

# Preventing and Therapeutic Effect of Propolis in Radiotherapy Induced Mucositis of Head and Neck Cancers: A Triple-Blind, Randomized, Placebo-Controlled Trial

Abbas Javadzadeh Bolouri,<sup>1</sup> Atessa Pakfetrat,<sup>1</sup> Arghavan Tonkaboni,<sup>2,\*</sup> Seyed Amir Aledavood,<sup>3</sup> Mohsen Fathi Najafi,<sup>4</sup> Zahra Delavarian,<sup>5</sup> Mohammad Taghi Shakeri,<sup>6</sup> and Azade Mohtashami<sup>3</sup>

<sup>1</sup>Department of Oral Medicine, School of Dentistry, Mashhad University of Medical Sciences, Mashhad, IR Iran

<sup>2</sup>Department of Oral Medicine, School of Dentistry, Tehran University of Medical Sciences, Tehran, IR Iran

<sup>3</sup>Cancer Research Center, Mashhad University of Medical Sciences, Mashhad, IR Iran

<sup>4</sup>Department of Veterinary Research and Biotechnology, Razi Vaccine and Serum Research Institute, Mashhad, IR Iran

<sup>5</sup>Department of Oral Medicine, Oral and Maxillofacial Diseases Research Center, Faculty of Dentistry, Mashhad University of Medical Sciences, Mashhad, IR Iran

<sup>6</sup>Department of Community Med and Public Health, Ghaem Hospital, Mashhad University of Medical Sciences, Mashhad, IR Iran

\*Corresponding author: Arghavan Tonkaboni, Department of Oral Medicine, School of Dentistry, Tehran University of Medical Sciences, Tehran, IR Iran. Tel: +98-9123802173; +98-2155851141, Fax: +98-2166525925, E-mail: a.tonkaboni@yahoo.com

Received 2015 September 7; Accepted 2015 September 22

## Abstract

**Background:** Mucositis is one of the acute complications of radiotherapy which can ulcerate oral mucosa and cause severe pain and discomfort which can affect oral normal function. Propolis is a natural source of flavenoid which has antiulcer, antibacterial, antifungal, healing and anti-inflammatory effects. Using such an affordable compound without any bad smell or taste that has reasonable price can help the radiotherapy undergoing patients.

**Objectives:** Our goal is assessing the preventing and therapeutic effect of propolis in radiotherapy induced mucositis in patients with head and neck cancer.

**Patients and Methods:** In a randomized triple blind clinical trial, 20 patient were selected randomly to swish and swallow 15 ml of water based extract of propolis mouth wash 3 times a day in the case group (n=10) and 15 ml placebo mouth wash in control group (n=10). we use NIC-CTC scale for determining mucositis grading.

**Results:** We use T-test, Man-Whitney, Chi-square, and Friedman as analyzing tests. Case group had significantly ( $P < 0.05$ ) lower grade of mucositis in all of the follow-ups, but xerostomia is not significantly different in two groups ( $P > 0.05$ ).

**Conclusions:** This is a pilot study which shows water based extract of propolis efficiently prevents and heals radiotherapy induced mucositis.

**Keywords:** Cancer, Mouthwashes, Mucositis, Propolis, Radiotherapy

## 1. Background

Malignancies are the second cause of death in developed countries, and among malignancies head and neck cancers are the six most prevalent neoplasms worldwide, with an estimated 900,000 new cases diagnosed annually. Oral squamous cell carcinoma is the most common cancer in oropharyngeal area (1-4).

Cancer treatments have been changed through the last 3 decades and include surgery, chemotherapy, and radiotherapy. Oral complications are commonly experienced by patients undergoing cancer therapy (5). Radiotherapy complications are affected by radiation dose, fraction size, volume of irradiated tissue, type of ionizing irradiation and fraction scheme (6). Ra-

diotherapy of oral cavity can induce mucositis which is the most common acute complication (7). Ionizing rays can cause inflammation of oropharyngeal mucosa especially in the 2nd and 3rd weeks and leads to ulcers and erosions which are so painful and cause problems in eating; therefore, cancer treatment protocol might be postponed due to the patients' malnutrition (8, 9). Mucositis will occur in all patients under radiotherapy (10). Age, gender, oral hygiene, radiation dose, smoking, systemic disease and radiotherapy techniques are risk factors (10). The first sign of mucositis is a white patch caused by interruption in desquamation, then mucosa with erythema is noted within 1 to

2 weeks, and throughout the course of therapy pseudomembrane formation represents ulceration which is accompanied by pain and soreness (11). Extraction of hopeless teeth, relining of dentures, and ameliorating of oral hygiene can decrease the severity of mucositis. Although different therapy modalities such as amifostin, G-CSF, KGF, Mg milk, amphogel, kaopectate, benzydamine hcl, capcasin, sucralfate and low level laser are suggested by researchers. There is still no definite and standard way to treat or prevent this condition. Xerostomia as another radiation complication could deteriorate the condition.

