**DOI:** 10.5152/jarem.2018.2008

**Manuscript Type:** Original Article

**Turkish Title:** Glutaminin Travmatik Oral Mukozal Lezyonların İyileşmesi Üzerine Etkileri; Deneysel Çalışma
**Turkish Running Head:** Oral Mukozal Lezyonlarda Glutaminin Etkileri

**Title:** Effects of Glutamine on Healing of Traumatic Oral Mucosal Lesions; an Experimental Study
**Running Head:** Effects of Glutamine on Oral Mucosal Lesions

**Authors:** Sedat Akbaş[1], Enes Karabulut[2], Ayşe Pervin Sutas Bozkurt[3], Övgü Aydın[4], Dilek Ergün Düzgün [5], Gürcan Güngör[3]
**Institutions:**
[1]Department of Anesthesiology and Reanimation, Inonu University School of Medicine, Malatya, Turkey
[2]Department of Clinical Trials, Turkish Medicines and Medical Devices Agency, Ankara, Turkey
[3]Department of Anesthesiology and Reanimation, İstanbul University-Cerrahpaşa, Cerrahpaşa School of Medicine, İstanbul, Turkey
[4]Department of Pathology, İstanbul University-Cerrahpaşa, Cerrahpaşa School of Medicine, İstanbul, Turkey
[5]Department of Biophyshics, İstanbul University-Cerrahpaşa, Cerrahpaşa School of Medicine, İstanbul, Turkey

**Address for Correspondence:** Sedat Akbaş [drsedatakbas@gmail.com]

**Cite this article as:** Akbaş S, Karabulut E, Sutas Bozkurt AP, Aydın Ö, Ergün Düzgün D, Güngör G. Effects of Glutamine on Healing of Traumatic Oral Mucosal Lesions; an Experimental Study. JAREM 2018; DOI: 10.5152/jarem.2018.2008

**Received:** 27.02.2018

**Accepted:** 09.07.2018

**ÖZ**

**Amaç:** Glutaminin (GLN); asit–baz dengesi, protein dönüşümü ve amonyak metabolizmasının düzenlenmesinde, katabolik durumlarda ve immun sistemin güçlenmesinde önemli görevleri bulunmaktadır. Klinik ve ekonomik olarak birçok problem yol açan oral mukozal lezyonların önlenmesi, bakımı ve tedavisi önemlidir. Bu deneysel hayvan modelinde; topikal ve sistemik yolla (enteral, parenteral) uygulanan GLN'nin travmatik oral mukozal lezyonlarda pozitif ve farklı etkilerinin ortaya çıkarılması amaçlandı.

**Yöntemler:** Bu çalışmaya 21 Wistar Albino sıçan dâhil edildi ve 4 gruba ayrıldı. Tüm sıçanlara intraperitoneal anestezi uygulamasından sonra ağız içerisinde travmatik oral mukozal lezyon oluşturuldu. Kontrol grubunda; travmatik oral mukozal lezyon oluşturuldu ve tedavi uygulanmadı. Çalışma gruplarında; GLN tedavisi parenteral, enteral ve topikal yollarla uygulandı. Travmatik oral mukozal lezyonun iyileşmesi makroskopik olarak gözlendi. Sıçanlar sakrifiye edildikten sonra biyopsiler alınarak histopatolojik ve biyokimyasal olarak değerlendirildi.

**Bulgular:** Biyopsilerin histopatolojik incelenmesinde; akut inflamasyon açısından kontrol grubu ile parenteral/topikal gruplar arasında, epitelyal proliferasyon açısından kontrol ve parenteral gruplar arasında, fibrosis açısından kontrol ve topikal gruplar arasında istatistiksel olarak anlamlı farklılık saptandı. Biyokimyasal analizlerde; yalnızca malondialdehit düzeyleri açısından kontrol ve enteral gruplar arasında anlamlı bir farklılık görüldü ($p<0,02$).

**Sonuç:** GLN takviyesinin travmatik oral mukozal lezyonların tedavisinde olumlu etkisi olduğu görüldü. GLN'nin parenteral yol ile verilmesi topikal ve enteral yol ile verilmesine kıyasla daha iyi sonuç verdiği görüldü. Travmatik oral mukozal lezyonlarda topikal veya enteral yol ile GLN desteğinin iyi bir alternatif olduğu düşünüldü.

**Anahtar kelimeler:** Deneysel çalışma, glutamin, oral mukozit, travmatik yara





This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as: Akbaş S, Karabulut E, Sutas Bozkurt AP, Aydın Ö, Ergün Düzgün D, Güngör G. Effects of Glutamine on Healing of Traumatic Oral Mucosal Lesions; an Experimental Study. JAREM 2018; DOI: 10.5152/jarem.2018.2008

**ABSTRACT**

**Objective:** Glutamine (GLN) has an important function in regulation of acid–base balance, protein turnover, ammonia metabolism, catabolic situations and enhancing immune system. Prevention, treatment and care of oral mucosa lesions are very important for causing too many kinds of clinical and economic problem. In this experimental study, we aimed to reveal positive and different effects of GLN on traumatic oral mucosal lesions by using topical or systemic (enteral, parenteral) route.

**Methods:** Twenty-one Wistar Albino rats were included to this experimental study and divided into 4 groups. Traumatic oral mucosal lesions were created to all rats after intraperitoneal administration of anesthesia. In control group; lesions were performed and no treatment was applied. In study groups; GLN treatment was applied by parenteral, enteral and topical routes. Healing of the lesions were observed macroscopically by high resolution photographs. Rats were sacrificed and biopsies were taken for histopathological and biochemical evaluations.

