

Original Article

## Salivary superoxide dismutase activity in the consumers of paan containing tobacco

Leila Farhadmollashahi (DDS)<sup>1</sup>, Alireza Nakhaie (PhD)<sup>2</sup>, Marieh Honarmand (DDS)<sup>1✉</sup>,  
Fateme Arbabi-Kalati (DDS)<sup>3</sup>, Payam Ghahari (DDS)<sup>4</sup>

1. Assistant Professor, Dental Research Center, Department of Oral Medicine, Faculty of Dentistry, Zahedan University of Medical Sciences, Zahedan-Iran.
2. Associate Professor, Department of Biochemistry, Faculty of Medicine, Zahedan University of Medical Sciences, Zahedan- Iran.
3. Associate Professor, Department of Oral Medicine, Faculty of Dentistry, Zahedan University of Medical Sciences, Zahedan- Iran.
4. Specialist of Oral Medicine, Zahedan-Iran.

✉ **Corresponding Author:** Marieh Honarmand, Faculty of Dentistry, Zahedan University of Medical Sciences, Zahedan- Iran.  
**Email:** Marieh.honarmand@gmail.com **Tel:** +989151430868

---

### Abstract

**Introduction:** The habit of smokeless tobacco chewing is one of the known risk factors for oral cancer among the residents of southeast of Iran. Most likely, the antioxidant defense system in dealing with free radicals induced paan and prevention of oral cancer is important. In this study, the activity of super oxide dismutase is compared in the saliva of paan consumers and non-consumers.

**Methods:** In this study, Unstimulated saliva of 87 subjects (47 paan consumers and 40 non-consumers) who referred to the Oral Medicine Department of Dentistry School of Zahedan was collected. The activity of super oxide dismutase enzyme was measured by standard biochemical methods (Mc Cord and Fridovich) and the obtained data were analyzed by statistical software SPSS-15 through non-parametric Mann-Whitney test.

**Results:** The mean activity of super oxide dismutase was significantly higher in the paan consumers group ( $4.4 \pm 1.6$  u/mg) compared to non-consumers ( $3.59 \pm 1.8$  u/mg,  $p=0.027$ ).

**Conclusions:** The results of this study demonstrate that consumption of paan leads to increased activity of salivary super oxide dismutase.

**Keywords:** Antioxidants, Saliva, Smokeless tobacco, Superoxide dismutase

---

Received: 28 Oct 2013

Accepted: 2 Feb 2014

*Citation for article:* Farhadmollashahi L, Nakhaie AR, Honarmand M, Arbabikalati F, Ghahari P. Salivary superoxide dismutase activity in the consumers of paan containing tobacco. *Caspian J Dent Res* 2014; 3: 8-13.

## فعالیت سوپراکسید دسموتاز بزاقی در مصرف کنندگان پان محتوی تنباکو

لیلا فرهادملاشاهی، علیرضا نخعی، ماریه هنرمند\*، فاطمه اربابی کلاتی، پیام قهاری

### چکیده

**مقدمه:** جویدن تنباکوی غیر تدخینی به عنوان یکی از ریسک فاکتورهای شناخته شده سرطان دهان در ساکنان جنوب شرقی ایران میباشد. احتمالاً سیستم دفاع آنتی اکسیدانی در مقابله با رادیکالهای آزاد ناشی از مصرف پان و همچنین در جلوگیری از ایجاد سرطان دهان مهم است. در این مطالعه فعالیت آنزیم سوپر اکسید دسموتاز در بزاق افراد مصرف کننده پان و افراد غیر مصرف کننده، مقایسه شده است.

**مواد و روش ها:** در این تحقیق بزاق غیر تحریکی ۸۷ فرد مراجعه کننده (۴۷ مصرف کننده پان و ۴۰ نفر غیر مصرف کننده) به بخش بیماری های دهان دانشکده دندان پزشکی زاهدان جمع آوری شد. میزان فعالیت آنزیم سوپر اکسید دسموتاز بر پایه روش استاندارد بیوشیمیایی (Mc Cord and Fridovich) اندازه گیری شد و اطلاعات به دست آمده توسط نرم افزار آماری SPSS 15 و توسط آزمون نان پارامتریک mann-hitney، آنالیز شد.