Propolis is a resinous material which is made by honey bee. It can be red, brown or green depending on the plants of the area. It has a special scent which is due to fat and protein. The ingredients make propolis adhesive to skin (12). The most important part is polyphenols including galangin, luteolin, campferol, capheic acid phenyl ethyl ester (CAPE), quercetin, artipelin, vitamin, sequiterpene quinines, coumarins, amino acids, steroids and inorganic compounds and aminoacids (12, 13). Propolis is used in homeopathic and herbal practice as an antiseptic, anti-inflammatory, antimycotic, and bacteriostatic agent (14). Animal studies suggested that polyphenolic compounds have antioxidant and a free radical scavenger effects and are responsible for protecting against radiation (15). When it is administered preventively, it has higher efficacy. The results suggest that propolis and related flavonoids given to mice before irradiation protected mice from lethal effects of whole-body irradiation and diminish primary DNA damage in their white blood cells as detected by the alkaline comet assay (16).

## 2. Objectives

Our goal is assessing the preventing and therapeutic effect of propolis in radiotherapy induced mucositis in patients with head and neck cancer.

## 3. Patients and Method

This triple blind placebo controlled randomized clinical trial was conducted in Omid hospital, cancer center, Mashhad university of medical sciences.

Patients were assessed according to the eligibility requirement. 20 patients involved with head and neck malignancies including 14 (70%) male and 6 (30%) female met the inclusion criteria and participate in the study and consecutively enrolled in this program (Box 1).

The patients could deny or cut the protocol whenever they wanted. All patients were advised to avoid alcohol, spicy and scour foods, smoking, and to maintain good oral hygiene during the course of radiotherapy. Radiotherapy was administered using Cobalt 60 radiotherapy unit at a dose of 2 Gy per day, five times a week up to total dose of 50 to 70 Gy.

The two under study mouthwashes (propolis or placebo)

were provided by SORENTEC company in 3% concentration and finally packaged in identical containers labeled as A and B. 20 patients fulfilling the inclusion criteria were divided into 2 groups by completely random method treated by propolis (group A) or placebo (group B). None of the patients nor researchers was aware of the medication inside. Only pharmacist knew and prepared the corresponding drug for every patient but had no contact with study participants.

Each patient received 2 bottles of mouth wash and a bottle of normal saline for oral rinsing before using the mouthwash. All the patients were instructed to rinse their mouths with 15 ml of placebo or propolis 3% and then to swallow 3 times a day for 5 weeks simultaneously with radiotherapy protocol from the first session.

Examinations of the oral mucosa were performed on all patients at the beginning of treatment and continued weekly up to the end of the study for 5 weeks.

The national institute of the cancer common toxicity criteria oral mucositis scale was used for grading mucositis and the xerostomia was assessed by 5 standardized questions, validated by other researchers (17, 18).

All of the scores for mucositis and xerostomia were assessed and recorded at the beginning and weekly, for 5 weeks (the end of radiotherapy) by researcher who were blinded for medication.

The weight of patients was measured at the beginning and at the end of the treatment. If there was any adverse event, treatment was discontinued and the subject was referred for necessary care.

### Box 1. Inclusion and Exclusion Criteria

#### Criteria

#### Inclusion Criteria

Age over 15 years old

At least half of the mouth to be under radiation

Radiotherapy was administered with dose of 50-70 cGy

Involving with head and neck cancer and undergoing radiotherapy

#### Exclusion Criteria

WBC < 3000 mm<sup>3</sup>

FBS > 150 mg/dL

If they had other serious systemic disease

Previously underwent radiotherapy, chemotherapy

Obtaining grade 3 mucositis accordingly

Statistical analysis was performed with SPSS 20. Data were analyzed using the independent sample t-test, Man

Whitney and Friedman tests with  $P < 0.05$  considered significant.

This project was approved by the Ethics committee of Mashhad university of medical science, Iran and all subjects signed informed consent.

#### 4. Results

The 20 patients including 6 females (70%) and 14 males (30%) aged 15 to 87 years old (mean age of 53.2 years) were enrolled and completed the study.