**Results:** Histopathological evaluations of the biopsies; there was a significant difference between control and parenteral/topical groups for acute inflammation, control and parenteral groups for epithelial proliferation, control and topical groups for fibrosis. For biochemical evaluations; only malonildialdehyde levels have a significant difference between control and enteral groups ($p<0.02$).

**Conclusion:** We observed a positive effect of GLN supplementation for the treatment of traumatic oral mucosal lesions. GLN administration by parenteral route had better results compared to the topical and enteral route. We therefore concluded that GLN supplements administered by topical or enteral route were a better alternative on traumatic oral mucosal lesions.

**Keywords:** Experimental study, glutamine, oral mucositis, traumatic wounds





This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as: Akbaş S, Karabulut E, Sutas Bozkurt AP, Aydın Ö, Ergün Düzgün D, Güngör G. Effects of Glutamine on Healing of Traumatic Oral Mucosal Lesions; an Experimental Study. JAREM 2018; DOI: 10.5152/jarem.2018.2008

## INTRODUCTION

Oral lesions are defined by inflammation of oral mucosa, compromised by damage of epithelial tissue with impairment of saliva barrier, damage of epithelial cells and frequent ulcerations (1). Oral mucosa cells with secreted substances constitute the first line of defense. The lesions cause to opportunistic oral infections by inflicting mucosa damage and fatal complications like bacteremia, fungemia and sepsis (1). The lesions, in which pain is required for opioid use, are the main cause of difficulties in chewing, swallowing and speaking, which then contribute dehydration, malnutrition, anorexia and cachexia (1).

The major risk factors for oral mucositis are age, sex, genetic factors, lack of oral hygiene, xerostomia, nutrition status, acute or chronic dental diseases, infections, malignancies, smoking, alcohol, the presence and severity of instrumentation inside the mouth, chemotherapy and radiotherapy and treatment-related causes (2). The treatment of oral mucositis includes the options of nutritional support, pain management, oral hygiene and palliation of xerostomia. Requirement for total parenteral nutrition and treatment of infections, long duration of febrile neutropenia, using higher doses of opioid analgesics for oral mucositis cause prolonged period of hospitalization and by that increases cost of treatment.

Amino acids and vitamins are used in order to support the immune system (3). Glutamine (GLN) is an amino acid that occupies an important corner for the synthesis of nucleotides in rapidly dividing cells (4). GLN, which has crucial role in wound healing, is not present in sufficient quantities of enteral nutrition and total parenteral nutrition because of solubility and stability problems even though it is the most abundant amino acid in the blood (4). Nutritional support with GLN has a significant role in



This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as: Akbaş S, Karabulut E, Sutas Bozkurt AP, Aydın Ö, Ergün Düzgün D, Güngör G. Effects of Glutamine on Healing of Traumatic Oral Mucosal Lesions; an Experimental Study. JAREM 2018; DOI: 10.5152/jarem.2018.2008

terms of shortening of hospitalization period, reducing the expenses of the treatment and providing quality life standards to patient (1).

We observed according to our clinical experience that patients with oral mucositis which were developed by trauma or diseases were healed faster by providing nutritional support with GLN. Therefore we aim to reveal, in experimental animal model, positive and different effects of GLN on traumatic oral mucosal lesions by topical or systemic (enteral, parenteral) route.

## METHODS

**Study Protocol:** This experimental study was approved by the Animal Experiments Local Ethics Committee and conducted at the Animal Experiments Laboratory. This study was conducted in accordance with the guideline for the use and care of laboratory animals.

**Animal Preparation:** Study was performed on 21 Wistar Albino type male rats weights ranging between 250-350 gr. Care for experimental animals was performed in 4 rat cages *(Euro type 3, polycarbonate stainless steel cage, 150 mm height, 375x215 mm bottom edges, 425x265 mm roof edge)* that provided rat chow and water without any restriction in the diet, at 21-22˚ C room temperature, period of 12-hour daylight/12-hour night, 3 or 6 rats to be in the same cage, before and during study.

**Experimental Protocol:** All experiments were performed under anesthesia and analgesia with 75 mg/kg ketamine hydrochloride (Ketalar®) and 5 mg/kg xylazine (Rompun®). Weight of animals, drugs and dosages are presented in Table 1. We have reached to left buccal mucosa inside the mouth with small retractor using a hook to upper and lower jaw teeth without damaging the structure of the



This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as: Akbaş S, Karabulut E, Sutas Bozkurt AP, Aydın Ö, Ergün Düzgün D, Güngör G. Effects of Glutamine on Healing of Traumatic Oral Mucosal Lesions; an Experimental Study. JAREM 2018; DOI: 10.5152/jarem.2018.2008

mouth and jaw and exposing any additional trauma. In left buccal mucosa of oral cavity, mucosal lesion was occurred by using black needle back plus (+) shape (12 mm×12 mm). The lesions created in all experiments were photographed from 3-5 cm distance to demonstrate that a standard damage form (Figure 1). Anesthesia and analgesia was performed to all study groups (parenteral, enteral and topical) for 5 days before each drug administration.

All experiments were allocated to 4 groups as control, parenteral, enteral and topical. Group C (n=3): The lesions were created and no treatment was applied. Group P (n=6); Glutamine (Dipeptiven®, Fresenius Kabi) slowly injected to the tail vein by insulin needle in a dose 0.4 mg $kg^{-1}.day^{-1}$. Group E (n=6); Feeding tube (No: 6, 10 cm) pushed forward from mouth to stomach. Glutamine (Resource Glutamin®, Nestle) administered without contacting the oral mucosa in a dose 1 $g.kg^{-1}.day^{-1}$. Group L (n=6); Glutamine (Resource Glutamin®, Nestle) administered by applying the ear bar to the lesion in a dose 1 $g.kg^{-1}.day^{-1}$. Drug administration was performed to all study groups by repeating the procedure for 5 days, being 2 times a day at the same time. Experimental Design is presented in Table 2.

