10.5005/jp-journals-10024-1479

ORIGINAL RESEARCH

Evaluation of Protective Effect of Propolis on Parotid Salivary Glands in Gamma-irradiated Rats

[1]Mina Motallebnejad, [2]Seyed Mohammad Abedi, [3]M Seyedmajidi, [4]AA Moghadamnia, [5]M Ashrafpour
[6]M Salehi, [7]D Moslemi, [8]A Ghorbani

ABSTRACT

Objective and background: One of the most significant side effects of radiotherapy for head and neck cancers is xerostomia as a result of salivary gland damage. Considering pharmacological effects of propolis, we evaluated its protective effect on salivary glands subjected to radiotherapy of head and neck cancer patients.

Materials and methods: Twenty-one male albino rats (8-11 W, 190 ± 5 gm) were divided into three groups of seven animals. Scintigraphy was performed in all the groups. Then groups 1 (S) and 2 (SR) received normal saline injections and group 3 (PR) received propolis injection over 3 days. After that groups 2 and 3 were exposed to gamma radiation and all the rats underwent scintigraphic assessment on third day and 70th day after irradiation. The lips and tongues of rats in groups 2 and 3 were examined for mucositis daily in first 10 days. At the end, the parotid glands of all rats were examined histologically.

Results: Scintigraphy results of third and 70th day after irradiation showed statistically significant differences between PR and SR as well as SR and S. However, there was no significant difference between the PR and S groups. Histopathologic assessment demonstrated significant difference between SR, PR and S.

Conclusion: These results suggest that propolis has protective effects on salivary gland function in animal models whilst it did not prevent radiation-induced histologic changes in tissues. Further investigations are needed to elucidate mechanisms of propolis actions.

Clinical significance: Regarding to the results of this study, propolis may be useful in reduction xerostomia due to radiation to salivary glands and may be helpful for head and neck cancer patients.

Keywords: Propolis, Scintigraphy, Xerostomia.

How to cite this article: Motallebnejad M, Abedi SM, Seyedmajidi M, Moghadamnia AA, Ashrafpour M, Salehi M, Moslemi D, Ghorbani A. Evaluation of Protective Effect of Propolis on Parotid Salivary Glands in Gamma-irradiated Rats. J Contemp Dent Pract 2014;15(1):8-11.

Source of support: Nil

Conflict of interest: None

[1,3]Associate Professor, [4]Professor, [2,5,6,8]Assistant Professor [7]Radiation Oncologist

[1]Department of Oral and Maxillofacial, Dental Material Research Center, Dental School, Babol University of Medical Sciences Babol, Iran

[2]Department of Radiology, Nuclear Medicine, Faculty of Medicine Mazandaran University of Medical Sciences, Sari, Iran

[3]Department of Oral and Maxillofacial Radiology, Dental Material Research Center, Dental School, Babol University of Medical Sciences, Babol, Iran

[4,5]Department of Pharmacology, Medical School, Babol University of Medical Sciences, Babol, Iran

[6,8]Department of Oral and Maxillofacial Medicine, Dental School Mazandaran University of Medical Sciences, Sari, Iran

[7]Department of Radiology and Radiation Therapy, Shahid Rajace Hospital, Babol University of Medical Sciences, Babol, Iran

Corresponding Author: A Ghorbani, Assistant Professor Department of Oral and Maxillofacial Medicine, Kian 90 Building Isar Alley, Piroozi Street, Sari, Mazandaran, Iran, Phone: +98-9111513085, Fax: +98-1513207898, e-mail: anahita1802@yahoo.com

INTRODUCTION

One of the most significant complications of radiation therapy for head and neck cancers is salivary gland dysfunction and dry mouth.[1-3] If a less than-desired dose is used to reduce damage to healthy tissues, it may result in diminished quality of life and higher morbidity rate for these individuals.[3] In radiation therapy for head and neck cancer, the major salivary glands often receive a high radiation dose. Acute and chronic responses displayed by salivary glands after radiation are related to dose of radiation and the amount of the glands in the direct line of the beam.[4,5]