There were no differences between 2 groups in age, gender, being edentulous, type of tumor radiation dose and weight. ( $P > 0.05$ ) weight loss can be a good marker of their malnutrition status.

The mucositis score of NCI-CTC at the end of each week in the propolis group was significantly lower than placebo group (Figure 1).

In propolis group 8 patients showed no evidence of mucositis during the radiotherapy course. (Figures 2-6).

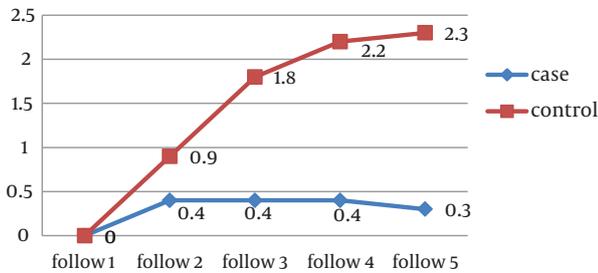


Figure 1. Severity of Mucositis in Each Follow-Up

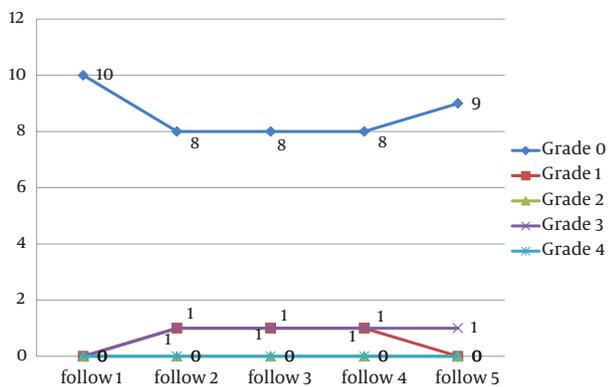


Figure 2. Grading of Mucositis in This Study

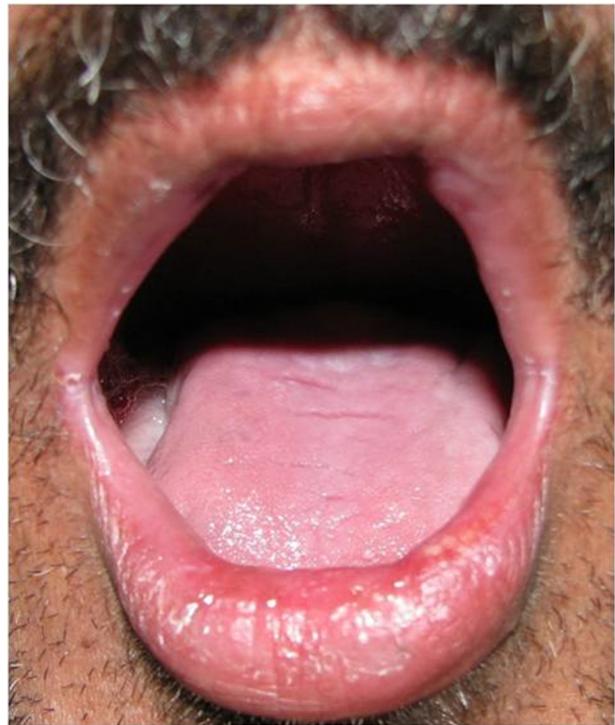


Figure 3. Case Group in Second Week



Figure 4. Control Group in Second Week



Figure 5. Case Group in 4th Week



Figure 6. Control Group in 4th Week

Table 1. Mean Rank and Number of Both Groups

Group	Mean Rank	Number
<b>Follow up 1</b>		
Case	2.65	10
Control	1.30	10
<b>Follow up 2</b>		
Case	3.15	10
Control	2.35	10
<b>Follow up 3</b>		
Case	3.15	10
Control	3.45	10
<b>Follow up 4</b>		
Case	3.15	10
Control	3.90	10
<b>Follow up 5</b>		
Case	2.90	10
Control	4.00	10

The mean rank of NCI-CTC at the end of each week in two groups was estimated (Table 1).

Weight loss was measured in both groups. The mean weight loss in the case group was 200 g (9 patients showed no weight loss), while in the control group the mean weight loss was 3.4 kg, and the difference was significant ( $P = 0.029$ ).

## 5. Discussion

Benderli Cihan and Deniz in 2011 reported propolis as an effective drug in reducing the severity of radiation induced oral mucositis in rats (19). Ghassemi and Zabihi also confirmed the effect of ethanolic extract of propolis on radiation induced mucositis in rats (20).