**یافته ها:** میانگین میزان فعالیت آنزیم سوپر اکسید دسموتاز در گروه مصرف کننده پان ( $4/4 \pm 1/6$  u/mg) به طور معنی داری بالاتر از گروه غیر مصرف کننده ( $3/59 \pm 1/8$  u/mg،  $p=0/027$ ) بود.

**نتیجه گیری:** نتایج این مطالعه نشان میدهد که مصرف پان موجب افزایش فعالیت سوپر اکسید دسموتاز بزاقی میشود.

**واژگان کلیدی:** آنتی اکسیدان، بزاق، تنباکوی بدون دود، سوپر اکسید دسموتاز

### Introduction

Paan is a combination of areca nut, slaked lime, catechu, tobacco, sweeteners, and spices (1, 2) and contains various carcinogenic compounds including reactive oxygen species, Arecoline (3), Tobacco specific nitrosamines (3, 4).

It is a risk factor for oral cancer, hypertension, dyslipidemia, miscarriage, low birth weight, diabetes, and asthma exacerbation (5, 6). Based on recent research, long-term use of smokeless tobacco can produce free radicals (7). They include superoxide anion ( $O_2^-$ ), hydroxyl radical ( $HO^\bullet$ ), peroxy radicals ( $ROO^\bullet$ ), and hydrogen peroxide ( $H_2O_2$ ) (8).

Free radicals are also called reactive oxygen species (ROS) which form following chewing areca nut and catechu at  $pH > 9.5$  (9). Free radicals can change the structure of intracellular and extracellular components such as proteins, lipids, and DNA and interfere with cell function (10).

Antioxidants are the body's defense system that neutralizes the destructive effects of ROS and minimize damage to cells. As the first defensive line, saliva has a protective antioxidant system that fights against oxidant-induced damage (11). One of the most important antioxidant enzymes which regulate

oxidation-reduction process of cell normal and tumorigenic conditions superoxide dismutase (SOD) (12). There are three types of superoxide dismutase including Fe-SOD, Mn-SOD and Cu-Zn SOD. SOD contains copper and zinc and is found in all body tissues as well as in some body fluids, in particular saliva (13).

SOD converts  $O_2$  to  $H_2O_2$  during its catalytic activity. (14) So far, several studies with contradictory results were carried out on the antioxidant enzymes such as SOD in the saliva of smokers (13-17), while no study was performed in this regard on smokeless tobacco consumers.

In the present study, we intended to compare the activity of SOD; the body's most important antioxidant enzyme, in the saliva of paan consumers and non-consumers. This study could lay the ground for research on the prevention of adverse effects of paan in oral cavity through antioxidant defense system of saliva.

## Methods

**Subjects:** According to previous studies (16) the sample size in confidence interval 95% and power of test 80% was determined. In this cross sectional study, 47 paan consumers who used daily at least one packet of 10 gram paan for at least one year and 40 age and sex matched non-consumers referred to Dentistry School of Zahedan were selected through simple sampling method. Any factors that might lead to imbalance oxidant/antioxidant system in the exclusion and inclusion criteria were considered.

### Inclusion criteria:

1. Healthy individual
2. Desire to participate in the study

### Exclusion criteria:

1. Suffering from any systemic disease.
2. Consumption of immunosuppressive and non-steroidal anti-inflammatory drugs, antioxidants and vitamin supplements in last three months.
3. Smoking and consumption of alcohol.
4. Oral cavity diseases such as aphthous, leukoplakia, periodontitis (pocket > 3mm), etc.

All participants were informed about the study and a written consent was obtained regarding their participation in the project. The study, was approved by the Ethics Committee of Zahedan University of Medical Sciences.

## Collection of saliva

The participants were asked to avoid eating, drinking, and brushing 2 hours before sampling. All samples were collected between 9 am to 11 am.