**Macroscopic Evaluation:** After the lesions were created, we photographed them by high resolution machine before the drug administration during 5 days. The healing process of the lesions were macroscopically observed in respect to presence of redness, edema, bleeding and scar tissue.

**Getting Tissue Samples:** Euthanasia was performed after 5 days once the lesion had been created by injecting intraperitoneally Ketamine 200 mg/kg (Ketalar®), Thiopental Sodium 150 mg/kg (Pental®). Scar tissue dissected and divided into two parts. One of the pieces was stored for histopathological evaluation, the other one was for analysis of oxidative stress factors.



This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as: Akbaş S, Karabulut E, Sutas Bozkurt AP, Aydın Ö, Ergün Düzgün D, Güngör G. Effects of Glutamine on Healing of Traumatic Oral Mucosal Lesions; an Experimental Study. JAREM 2018; DOI: 10.5152/jarem.2018.2008

**Histopathological Evaluation:** Biopsy specimens were embedded in paraffin, cut and stained with hematoxylin and eosin after 48 hours of formalin fixation. These specimens were analyzed by a pathologist blinded to the experimental study. First, the cross-sections were scanned and then the areas of pathologic changes were characterized. Epithelial proliferation, acute inflammation, vascular proliferation and fibrosis that are important for wound healing were evaluated for based on pathological scoring criteria generated by a pathologists. Modified and updated form of Shafer histopathological scoring criteria (5) for all parameters were assessed on 2 scores based on intensity and diffuseness of the cells; Score 1: nothing to appear on section or less than 10%, Score 2: 10% and above in section.

**Biochemical Analysis:** Tissue samples taken for biochemical analysis were exposured to spectrophotometric measurements by supernatant Elisa method with 450 nm wavelength. Easybiofarm/China commercial kit was used for Malonil Dialdehyde (MDA: Lipid peroxidation product), superoxide dismutase (SOD: antioxidant), Glutathione peroxidase (GSH-Px: enzymes of antioxidant defense system) and OH-proline (HYP) measurements.

**Statistical Analysis:** Histopathological and biochemical evaluation results were evaluated using SPSS-9 statistical program. Fisher's Exact Chi-square test and Mann-Whitney U test were used for all groups in comparison to a binary of histopathological evaluation parameters. $p<0.05$ was considered statistically significant in resulted values. For biochemical evaluation parameters, differences among groups were analyzed by Kruskal-Wallis test, using a Mann-Whitney U test with a Bonferroni correction for pairwise comparisons. $p<0.02$ was considered statistically significant in resulted values.

## RESULTS

**Macroscopic Findings:** Control group had redness, edema and bleeding at 48-72 hours and scar tissue at 4th and 5th days. Parenteral group had redness, less edema and bleeding at 24-36 hours, scar tissue at 3th and 5th days. Enteral group had redness, edema and bleeding at 24-48 hours, scar tissue at 3th and 5th days. Topical group had redness and edema at 24-48 hours, scar tissue at 3th and 5th days.

**Histopathologic Findings:** There was significant difference between control group and parenteral group in terms of epithelial proliferation (*p=0.012*) (Figure 2). There was significant difference between control group, parenteral and enteral groups in terms of acute inflammation (*p=0.018*) (Figure 3). Histopathological Evaluation is presented in Table 3. There was significant difference between control group and topical group in terms of fibrosis (*p=0.029*) (Figure 4).

**Oxidative Stress Analysis:** There was no significant difference between groups for values of SOD, GSH-Px and HYP. In terms of MDA values; there was significant difference between control group and enteral group (*p<0.02*) (Figure 5, *p<0.02* and * shows significant difference between control and enteral group). Oxidative Stress Analysis of MDA, SOD, GSH-Px and HYP are presented in Table 4.

## DISCUSSION

In this experimental animal model, the effects of GLN by topical or systemic (enteral, parenteral) routes were demonstrated in the treatment of traumatic oral mucosal lesions. The healing process of the lesions last days, months or even years is a dynamic process. GLN seems to be more effective in initial 3-5 days in which has a turnover time of mucosal cells after the mucosal damage (6).



This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as: Akbaş S, Karabulut E, Sutas Bozkurt AP, Aydın Ö, Ergün Düzgün D, Güngör G. Effects of Glutamine on Healing of Traumatic Oral Mucosal Lesions; an Experimental Study. JAREM 2018; DOI: 10.5152/jarem.2018.2008

A normal wound healing comprises the process of inflammatory and proliferative phases, maturation and remodeling. Many topical and systemic agents were used for shortening the process and studied to accelerate the wound healing (7). In normal wound healing, evaluation of the interaction for clinical, cellular and other factors has an important role on planning and development of convenient therapeutic method (8). The macroscopic evaluation in our study, control group had redness, edema and bleeding at the 3rd day and scar tissue at the 4th and 5th day after created the lesions. But the study groups had redness, edema and bleeding at the 2nd day and scar tissue occurred less extent. On the other hand, there was a significant difference between control group and parenteral group in terms of epithelial proliferation (*p=0.012*). Positive effect of GLN on epithelial proliferation was observed while there was no significant difference between control group and other study groups and study groups were compared with each other. The results of enteral and topical group were similar. Although the best results in study groups were observed in parenteral group. Enteral administration of GLN instead of parenteral route has been shown in studies to reduce mucosal atrophy, bacterial translocation and the incidence of sepsis (9).