To authors' knowledge, there is no available human study report about the effects of propolis on mucositis until this paper was published. This study showed encouraging results for the prevention and treatment of radiation induced mucositis.

The effective dose of propolis suggested in two mentioned animal study was 100 mg/kg/day (19, 20). In this study we used polyphenol 1.4 mg/kg/day which is equal to 70 mg propolis per day. We provide mouth wash containing this exact daily dose in a swish and swallow way to benefit from both topical and systemic effects (21).

Propolis has a broad biological effect that help prevent-

ing and treating mucositis. It is antifungal, antibacterial and antiviral agent, besides healing, anti-ulceration, anti-inflammatory, antioxidant effects which are due to rich flavenoid agents in propolis (19). The most important effect of flavenoids is oxygen free radical scavenging and several studies have confirmed the role of deactivation of free radical (21).

Some researchers proved the hemathopoetic recovery effects of propolis derived compounds. The results of this research confirmed this hypothesis on preventing and therapeutic effects of oral mucositis, but the greatest limitation at this study was small sample size, and we assessed just one exact concentration in mouthwash (22).

While assessing the radioprotective effect of quercetin and the ethanolic extract of propolis, (EEP) Benković showed that the alkaline comet assay proved that both natural compounds, especially when given as pre-treatment, protect against primary leukocyte DNA damage in mice. In the tested concentrations, EEP and quercetin were not genotoxic to non-irradiated mice. AET, however, resulted in a slight, but not significant increase in DNA damage. Although the results of this study show the radioprotective potential of the test compounds, further investigation is needed to clarify the underlying protection mechanisms (23).

Propolis is a product with rich flavenoid that could be suggested as a preventing and economic treatment agent in the medical approach of radiation induced oral mucositis. Different ways and doses at propolis on larger size sample were suggested for future studies.

In conclusion this study suggests that propolis water based mouthwash is safe and effective in the prevention and treatment for radiotherapy induced mucositis. Doing more studies with wider scope of samples different concentration of propolis from all over the world is highly recommended.

## Acknowledgment

This paper is extracted from a thesis by Dr Tonkaboni, and is supported by Research Deputy of Mashhad University of Medical Sciences. The authors would like to thank the vice chancellor for his assistance and the Research Committee for their support.

## Footnotes

**Authors' Contribution:** Abbas Javadzadeh Bolouri, Atessa Pakfetrat and Arghavan Tonkaboni designed the study, Mohsen Fathi Najafi produced the mouthwashes with regard to the opinion of the group Arghavan Tonkaboni perform the study, wrote the paper and contributed to the data entry, literature review and writing-up process. Azade Mohtashami assist in performing and contributed to the study design and Mohammad Taghi Shakeri contributed to analysis. Seyed Amir Aledavood was the main consultant in selecting the patients. All authors read and approved the final manuscript.

**Conflict of Interest:** There is no conflict of interest in this article.

**Financial Disclosure:** This study was supported by vice chancellor for research of Mashd university of medical sciences.