During sample collection, whilst seated and slightly bent forward, the subjects evacuated their saliva 1-2 times per minute for at least 5 minutes in sterile tubes (17). The test tubes were coded and sent immediately to Biochemistry Lab of Zahedan University of Medical Sciences. Then in the laboratory, they were centrifuged (Clement 2000) for 10 minutes at a speed of 2000 rpm. The supernatant was separated and maintained at  $-70^{\circ}C$ .

## Assay of SOD activity

The required materials for experimentation were purchased from Merck, Germany. The enzyme activity was measured according to McCord and Fridovich method (18). 50  $\mu$ L of sample was mixed with 2.9 ml of the solution was prepared via mixing of 100 ml PBS 50 mM (pH 7.4) containing EDTA 0.1 mM and 2  $\mu$ M cytochrome C, with 10 ml sodium hydroxide 0.001 N containing 5  $\mu$ M oxanthine, and the reaction was started by adding of 50  $\mu$ L the solution containing xanthine oxidase 0.2U/ml and EDTA 0.1 mM.

The absorbance of each sample was measured with a spectrophotometer (Pharmacia-Biotech) at 550 nm wavelength. In controls, 50 ml of distilled water was used instead of sample.

After the calculation of changes in absorbance in each sample for four minutes, the mean of absorbance changes were calculated for every minute. Then, the activity of each sample (in U/mg) was calculated based on molar absorption coefficient of cytochrome C and the amount of protein present in each sample.

## Statistical analysis

The data obtained from paan consumer and non-consumer groups were analyzed by SPSS-15 statistical software through descriptive statistics for mean and standard deviation and Mann-Whitney non-parametric test.  $p \leq 0.05$  was considered statistically significant.

## Results

Paan consumers included 29 males and 18 females with a mean age of 27 years and non-consumers included 22 males and 18 females with a mean age of 31 years. Table 1 shows that the mean SOD activity was significantly higher in the paan consuming group

( $p \leq 0.05$ ). It was also found that there was not a significant difference between age and gender of two groups; this was expected regarding matching of variables of the two groups ( $p > 0.05$ ).

**Table 1. Superoxide dismutase activity and demographic characteristic in subjects of the study groups**

Study groups	Consumers (Mean±SD)	Non consumers (Mean±SD)	P value
SOD activity (u/mg)	4.4±1.6	3.59±1.8	0.027
Age (year)	27±11	31±9	0.176
Male	29	22	0.63
Female	18	18	

## Discussion

The results of the present study showed that the activity of super oxide dismutase in saliva was significantly higher in paan consumers compared with non-consumers. It seems that the increase of this enzyme, as a component of the antioxidant defense system in saliva, is to reduce the damaging effects of free radicals produced by the consumption of paan. Exposing to the compounds in the paan induce microsomal cytochrome P<sub>450</sub> as a source of reactive oxygen species (ROS).

Super oxide anion and hydrogen peroxide are formed especially following disruption and uncoupling of cytochrome P<sub>450</sub> in the catalytic cycle (19). Oxidant toxicity caused by smoking may lead to increase antioxidant enzymes such as SOD (17). Super oxide dismutase converts super oxide anion to hydrogen peroxide which then H<sub>2</sub>O<sub>2</sub> is removed by glutathione peroxidase (GPX) or catalase (9).

No study was performed on enzymatic antioxidants such as SOD in saliva of paan consumers, thus, it is difficult to compare the results of this study with other studies; this issue may be considered as a limitation of our study.

The results of the present study are consistent with the research of Bahar vandand et al. who found that smoking increases the activity of salivary super oxide dismutase (13).

Several investigators also showed that the mean levels of SOD were significantly higher in the saliva of smokers than non smokers (14,17,20). Our results were inconsistent with the studies of Abdolsamadi and

Agnihotri. In the study of Abdolsamadi et al., the activity of salivary SOD was significantly lower in smokers compared with non-smokers (16). Agnihotri and et al., showed that the activity of SOD in saliva and gingival crevicular fluid of smokers was reduced compared with the control group and was lower in heavy smokers than light smoker (15). The difference between these results and ours may be due to the measurement of this enzyme in subjects with periodontal disease.

Zappacosta and et al., studied the level of glutathione, uric acid, and total antioxidant activity in saliva of smokers (before and after smoking a cigarette) and non-smokers.