The use of GLN by enteral route is more effective rather than parenteral route which has limited effects on intestinal cells (10). But in our study, we observed that parenteral results had better healing process. Muscle and plasma GLN levels are reduced in the period of catabolic stress. GLN is an important energy source for continuity, maintenance and repair of all gastrointestinal mucosa and reducing the intestinal mucosal injury. Inflammation is a response that occurs against the tissue damage which is caused by infections and physical or chemical agents in organisms. Erbil et al reported that GLN had benefits for the inflammation process of intestinal cells (11). In our study,

acute inflammatory cells were observed more widespread and intense in control group compared to the study groups. Longer presence of acute inflammatory cells were considered the damage caused by a longer time to clean. In study groups, topical group compared to parenteral and enteral group; contrary to our expectations, acute inflammatory cells located for a longer time and more intensive. Therefore wound healing was observed starting later. Topical GLN was not the reason of shortening the time of acute inflammation. Therefore, we observed that the difference between enteral and topical group was the little inevitable trauma on the mucous layer in topical application which made the acute inflammation continue. A better result in parenteral group was considered due to the fact that the optimal level without plasma GLN had been exposed to presystemic elimination. We thought that the optimal plasma level of GLN reached by systemic way the same as enteral just after topical application because of swallowing reflex.

Enteral supplementation cannot be used routinely due to the instability of GLN solution. Topical GLN provides the growth of gastrointestinal cells and so that prevents evolving intestinal atrophy in total parenteral nutrition patients (12). It has been considered that topical GLN would be useful for enhancing mucosal contact. But we did not have the same result in our study. Skubitz et al reported that topical-oral GLN after chemotherapy reduced the grade and duration of mucositis. Likewise, Anderson et al reported topical-oral GLN reduced the grade of oral mucositis and decreased the oral pain as soon as 4-5 days (13).

Revascularization in wound healing process is very important in terms of nutrition of damaged tissue area. In our study, there was no significant difference between control group and study groups in

terms of vascular proliferation. Although we expected that it would be better for vascular proliferation for topical GLN, there was no difference comparing to study groups.

Normal wound healing process does not always have an expected conclusion, it may results as a scar tissue development and failure to catch up with old properties of healed area. It is important in clinical practice that wound healing resulted in shorter time and less sequelae. In our study, there was significant difference among control group and topical group in terms of fibrosis (p=0.029). We observed that control group had widespread and intensive fibrosis, whereas in all subjects of topical group had less grade of fibrosis. Thus, we considered that GLN has allowed the reepithelialization by delaying or preventing fibrosis. San-Miguel et al reported that GLN significantly prevents occurrence of intensive fibrosis on studies for the effects of antifibrinogen (14).

Wound healing is a pathophysiological process including the cellular interaction and biochemical events. Reactive oxygen species in cells continuously produced during normal metabolism and the cells protect themselves by antioxidant mechanisms from the damage of products. But the tissues are faced to oxidative damage if that balance corrupted (15). Increasing the levels of SOD and MDA is claimed in the presence of oxidative stress. The increase of SOD activity demonstrates the capacity of the cellular antioxidant activity, increase of MDA demonstrates the degree of severity of the cellular damage (16). SOD and MDA are observed in featured parameters efficacy of antioxidant therapy and the assessment of the presence of oxidative stress. It is known that the activity of SOD and GSH-Px are increased in the presence of oxidative stress (17). Steiling et al reported in experimental study of wound healing in mice, the levels of SOD and GSH-Px were increased with oxidative stress in scar tissue. In addition, increased expression of antioxidant enzymes during healing was evaluated for the

purpose of adapting to the increased oxidative stress (18). In our study, SOD levels of control group compared to the study groups were found lower. High level of SOD was interpreted as better eliminates the free radicals by increased enzymatic activity. SOD levels in topical group compared to parenteral and enteral group was observed higher and we concluded that GLN administrated by topically more increased the SOD levels. Marquez et al reported in experimental mucosal injury study improved to secondary portal hypertension that GLN supplementation prevented the reduction in the SOD enzyme activity (19). In our study, high levels of GSH-Px were interpreted as better elimination of the free radicals by increasing the enzymatic activity with in parallel observation of macroscopic and microscopic viewing.

Increased free radicals or decreased antioxidant defense mechanism leads to increased level of serum MDA. In our study; there was significant difference between control and enteral group in terms of MDA levels ($p<0.02$). We observed that there was no increase an antioxidant enzyme compatible with increasing MDA. These results can be interpreted as corruption compliance to increased oxidative stress.

In order to evaluate wound healing, one of the method commonly used is identifying the collagen levels. Generally, determination of the OH-proline level which is abundant in collagen structure and less occurred in the structure of other proteins to identify the quantity of collagen tissue are commonly used (20). In our study, we observed that tissue integrity protected on the best level in parenteral group which was not allowed for the reepithelialization.



This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as: Akbaş S, Karabulut E, Sutas Bozkurt AP, Aydın Ö, Ergün Düzgün D, Güngör G. Effects of Glutamine on Healing of Traumatic Oral Mucosal Lesions; an Experimental Study. JAREM 2018; DOI: 10.5152/jarem.2018.2008

**Limitation**

The limitation of the present study was small number of samples that consequently would lead to partial inconclusive results. For further studies, it is suggested to use larger sample size to obtain more precise results.

**CONCLUSION**

We observed a positive effect of GLN supplementation for the treatment of traumatic oral mucosal lesions. GLN administration by parenteral route had better results compared to the topical and enteral route. We therefore concluded that GLN supplements administered by topical or enteral route were a better alternative on traumatic oral mucosal lesions.