## References

1. Little JW. *Dental management of the medically compromised patient*. Mosby; 1997.
2. Stewart BW, Kleihues P, International Agency for Research on Cancer. *World cancer report*. IARC press Lyon; 2003.
3. Silveira A, Gonçalves J, Sequeira T, Ribeiro C, Lopes C, Monteiro E, et al. Oncologia de Cabeça e Pescoço: enquadramento epidemiológico e clínico na avaliação da Qualidade de Vida Relacionada com a Saúde. *Revista Brasileira de Epidemiologia*. 2012;15(1):38–48. doi:10.1590/s1415-790x2012000100004. [PubMed: 22450491]
4. Petersen PE. The World Oral Health Report 2003: continuous improvement of oral health in the 21st century—the approach of the WHO Global Oral Health Programme. *Community Dent Oral Epidemiol*. 2003;31 Suppl 1:3–23. [PubMed: 15015736]
5. Scully C, Epstein JB. Oral health care for the cancer patient. *Eur J Cancer B Oral Oncol*. 1996;32B(5):281–92. [PubMed: 8944831]
6. Vissink A, Burlage FR, Spijkervet FKL, Jansma J, Coppes RP. Prevention and Treatment of the Consequences of Head and Neck Radiotherapy. *Crit Rev Oral Biol Med*. 2003;14(3):213–25. doi:10.1177/154411130301400306. [PubMed: 12799324]
7. Suemaru K, Cui R, Li B, Watanabe S, Okihara K, Hashimoto K, et al. Topical application of royal jelly has a healing effect for 5-fluorouracil-induced experimental oral mucositis in hamsters. *Methods Find Exp Clin Pharmacol*. 2008;30(2):103–6. doi:10.1358/mf.2008.30.2.1159655. [PubMed: 18560624]
8. Berger AM, Fall-Dickson Jane M. Oral complications. In: Vincent T, editor. *Devita: Principles and practice of oncology*. Philadelphia: Lippincott Williams & Wilkins; 2005. pp. 2523–35.
9. Burket LW, Greenberg MS, Glick M, Ship JA. *Burket's oral medicine*. PMPH-USA; 2008.
10. Vera-Llonch M, Oster G, Hagiwara M, Sonis S. Oral mucositis in patients undergoing radiation treatment for head and neck carcinoma. *Cancer*. 2006;106(2):329–36. doi:10.1002/cncr.21622. [PubMed: 16342066]
11. Hall EJ, Giaccia AJ. *Radiobiology for the Radiologist*. Lippincott Williams & Wilkins; 2006.
12. Farrell CL, Bready JV, Rex KL, Chen JN, DiPalma CR, Whitcomb KL, et al. Keratinocyte growth factor protects mice from chemotherapy and radiation-induced gastrointestinal injury and mortality. *Cancer Res*. 1998;58(5):933–9. [PubMed: 9500453]
13. Khalil ML. Biological activity of bee propolis in health and disease. *Asian Pac J Cancer Prev*. 2006;7(1):22–31. [PubMed: 16629510]
14. Kukreja B, Dodwad V. Propolis mouthwash: A new beginning. *J Indian Soc Periodontol*. 2011;15(2):121. doi:10.4103/0972-124x.84379. [PubMed: 21976834]
15. Scheller S, Gazda G, Krol W, Czuba Z, Zajusz A, Gabrys J, et al. The ability of ethanolic extract of propolis (EEP) to protect mice against gamma irradiation. *Z Naturforsch C*. 1989;44(11-12):1049–52. [PubMed: 2698623]
16. Orsolic N, Benkovic V, Horvat-Knezevic A, Kopjar N, Kosalec I, Bakmaz M, et al. Assessment by survival analysis of the radioprotective properties of propolis and its polyphenolic compounds. *Biol Pharm Bull*. 2007;30(5):946–51. [PubMed: 17473440]
17. You WC, Hsieh CC, Huang JT. Effect of Extracts from Indigowood Root (*Isatis indigotica* Fort.) on Immune Responses in Radiation-Induced Mucositis. *J Alternat Complement Med*. 2009;15(7):771–8. doi:10.1089/acm.2008.0322.
18. Lee SH, Kim TH, Kim JY, Park SY, Pyo HR, Shin KH, et al. Evaluation of parotid gland function following intensity modulated radiation therapy for head and neck cancer. *Cancer Res Treat*. 2006;38(2):84–91. doi:10.4143/crt.2006.38.2.84. [PubMed: 19771265]
19. Benderli Cihan Y, Deniz K. [Effect of propolis against radiation-

- induced oral mucositis in rats]. *Kulak Burun Bogaz Ihtis Derg.* 2011;**21**(1):32-41. [PubMed: 21303315]
20. Ghassemi L, Zabihi E, Mahdavi R, Seyedmajidi M, Akram S, Motallebnjad M. The effect of ethanolic extract of propolis on radiation-induced mucositis in rats. *Saudi Med J.* 2010;**31**(6):622-6. [PubMed: 20563358]
21. Olaitan PB, Adeleke OE, Ola IO. Honey: a reservoir for microorganisms and an inhibitory agent for microbes. *Afr Health Sci.* 2007;**7**(3):159-65. doi:10.5555/afhs.2007.7.3.159. [PubMed: 18052870]
22. Benkovic V, Orsolcic N, Knezevic AH, Ramic S, Dikic D, Basic I, et al. Evaluation of the radioprotective effects of propolis and flavonoids in gamma-irradiated mice: the alkaline comet assay study. *Biol Pharm Bull.* 2008;**31**(1):167-72. [PubMed: 18175964]
23. Benkovic V, Knezevic AH, Dikic D, Lisicic D, Orsolcic N, Basic I, et al. Radioprotective effects of quercetin and ethanolic extract of propolis in gamma-irradiated mice. *Arh Hig Rada Toksikol.* 2009;**60**(2):129-38. doi:10.2478/10004-1254-60-2009-1908. [PubMed: 19581205]