Different procedures, such as lower exposure volume, biological agents, that stimulate progenitor cells, gene-factor techniques and drug therapy used to prevent or minimize harmful side effects of radiation on salivary glands.[6] Many radioprotective agents have been tested in laboratories to determine their efficacy in preventing radiation damage to normal cells and tissues. Propolis is a resinous mixture that honey bees collect. It composed of flavonoids, resin, polyphenol, phenolic acid, phenolic aldehyde, wax and pollen. All of these compounds are responsible for its biological and pharmacological properties.[7] Some of the proposed properties are anti-inflammatory,[7] bacteriostatic and bactericidal agent,[8] antifungal,[9] antiviral,[9] antioxidant,[10] immune system stimulant, and wound healing actions.[11,12] Bee propolis ingredient seems to stop cancer cell growth[13] and control the growth

of human tumors.[11] Previous studies have mentioned some characteristics of propolis, such as radioprotective effects, improving radiation complications if administered prophylactically (before radiation), anti-inflammatory effects, reducing and delaying radiation-induced mucositis in an animal model, anti-tumoral and antioxidant effects.[14-17]

According to pharmacological characteristics of propolis and lack of data about its effects on salivary glands as well as its reasonable price and good historical safety profile, we decided to use propolis in our study.

MATERIALS AND METHODS

This study was conducted using male Wistar albino rats. Rats were randomly selected from samples housed at the Animal Care and Research Unit. Twenty-one male Wistar albino rats aged 8 to 11 weeks and weighting 190 ± 5 gm were used for this study. All rats were kept on the same temperature (22 ± 2°C) and 12:12 hours light:dark cycle with the same food and water before the experiment. Rats were divided into three groups of seven animals: group 1—control group that received normal saline injections without irradiation (S); group 2—control group that received normal saline injections before irradiation (SR); group 3—experimental group received 400 mg/kg propolis injections before irradiation (PR).

Rats were marked and then anesthetized with ketamine intraperitoneal (60 mg/kg, IP) and scintigraphy was performed for 15 minutes (scintigraphy evaluation). Then S and SR groups received normal saline injections and PR group received 400 mg/kg/d propolis injection for 3 days. The solutions were injected intraperitoneal. At the third day of injections, SR and PR groups received radiation. Rats were anesthetized intraperitoneal with ketamine (100 mg/kg) prior to irradiation. Rats were fixed on a special plate and were irradiated with gamma-ray (cobalt60) 15 Gy, on the whole cranium for 7 minutes and 39 seconds. For the next 10 days, lips and tongues of the rats were examined daily to assess mucositis according to Parkin's clinical scale.[18] Three days after irradiation all groups were anesthetized with ketamine (IP), weighed and parotid gland scintigraphy was performed. Due to chronic effects of radiation, scintigraphy was performed for all the rats after 70 days to assess parotid function.[19] Then rats were anesthetized with ketamine (100 mg/kg, IP) and sacrificed. The parotid salivary glands were removed and weighed and evaluate microscopically. Moreover, histological examination with hematoxylin and eosin (H&E) staining was performed and the percentage of connective tissue cells, acinar cells, ductal cells, fat cells and vascular tissue were assessed.[19]

RESULTS

During the experiment, three rats died. Two rats of the S group died in first session of scintigraphy and one of the SR group died after 2 months in the last session of scintigraphy.

Scintigraphy

The results of scintigraphy which performed 3 days after irradiation showed significant salivary gland hypofunction in the SR groups compared with S and PR groups ($p < 0.0001$). There was no significant difference between S and PR groups. In third scintigraphic assessment (70 days after irradiation), SR group showed significantly lower salivary gland function compared with S group ($p < 0.0001$). The results of scintigraphy of PR and SR groups are relatively the same ($p < 0.0001$) (Table 1).

Salivary Gland Weight Measurement

Rats were sacrificed and parotid glands were dissected out and weighed. The weights of S, SR and PR groups are 0.15 ± 0.02, 0.17 ± 0.01 and 0.12 ± 0.002 gm respectively. The weight of parotid glands in PR group was significant lower than S group ($p < 0.0001$).

Histopathologic Examination

According to the percentage of acinar cells, ductal cells, vascular and connective tissue cells, and fat cells demonstrated in Table 2, there was a significant reduction in acinar cells after radiation in both SR and PR groups in comparison with S group ($p < 0.0001$). In both SR and PR groups, increased percentage of connective tissue cells was observed compared to control group ($p < 0.0001$). No statistically significant difference was observed between SR and PR groups ($p = 0.949$). Difference between ductal, fat and vascular tissue cells was not significant between three groups.