**REFERENCES**


1. Köroğlu G. Hemşirelerin kemoterapiye bağlı gelişen oral mukozite ilişkin bilgilerin saptanması. Gazi Üniversitesi, Sağlık Bilimleri Enstitüsü, Ankara. Yüksek Lisans Tezi, 2007.
2. Naidu MU, Ramana GV, Rani PU, et al. Chemotherapy-induced and/or radiation therapy-induced oral mucositis--complicating the treatment of cancer. Neoplasia. 2004 Sep; 6(5): 423–431.
3. Ohtani M, Kawada S, Seki T, Okamoto Y. Amino acid and vitamin supplementation improved health conditions in elderly participants. J Clin Biochem Nutr. 2012 Mar; 50(2): 162-8.
4. Rubio IT, Cao Y, Hutchins LF, et al. Effect of glutamine on methotrexate efficacy and toxicity. Ann Surg. 1998 May; 227(5):772-8

5. Shafer W, Hine M, levy B. A textbook of oral pathology. 4th ed. Philadelphia: Saunders; 1983. p. 594.
6. Klimberg VS, Salloum RM, Kasper M, et al. Oral glutamine accelerates healing of the small intestine and improves outcome after whole abdominal radiation. Arch Surg. 1990;25:1040-1045.
7. Li J, Chen J, Kirsner R. Pathophysiology of acute wound healing. Clin Dermatol. 2007 Jan-Feb; 25(1): 9-18.
8. Mutsaers SE, Bishop JE, McGrouther G, Laurent GJ. Mechanisms of tissue repair: from wound healing to fibrosis. Int J Biochem Cell Biol. 1997 Jan; 29(1): 5-17.
9. L-Glutamine. Altern Med Rev 2001;6(4): 406-410
10. Hall JC, Heel K, McCauley R. Glutamine. Br J Surg 1996; 83: 305
11. Ersin S, Tuncyurek P, Esassolak M, et al. The prophylactic and therapeutic effects of glutamine and arginine-enriched diets on radiation-induced enteritis in rats. J Surg Res. 2000;89:121-125
12. Li J, Langkamp-Henken B, Suzuki K, et al. Glutamine prevents parenteral nutrition- induced increases in intestinal permeability. JPEN 1994; 18: 303–307.
13. Savarese DM, Savy G, Vahdat L, et al. Prevention of chemotherapy and radiation toxicity with glutamine. Cancer Treat Rev. 2003 Dec; 29(6): 501-13.
14. San-Miguel B, Crespo I, et al. Glutamine prevents fibrosis development in rats with colitis induced by 2,4,6-Trinitrobenzene Sulfonic Acid. J Nutr. 2010 Jun; 140(6): 1065-71.



This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as: Akbaş S, Karabulut E, Sutas Bozkurt AP, Aydın Ö, Ergün Düzgün D, Güngör G. Effects of Glutamine on Healing of Traumatic Oral Mucosal Lesions; an Experimental Study. JAREM 2018; DOI: 10.5152/jarem.2018.2008

15. Serarslan G, Altug ME, Kontas T. Effect of caffeic acid phenethyl ester on plasma lipid peroxidation, antioxidant status and nitric oxide levels in incisional wound model. Türkderm, 2007; 41: 11-4.
16. Eris O, Cankayali I, Sezer E, et al. The effect of N-Acetylcysteine on oxidative stress in induced hemorrhagic shock model in rats. J Turk Anaesth Int Care 2009; 37(4):208-216.
17. Matés JM. Effects of antioxidant enzymes in the molecular control of reactive oxygen species toxicology. Toxicology. 2000 Nov 16; 153(1-3): 83-104.
18. Steiling H, Munz B, Werner S, Brauchle M. Different types of ROS-scavenging enzymes are expressed during cutaneous wound repair. Exp Cell Res. 1999 Mar 15; 247(2): 484-94.
19. Marques C, Mauriz JL, Simonetto D, et al. Glutamine prevents gastric oxidative stress in an animal model of portal hypertension gastropathy. Ann Hepatol. 2011 Oct-Dec; 10(4): 531-9.
20. Hendriks T, Mastboom WJ. Healing of experimental intestinal anastomoses. parameters for repair. Dis Colon Rectum. 1990 Oct; 33(10): 891-901.





This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as: Akbaş S, Karabulut E, Sutas Bozkurt AP, Aydın Ö, Ergün Düzgün D, Güngör G. Effects of Glutamine on Healing of Traumatic Oral Mucosal Lesions; an Experimental Study. JAREM 2018; DOI: 10.5152/jarem.2018.2008

