation obtained from blood examinations showed that OLE has anti-inflammatory property in the current study. Anti-inflammatory effect of OLE may contribute to its therapeutic effect in OM. Future studies should measure IL-1 and TNF-α following OLE treatment to address this possibility. In addition, an antioxidant activity of OLE may contribute to its beneficial effect in OM. The MDA reduction in oral mucosa observed in OLE treated groups supports this possibility. Furthermore, olive leaves contain variety of compounds with antibacterial, antifungal, antiviral, anti-inflammatory and anti-oxidant activities including verbascoside, luteolin-7-glucoside, apigenin-7-glucoside, diosmetin-7-glucoside, luteolin, diosmetin, vanillin, vanillic acid, caffeic acid, tyrosol and hydroxytyrosol. [26, 29-32] These components may have synergic effects on the wound healing of mucositis ulcers in addition to its main component oleouropein.

Conclusion

In conclusion, this study demonstrated that daily application of OLE ointment have a healing effect on chemo-induced OM in hamsters possibly by reducing mucosal inflammation and oxidative stress as suggested by the histopathology and tissue MDA findings and systemic evaluations. Despite of some side effects on platelet count and MCV, OLE is a suitable choice for the treatment of OM based on the anti-inflammatory, antioxidant, and wound healing properties.

Acknowledgements

This article was extracted from the thesis of Dr. Najmeh Showraki, an oral medicine resident of Shiraz University of Medical Sciences. This work was financially supported by a grant (92-01-03-7082) from Vice Chancellor of Research Affair of Shiraz University of Medical Sciences.

Conflict of Interest

No potential conflict of interest relevant to this article was reported.

References

[1] Karthaus M, Rosenthal C, Ganser A. Prophylaxis and treatment of chemo- and radiotherapy-induced oralmucositis- are there new strategies? Bone Marrow Transplant. 1999; 24: 1095-108.

[2] Naidu MU, Ramana GV, Rani PU, Mohan IK, Suman A, Roy P. Chemotherapy-induced and/or radiation therapy- induced oral mucositis--complicating the treatment of cancer. Neoplasia. 2004; 6: 423-431.

[3] Sonis ST. Oral mucositis. Anticancer Drugs. 2011; 22: 607-612.

[4] Napenas JJ, Shetty KV, Streckfus CF. Oral mucositis: review of pathogenesis, diagnosis, prevention, and management. Gen Dent. 2007; 55: 335-544.

[5] Leitão RF, Ribeiro RA, Lira AM, Silva LR, Bellaguarda EA, Macedo FD, et al. Glutamine and alanylglutamine accelerate the recovery from 5-fluorouracil-inducedexperimental oral mucositis in hamster. Cancer Chemother Pharmacol. 2008; 61: 215-222.

[6] Lima V, Brito GA, Cunha FQ, Rebouças CG, Falcão BA, Augusto RF, et al. Effects of the tumour necrosis factor-alpha inhibitors pentoxifylline and thalidomide in short-term experimental oral mucositis in hamsters. Eur J Oral Sci. 2005; 113: 210-217.

[7] Davarmanesh M, Miri R, Haghnegahdar S, Tadbir AA, Tanideh N, Saghiri MA, et al. Protective effect of bilberry extract as a pretreatment on induced oral mucositis in hamsters. Oral Surg Oral Med Oral Pathol Oral Radiol. 2013; 116: 702-708.

[8] Pavesi VC, Lopez TC, Martins MA, Sant'Ana Filho M, Bussadori SK, Fernandes KP, et al. Healing action of topical chamomile on 5-fluoracil induced oral mucositis in hamster. Support Care Cancer. 2011; 19: 639-646.

[9] Tanideh N, Namazi F, Andisheh Tadbir A, Ebrahimi H, Koohi-Hosseinabadi O. Comparative assessment of the therapeutic effects of the topical and systemicforms of Hypericum perforatum extract on induced oral mucositis in golden hamsters. Int J Oral Maxillofac Surg. 2014; 43: 1286-1292.

[10] Tanideh N, Tavakoli P, Saghiri MA, Garcia-Godoy F, Amanat D, Tadbir AA, et al. Healing acceleration in hamsters of oral mucositis induced by 5-fluorouracil with topical Calendula officinalis. Oral Surg Oral Med Oral Pathol Oral Radiol. 2013; 115: 332-338.

[11] 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 Zizyphusjujuba extract on oral mucositis induced by 5-fluorouracil: A golden hamster model. J Clin Exp Dent. 2015; 7: e304-309.

[12] Mardani M, Afra SM, Tanideh N, Tadbir AA, Modarresi F, Koohi-Hosseinabadi O, et al. Hydroalcoholic extract of Carum carvi L. in oral mucositis: a clinical trial in male golden hamsters. Oral Dis. 2016; 22: 39-45.