Mucositis

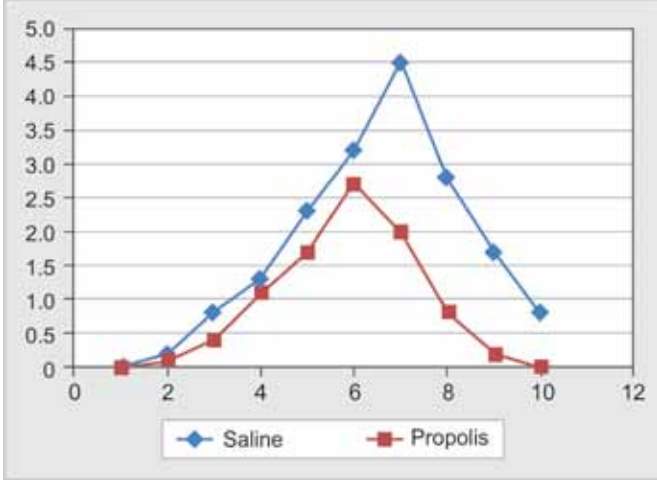
As shown in Graph 1, the intensity of mucositis in the SR group was greater than PR, group during 10-day evaluation

Table 1: Mean ± SD salivary gland uptake to background ratio of the three study groups

Group	*1st scintigraphy*	*2nd scintigraphy*	*3rd scintigraphy*
S	1.72 ± 0.06	1.70 ± 0.04	1.71 ± 0.06
SR	1.69 ± 0.06	1.53 ± 0.04	1.52 ± 0.06
PR	1.75 ± 0.04	1.72 ± 0.06	1.72 ± 0.06

Table 2: Percentage of acinar, connective tissue, ductal, vascular and fat cells in three groups (check the names used in these lines)

Groups	*Acinar cell (%)*	*Connective tissue (%)*	*Ductal cell (%)*	*Vascular cell (%)*	*Fat cell (%)*
S	88	6.44	1.7	4.09	0
SR	41.53	44.94	1.78	8.36	3.4
PR	42.83	42.34	5.78	8.91	0.14
p-value	0.001	0.001	0.260	0.397	0.149



Graph 1: Trend of mucositis in SR and PR groups during 10-day evaluation

according to Parkin's clinical scale. Difference was statistically significant on the seventh, eighth, ninth ($p < 0.0001$) and tenth day ($p = 0.008$).

DISCUSSION

Since radiotherapy and chemotherapy cause damage to tissues surrounding the radiation site, development of radioprotective agents has been the subject of intense search in cancer research centers. Radioprotective agents should be nontoxic and have efficacy in protecting tissues against gene alteration, mutations, immune system damage and teratogens. Salivary glands, which frequently reside within the irradiation field, mostly become damaged in head and neck radiation therapy.[20]

In the present research, we evaluated propolis effects on histopathology and function of salivary glands after radiotherapy for the first time.

Results achieved from the analysis of scintigraphic data showed no significant difference between control and propolis group. In normal saline group, there was a significant decrease in salivary gland function in comparison with the other two groups so it can indicate that propolis has early and late radioprotective effects. Intracellular signal transduction distortion as a result of exocrine cellular membrane disruption is responsible for salivary flow reduction after irradiation whereas loss of acinar tissue is not the reason.[19] Loss of high-affinity agonists binding to muscarinic receptors, for watery secretion, in early stages after irradiation could be a part of cellular membrane disruption.[19] This study demonstrated that propolis probably could protect cellular membrane and subsequently intracellular signal transduction.

Despite propolis protective effects on salivary gland function, weight measurement results were not consistent with it. Salivary glands change commensurate with rats change. Rats weight gain was not considerable after irradiation because of mucositis and impairment in nutrition but rats in control group gained more weight so salivary gland measurement is not considered to bean appropriate criterion for evaluating propolis effects after radiation therapy.

Other studies have demonstrated that radiation causes loss of acinar tissue and increase in connective tissue.[19] Similar to our findings in control group. Same changes in propolis group and control group indicate that propolis is ineffective in this case. In the present study, salivary gland histopathology was performed 70 days after irradiation. It can be concluded that for evaluating propolis effects a long-term study with larger groups should be performed and salivary gland should be checked and compared at 1 month, 6 months and 1 year.

Our result showed that propolis has clinical effects on mucositis. This finding is supported by Ghassemi et al in and Molania et al.[21,22]

As this study is the first to provide evidence showing propolis effects on function and histopathologic changes of salivary glands, future investigations with different doses of propolis, different approaches of propolis administration and long-term surveys are suggested.

CONCLUSION

These results suggest that propolis has protective effects on salivary gland function in animal models whilst it did not prevent radiation-induced histologic changes in tissues. Further investigations are needed to elucidate mechanisms of propolis actions.

CLINICAL SIGNIFICANCE

Regarding to the results of this study, propolis may be useful in reduction xerostomia due to radiation to salivary glands and may be helpful for head and neck cancer patients.