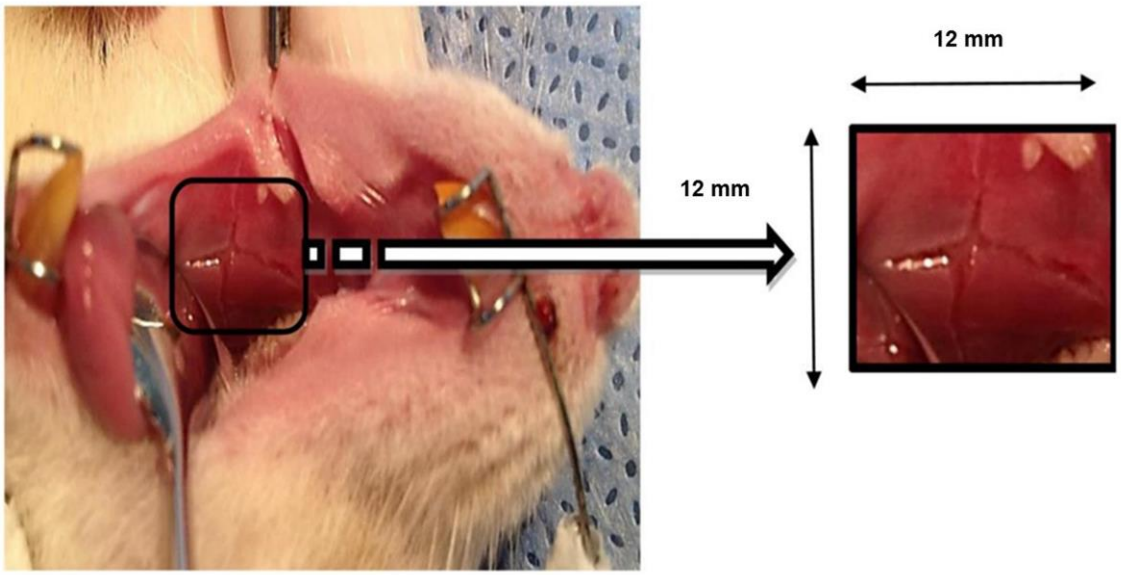


**Figure 1.** Making Oral Mucosal Lesion

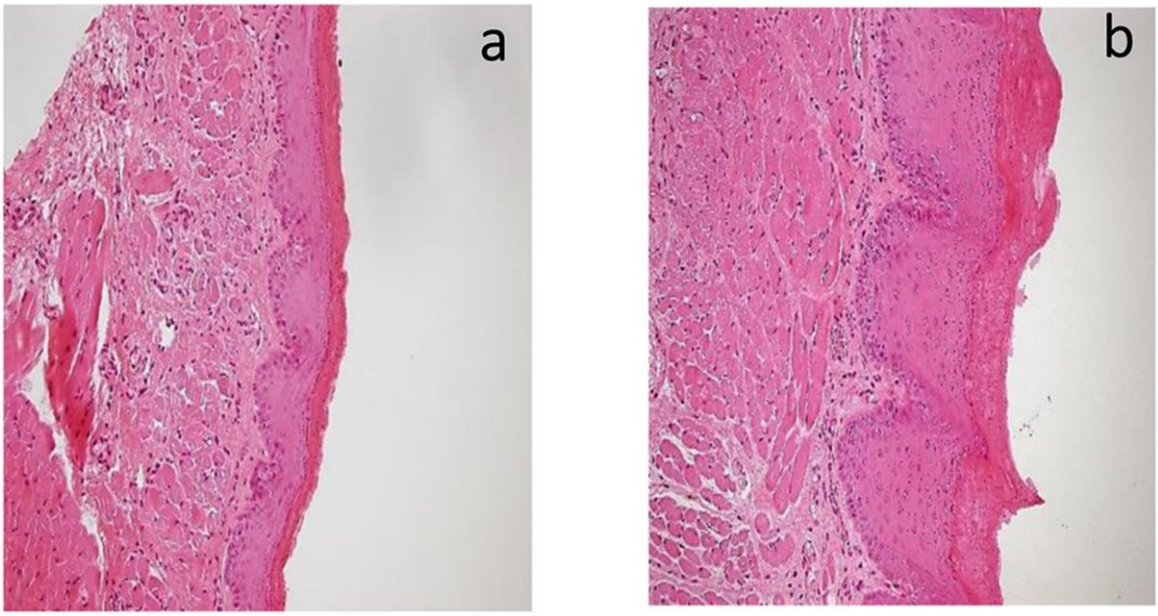


**Figure 2.** Epithelial Proliferation (a. Control Group, b. Parenteral Group)



This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as: Akbaş S, Karabulut E, Sutas Bozkurt AP, Aydın Ö, Ergün Düzgün D, Güngör G. Effects of Glutamine on Healing of Traumatic Oral Mucosal Lesions; an Experimental Study. JAREM 2018; DOI: 10.5152/jarem.2018.2008