In this study, no statistically significant difference was seen between the two groups in terms of uric acid concentration and total antioxidant activity of saliva, however, the glutathione level was significantly higher in smokers and decreased significantly after smoking (21). Reznick and et al., studied the activity of antioxidant enzymes *in vivo* and *in vitro*.

In *in vivo* study, the activities of antioxidant enzymes decreased during the first half an hour after consumption but then returned to 90-100% of previous status due to new saliva secretion (22).

In consistency in the results of the studies could be due to the differences in the type of consumed tobacco, duration and consumption pattern, method of enzyme assessment, subjects' age, research sample (saliva or blood), research method (*in vitro* or *in vivo*) and type of antioxidant agent.

Shrestha and et al., compared the status of non-enzymatic antioxidants between the consumers of masala paan (containing tobacco) and control group. In this study, the levels of vitamin C, vitamin E, and albumin were significantly lower in paan consumers than in control group (19).

The difference between these results and ours may be due to the measurement of these antioxidants in plasma of subjects as well as studying the non-enzymatic antioxidants in the mentioned research. Karincoglu and et al., studied antioxidant enzymes of catalase and SOD in saliva of patients with aphthous and healthy subjects. Salivary SOD and catalase levels were significantly higher in the patients group than the controls, but the serum levels of SOD and CAT were decreased in them.

They argued that the salivary defense mechanisms which act through antioxidant system cause the whole

body to send its stored antioxidants to the site of injury during aphthous occurrence, resulting in the increase of salivary antioxidant agents (18). This research corresponds to the present study since in both, the activity of salivary SOD increases, however during aphthous, the enzymatic changes precede the appearance of the lesion, while paan consumption alters the antioxidant system in consumers.

Goku and et al., showed that the antioxidant enzymes SOD and catalase were significantly reduced in tissue samples of oral squamous cell carcinoma group than the control. While, the SOD levels in the erythrocytes were higher in patients in comparison with the control group.

They emphasized that the imbalance of oxidant/antioxidant system as a risk factor in cancer may be considered (23). Finally, it can be mentioned that the periodic assessment of salivary antioxidant system in paan consumers can play an important role in the early treatment of paan damaging effects in oral cavity.

## Conclusions

The results of this study showed that salivary superoxide dismutase enzyme activity in paan consumers is higher than the non-consumers.

## Acknowledgments

We would like to thank the Zahedan University of medical sciences for financial support of this research project.

**Funding:** This study a part thesis and research project (Grant No: 5539-T) supported and funded by Zahedan University of Medical Sciences.

**Conflict of interest:** There was no conflict of interest.

## References

1. Somatunga LC, Sinha DN, Sumanasekera P, Galapatti K, Rinchen S, Kahandaliyanage A, et al. Smokeless tobacco use in Srilanka. *Indian J cancer* 2012; 49: 357- 63.
2. Auluck A, Hislop G, Poh C, Zhang L, Rosin MP. Areca nut and betel quid chewing among South Asian immigrants to Western countries and its implications