ACKNOWLEDGMENT

We appreciate the staff of Nuclear Medicine Department of Mazandaran University of Medical Sciences. This project was financially supported by Deputy of Research and Technology of Babol University of Medical Sciences, Iran.

REFERENCES

1. Mossman KL. Frequent short-term oral complications of head and neck radiotherapy. Ear Nose Throat J 1994;73:16-320.
2. Parsons JT. The effect of radiation on normal tissues of the head and neck. In: Cassisi JH, editor Management of head and neck cancer. Philadelphia: JB Lippincott 1984. p. 173-207.
3. Nguyen NP, Sallah S, Karlsson U, et al. Combined chemotherapy and radiation therapy for head and neck malignancies: Quality of life tissues, Cancer 2002;94:1131-1141.
4. Kashima HK, Kirkham WR, Andrew JR. Postirradiation sialadenitis: a study of the clinical features, histopathologic changes, and serum enzyme variations following irradiation of human salivary glands. Am J Roentgenol Rad Ther Nucl Med 1965;94:271-291.
5. Kaplan P. Mantle irradiation of the major salivary glands. J Prosthet Dent 1985;54:681-686.
6. Emami B, Lyman J, Brown A, et al. Tolerance of normal tissue to therapeutic irradiation. Int Radiat Oncol Biol Phys 1991;21:109-122.
7. Siro B, Szelekovzky S, Lakatos B, Mady G, Szathmari E, Karanyi Z. Local treatment of rhematic disease with propolis compounds. Orv Hetil 1996 Jun 23;137(25):1363-1364.
8. Lotfy M. Biological Activity of Bee propolis in health and disease. Asian Pac J Cancer Prev 2006;7:22-31.
9. Grange JM, Davey RW. Antibacterial properties of propolis (Bee glue). JR Soc Med 1990 March;83(3):159-160.
10. María I, Moreno N, Isla MI, Antonia R, Sampietro, Vattuong MA. Comparison of the free radical scavenging activity of propolis from several regions of Argentina 2000;18(2):320-331.
11. Siro B. Mady G, et al. Local treatment of rheumatic disease with propolis compound. Orv Hetil 1998 June 23;137(25):1363-1364.
12. Leguthoj HA. Propolis: It's properties and administration to patients treated for some surgical diseoses. Przegl Lek 2000; 57(4):191-194.
13. EL-Ghazaly MA, Khayyal MT. The use of aqueous propolis extract against roaliation-induced damage. Drug Exp Clin Res 1995;21(6):229-236.
14. Benderli Cihan Y, Deniz K. Effect of propolis against radiation-induced oral mucositis in rats. Kulak Burun Bogaz Ihtis Derg 2011 Jan-Feb;21(1):32-41.
15. Benković V, Knezević AH, Dikić D, Lisicić D. Radioprotective effects of quercetin and ethanolic extract of propolis in gamma-irradiated mice. Arh Hig Rada Toksikol 2009 Jun;60(2): 129-138.
16. Benković V, Orsolić N, Knezević AH, Ramić S. Evaluation of the radioprotective effects of propolis and flavonoids in gamma-irradiated mice: the alkaline comet assay study. Biol Pharm Bull 2008 Jan;31(1):167-172.
17. Yeun-Hwa GU. Antioxidant activity and anti-tumor immunity by Agaricus, propolis and Paffia in mice. Available at: http:// st.suzuka_u.ac.jp/information/bulletin/pdf/01-gu.pdf.
18. Limesand KH, Said SH, Anderson SM. Suppression of radiation-induced salivary gland dysfunction by IGF-1. Plos One 2009;4(3):e4663.
19. Konings AWT, Faber H, Cotteleer F, Vissink A, et al. Secondary radiation damage as the main cause for unexpected volume effects: a histopathologic study of the parotid gland. Int J Radiation Oncology Biol Phys 2006;64:98-105.
20. Benkovic V, Horvat Knezevic A, Dikic D, Orsolic N, et al. Radioprotective effects of propolis and quercetin in gamma-irradiated mice evaluated by the alkaline comet assay. Phytomedicine 2008;15:851-858.
21. Molania T, Moghadamnia AA, Pouramir M, Aghel S, Moslemi D, et al. Comparison the effect of cinnamaldehyde and propolis on gamma-irradiated mucositis and salivary antioxidant activity in rat. Daru Journal 2012;20:89.
22. Ghassemi L, Zabihi E, Mahdavi R. Seyedmajidi M. Akram S. Motallebnejad M. The effect of ethanolic extract of Propolis on radiation-induced mucositis in rats. Saudi Med J 2010 Jun;31(6):622-626.