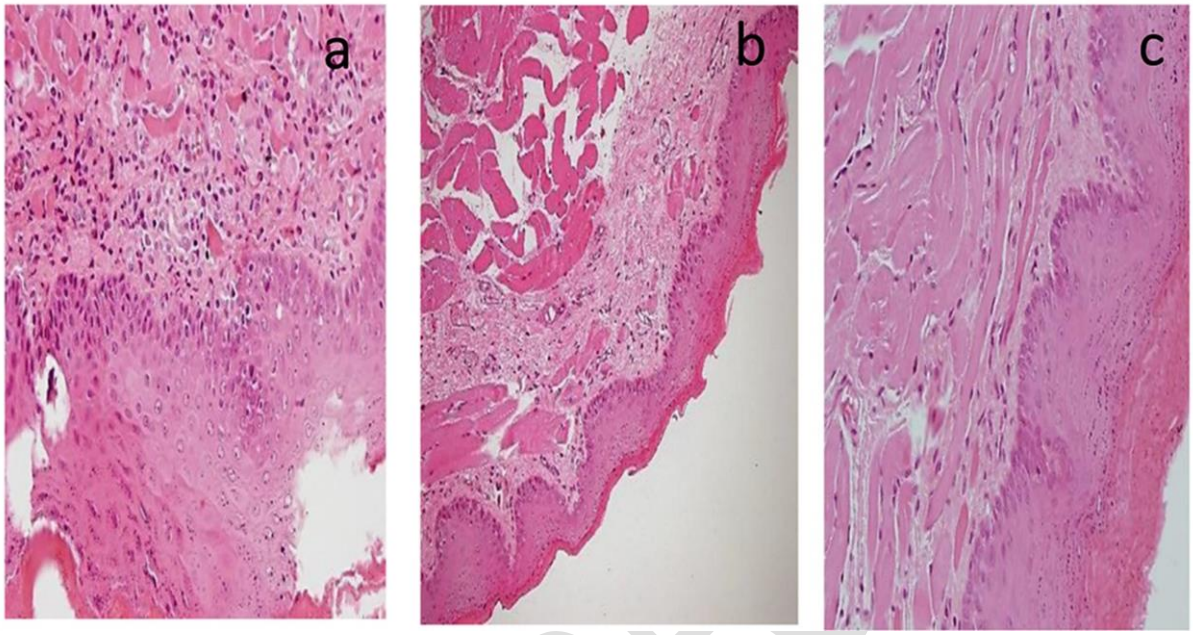


**Figure 3**. Acute Inflammation (a. Control Group, b. Parenteral Group, c. Enteral Group)





This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as: Akbaş S, Karabulut E, Sutas Bozkurt AP, Aydın Ö, Ergün Düzgün D, Güngör G. Effects of Glutamine on Healing of Traumatic Oral Mucosal Lesions; an Experimental Study. JAREM 2018; DOI: 10.5152/jarem.2018.2008

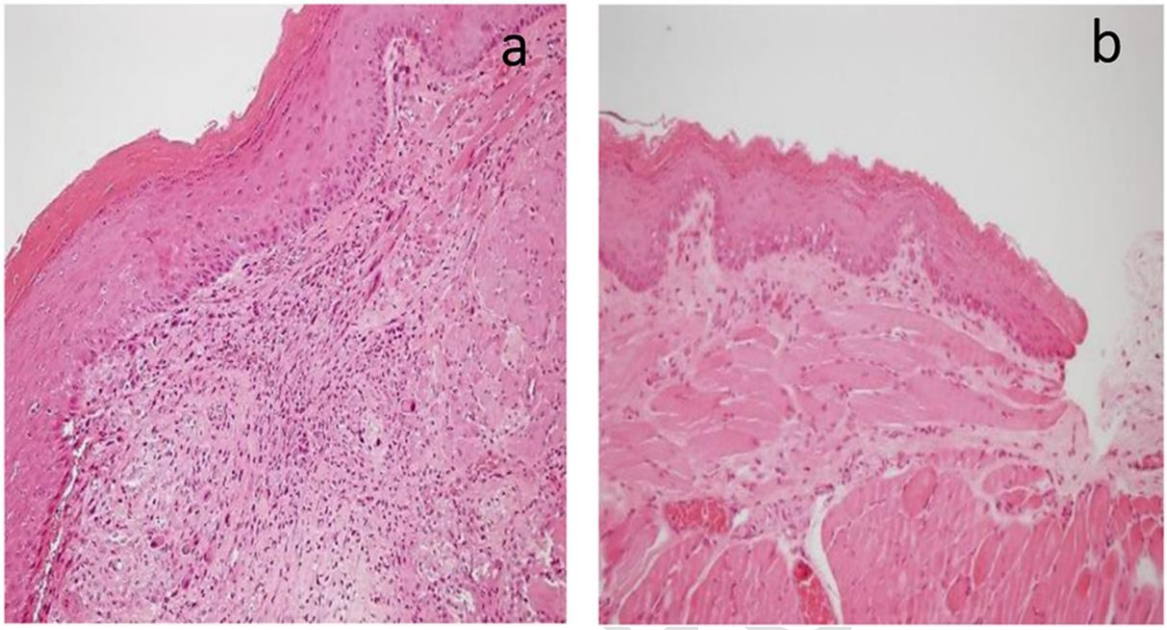


**Figure 4.** Fibrosis (a. Control Group, b. Topical Group)





This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as: Akbaş S, Karabulut E, Sutas Bozkurt AP, Aydın Ö, Ergün Düzgün D, Güngör G. Effects of Glutamine on Healing of Traumatic Oral Mucosal Lesions; an Experimental Study. JAREM 2018; DOI: 10.5152/jarem.2018.2008

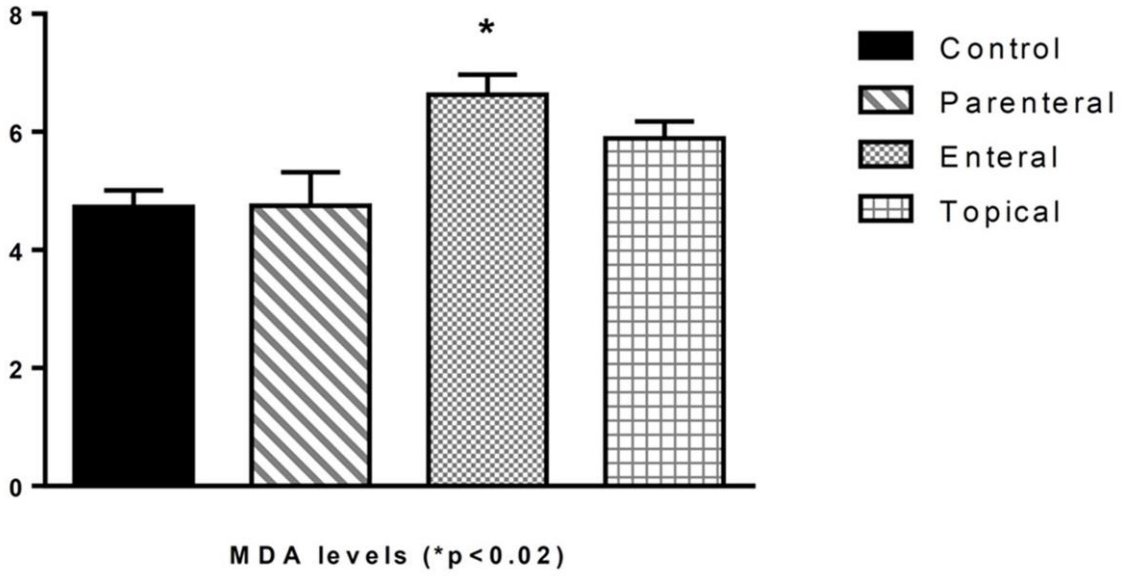


**Figure 5.** MDA levels, Mean Value and Standard Deviation (StdD)





This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as: Akbaş S, Karabulut E, Sutas Bozkurt AP, Aydın Ö, Ergün Düzgün D, Güngör G. Effects of Glutamine on Healing of Traumatic Oral Mucosal Lesions; an Experimental Study. JAREM 2018; DOI: 10.5152/jarem.2018.2008