- for oral cancer screening. *Rural Remote Health* 2009; 9: 1118.
3. Nair U, Bartsch H, Nair J. Alert for an epidemic of oral cancer due to use of the betel quid substitutes gutkha and pan masala: a review of agents and causative mechanisms. *Mutagenesis* 2004; 19: 251-62.
4. Talukdar FR, Ghosh SK, Laskar RS, Mondal R. Epigenetic, genetic and environmental interactions in esophageal squamous cell carcinoma from northeast India. *PLoS One* 2013; 8: e 609-96.
5. Rozi S, Akhtar S. Prevalence and predictors of smokeless tobacco use among high-school males in Karachi, Pakistan. *East Mediterr Health J* 2007; 13: 916-24.
6. Imam SZ, Nawaz H, Sepah YJ, Pabaney AH, Ilyas M, Ghaffar S. Use of smokeless tobacco among groups of Pakistani medical students - a cross sectional study. *BMC Public Health* 2007; 7: 231.
7. Sapkota A, Gajalakshmi V, Jetly DH, Roychowdhury S, Dikshit RP, Brennan P, et al. Smokeless tobacco and increased risk of hypopharyngeal and laryngeal cancers: a multicentric case-control study from India. *Int J Cancer* 2007; 121: 1793-8.
8. Strzelczyk JK, Wiczowski A. Oxidative damage and carcinogenesis. *Contemp Oncol (Pozn)* 2012; 16: 230-3.
9. Sirohi Y, Shetty DC, Urs AB, Rai HC. Profiling of antioxidant superoxide dismutase in saliva of oral submucous fibrosis patients to categorize its diagnosis in varying stages. *Pak J Med Res* 2011; 50: 120-2. [In Pakistan]
10. Bagchi M, Balmoori J, Bagchi D, Stohs SJ, Chakrabarti J, Das DK. Role of reactive oxygen species in the development of cytotoxicity with various forms of chewing tobacco and pan masala. *Toxicology* 2002; 179: 247-55.
11. Khan GJ, Mehmood R, Salah-ud-Din, Ihtesham-ul-Haq. Effects of long-term use of tobacco on taste receptors and salivary secretion. *J Ayub Med Coll Abbottabad* 2003; 15: 37-9.
12. Shetty SR, Babu SG, Kumari S, Karikal A, Shetty P, Hegde S. Salivary superoxide dismutase levels in oral leukoplakia and oral squamous cell carcinoma; a clinic oopathological study. *Oxid Antioxid Med Sci* 2013; 2: 69-71.
13. Baharvand M, Maghami AG, Azimi S, Bastani H, Ahmadih A, Taghibakhsh M. Comparison of

superoxide dismutase activity in saliva of smokers and nonsmokers. *South Med J* 2010; 103: 425-7.

14. Kanehira T, Shibata K, Kashiwazaki H, Inoue N, Morita M. Comparison of antioxidant enzymes in saliva of elderly smokers and non-smokers. *Gerodontology* 2006; 23: 38-42.

15. Agnihotri R, Pandurang P, Kamath SU, Goyal R, Ballal S, Shanbhogue AY, et al. Association of cigarette smoking with superoxide dismutase enzyme levels in subjects with chronic periodontitis. *J Periodontol* 2009; 80:657-62.

16. Abdolsamadi HR, Goodarzi MT, Mortazavi H, Robati M, Ahmadi-Motemaye F. Comparison of salivary antioxidants in healthy smoking and non-smoking men. *Chang Gung Med J* 2011; 34: 607-11.

17. Saggi TK, Masthan KMK, Dudanakar MP, Nisa SUI, Patil S. Evaluation of salivary Antioxidant Enzymes among smokers and nonsmokers. *World J Dent* 2012; 3: 18-21.

18. Karıncaoglu Y, Batcioglu K, Erdem T, Esrefoglu M, Genc M. The levels of plasma and salivary antioxidants in the patient with recurrent aphthous stomatitis. *J Oral Pathol Med* 2005; 34:7-12.

19. Shrestha R, Nepal AK, Lal Das BK, Gelal B, Lamsal M. Non-enzymatic antioxidant status and biochemical parameters in the consumers of Pan Masala containing tobacco. *Asian Pac J Cancer Prev* 2012; 13: 4353-6.

20. Belce A, Uslu E, Kucur M, Umut M, Ipbüker A, Seymen HO. Evaluation of salivary sialic acid level and Cu-Zn superoxide dismutase activity in type 1 diabetes mellitus. *Tohoku J Exp Med* 2000;192:219-25.

21. Zappacosta B, Persichilli S, De Sole P, Mordente A, Giardina B. Effect of smoking one cigarette on antioxidant metabolites in the saliva of healthy smokers. *Arch Oral Biol* 1999; 44: 485-8.

22. Reznick AZ, Klein I, Eiserich JP, Cross CE, Nagler RM. Inhibition of oral peroxidase activity by cigarette smoke: in vivo and in vitro studies. *Free Radic Biol Med* 2003; 34: 377-84.

23. Gokul S, Patil VS, Jailkhani R, Hallikeri K, Kattappagari KK. Oxidant-antioxidant status in blood and tumor tissue of oral squamous cell carcinoma patients. *Oral Dis* 2010; 16:29-33.