[13] Ahmed KM, Talabani N, Altaei T. Olive Leaf Extract as a New Topical Management for Oral Mucositis Following Chemotherapy: A Microbiological Examination, Experimental Animal Study and Clinical Trial. Pharm Anal Acta. 2013, 4: 269.

[14] Tanideh N, Nematollahi SL, Hosseini SV, Hosseinzadeh M, Mehrabani D, Safarpour A, Sepehrimanesh M, Koohi-Hosseinabadi O, Najibi A. The healing effect of Hypericum perforatum extract on acetic acid-induced ulcerative colitis in rat. Ann Colorectal Res. 2014; 2: e25188.

[15] El SN, Karakaya S. Olive tree (Olea europaea) leaves: potential beneficial effects on human health. Nutr Rev. 2009; 67: 632-638.

[16] Jemai H, Fki I, Bouaziz M, Bouallagui Z, El Feki A, Isoda H, et al. Lipid-lowering and antioxidant effects of hydroxytyrosol and its triacetylatedderivative recovered from olive tree leaves in cholesterol-fed rats. J Agric Food Chem. 2008; 56: 2630-2636.

[17] Mijatovic SA, Timotijevic GS, Miljkovic DM, Radovic JM, Maksimovic-Ivanic DD, Dekanski DP, et al. Multiple antimelanoma potential of dry olive leaf extract. Int J Cancer. 2011; 128: 1955-1965.

[18] Sabry OMM. Beneficial Health Effects of Olive Leaves Extracts. J Natural Scien Res. 2014; 4: 1-9.

[19] Singh I, Mok M, Christensen AM, Turner AH, Hawley JA. The effects of polyphenols in olive leaves on platelet function. Nutr Metab Cardiovasc Dis. 2008; 18: 127-132.

[20] Dekanski D, Janićijević-Hudomal S, Tadić V, Marković G, Arsić I, Mitrović D. M. Phytochemical analysis and gastroprotective activity of an olive leaf extract. Journal of the Serbian Chemical Society. 2009; 74: 367–377.

[21] Dekanski D., Ristić S., Mitrović D. M. Antioxidant effect of dry olive (Olea europaea L.) leaf extract on ethanol-induced gastric lesions in rats. Mediterranean Journal of Nutrition and Metabolism. 2009; 2: 205–211.

[22] Sonis ST, Tracey C, Shklar G, Jenson J, Florine D. An animal model for mucositis induced by cancer chemotherapy. Oral Surg Oral Med Oral Pathol. 1990; 69: 437-443.

[23] Atai Z, Ansari M, Torabi N. Efficacy of Olive Leaf Extract in the Treatment of Minor Oral Aphthous Ulcers. Am J Infect Diseas. 2007; 3: 24-26.

[24] Gong D, Geng C, Jiang L, Wang L, Yoshimuram H, Zhong L. Olive leaf extract facilitates healing of experimental cartilaginous injuries in rabbits. J Med Food. 2011; 14: 268-275.

[25] Koca U, Süntar I, Akkol EK, Yilmazer D, Alper M. Wound repair potential of Olea europaea L. leaf extracts revealed by in vivo experimental models and comparative evaluation of the extracts' antioxidant activity. J Med Food. 2011; 14: 140-146.

[26] Lee OH, Lee BY. Antioxidant and antimicrobial activities of individual and combinedphenolics in Olea europaea leaf extract. Bioresour Technol. 2010; 101: 3751-3754.

[27] Markin D, Duek L, Berdicevsky I. In vitro antimicrobial activity of olive leaves. Mycoses. 2003; 46: 132-136.

[28] Sudjana AN, D'Orazio C, Ryan V, Rasool N, Ng J, Islam N, et al. Antimicrobial activity of commercial Olea europaea (olive) leaf extract. Int J Antimicrob Agents. 2009; 33: 461-463.

[29] Ahmed KM. The effect of olive leaf extract in decreasing the expression of two pro-inflammatory cytokines in patients receiving chemotherapy for cancer. A randomized clinical trial. Saudi Dent J. 2013; 25: 141-147.

[30] Alirezaei M, Dezfoulian O, Neamati S, Rashidipour M, Tanideh N, Kheradmand A. Oleuropein prevents ethanol- induced gastric ulcers via elevation ofantioxidant enzyme activities in rats. J Physiol Biochem. 2012; 68:

583-592.

[31] Fares R, Bazzi S, Baydoun SE, Abdel-Massih RM. The antioxidant and anti-proliferative activity of the Lebanese Olea europaea extract. Plant Foods Hum Nutr. 2011; 66: 58-63.

[32] Silva S, Gomes L, Leitão F, Coelho AV, Boas LV. Phenolic compounds and antioxidant activity of Olea europaea L. fruits and leaves. Food Science and Technology International. 2006; 12: 385–396.