**Table 1: Weight of animals, Drugs and Dosages**

| | **Group Control** (n=3) Mean ± StdD[1] | **Group Parenteral** (n=6) | **Group Enteral** (n=6) | **Group Topical** (n=6) Mean ± StdD |
|---|---|---|---|---|
| Weight (mg) | 292.0 ± 10.58 | 303.3 ± 12.69 | 289.2 ± 18.03 | 297.0 ± 18.54 |
| Ketamine (Ketalar®) (mg) | 21.90 ± 0.79 | 22.73 ± 0.95 | 21.65 ± 1.32 | 22.22 ± 1.41 |
| Xylazine (Rompun®) (mg) | 1.46 ± 0.05 | 1.48 ± 0.07 | 1.43 ± 0.08 | 1.43 ± 0.10 |

1.StdD: Standard Deviation





This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as: Akbaş S, Karabulut E, Sutas Bozkurt AP, Aydın Ö, Ergün Düzgün D, Güngör G. Effects of Glutamine on Healing of Traumatic Oral Mucosal Lesions; an Experimental Study. JAREM 2018; DOI: 10.5152/jarem.2018.2008

**Table 2: Experimental Design**

| Groups | Number of Animals |
|---|---|
| **Group C: Control Group**<br>Making purely traumatic oral mucosal lesion | 3 |
| **Group P: Parenteral Group**<br>Administered by parenteral GLN(1) (0.4 $mg.kg^{-1}.day^{-1}$) | 6 |
| **Group E: Enteral Group**<br>Administered by (feeding tube) enteral GLN (1 $g.kg^{-1}.day^{-1}$) | 6 |
| **Group T: Topical Group**<br>Administered by (ear bar) topical GLN (1 $g.kg^{-1}.day^{-1}$) | 6 |

1.GLN: Glutamine





This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as: Akbaş S, Karabulut E, Sutas Bozkurt AP, Aydın Ö, Ergün Düzgün D, Güngör G. Effects of Glutamine on Healing of Traumatic Oral Mucosal Lesions; an Experimental Study. JAREM 2018; DOI: 10.5152/jarem.2018.2008

**Table 3: Histopathological Evaluation**

| | **Group Control** (n=3) (Score 1/ Score 2) | **Group Parenteral** (n=6) (Score 1/ Score 2) | **Group Enteral** (n=6) (Score 1/ Score 2) | **Group Topical** (n=6) (Score 1/ Score 2) | **P Value** |
|---|---|---|---|---|---|
| Epithelial Proliferation, n | 3/0* | 0/6* | 4/2 | 2/4 | **0.012*** |
| Acute Inflammation, n | 0/3* | 6/0* | 5/1* | 3/3 | **0.018*** |
| Vascular Proliferation, n | 1/2 | 0/6 | 0/6 | 1/5 | 0.486 |
| Fibrosis, n | 0/3* | 3/3 | 3/3 | 6/0* | **0.029*** |

n: number of experiment, score 1: nothing to appear or less than 10%, score 2: 10% and above in section.





This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as: Akbaş S, Karabulut E, Sutas Bozkurt AP, Aydın Ö, Ergün Düzgün D, Güngör G. Effects of Glutamine on Healing of Traumatic Oral Mucosal Lesions; an Experimental Study. JAREM 2018; DOI: 10.5152/jarem.2018.2008

**Table 4. Oxidative Stress Analysis of MDA, SOD, GSH-Px and HYP**

| | MDA[3] (ng/ml) | SOD[4] (ng/ml) | GSH-Px[1] (ng/ml) | HYP[2] (ng/ml) |
|---|---|---|---|---|
| **Control Group** (n=3) Mean ± StdD[5] | 4,72 ± 0,49 | 0,22 ± 0,22 | 106,00 ± 83,62 | 811,66 ± 110,71 |
| **Parenteral Group** (n=6) | 4,75 ± 1,37 | 1,34 ± 1,71 | 108,00 ± 11,41 | 1063,16 ± 274,08 |
| **Enteral Group** (n=6) Mean ± StdD | 6,63 ± 0,82 | 1,31 ± 1,23 | 89,66 ± 44,41 | 837,16 ± 231,41 |
| **Topical Group** (n=6) Mean ± StdD | 5,97 ± 0,64 | 3,46 ± 3,27 | 93,16 ± 26,50 | 765,83 ± 286,15 |

1. GSH-Px: Glutathione Peroxidase, 2.HYP: Hydroxyproline, 3.MDA: Malonil Dialdehyde, 4.SOD: Superoxide Dismutase, 5.StdD: Standard Deviation





This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as: Akbaş S, Karabulut E, Sutas Bozkurt AP, Aydın Ö, Ergün Düzgün D, Güngör G. Effects of Glutamine on Healing of Traumatic Oral Mucosal Lesions; an Experimental Study. JAREM 2018; DOI: 10.5152/jarem.2018.2008