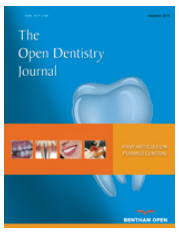
1874-2106/19



The Open Dentistry Journal

Content list available at: https://opendentistryjournal.com

RESEARCH ARTICLE

The Effect of Zinc Lactate added Tablets on Volatile Sulfur-containing Compounds in the Oral Cavity

Pier F. Porciani[1,*] and Simone Grandini[1]

[1]*Dental School, University of Siena, Siena, Italy*

Abstract:

Background:

Oral malodor is defined as breath that is offensive to others and its prevalence is around 35%.

Objective:

A controlled clinical double-blinded study was conducted to assess the efficacy of sugar-free tablets containing zinc lactate on oral Volatile Sulfur-containing Compounds (VSC) *versus* placebo tablets.

Methods:

All participants, who met the inclusion criteria, had to score a level of VSC ≥75 ppb at the basal measurement. Subjects were randomly assigned to one of the groups. The test tablet (0.7g) contained 0.255 mg of zinc lactate; the control tablet was identical but without the active agent. The OralChroma2© device was utilized to evaluate VSC. The levels were recorded at baseline, after sucking two tablets in succession and after 1 hour and 2 hours. Data were analyzed with SPSS and significance was set at α=0.05.

Results:

186 subjects completed the trial. The mean reduction from baseline at the end of tablets sucking was, respectively, 43% (p <0.001) in the control and 67% (p <0.001) in the test group, after 1 hour, it was 6% in the control (p=NS) and 25% (p <0.001) in the test group, after 2 hours, it was 3% in the control (p=NS) and 12% (p <0.001) in the test group. The comparison between the two groups after baseline adjustment showed a statistically significant difference for reductions at the end of the sucking period (p <0.001), after 1 hour (p <0.001) and after 2 hours (p <0.05).

Conclusion:

Tablets containing zinc lactate can statistically reduce the oral VSC levels immediately and for over 2 hours.

keywords: Zinc Lactate, Tablet, Halitosis, Volatile sulphur compounds, Halimeter, Food.

Article History	Received: July 15, 2019	Revised: August 28, 2019	Accepted: October 20, 2019

1. INTRODUCTION

Oral malodor is defined as breath that is offensive to others [1, 2] and a problem for millions of people as its prevalence is around 30% for moderate and 5% for severe bad breath [1]. It is one of the most frequent causes for dental visits after caries and periodontal disease [3], therefore, dentistry is giving to this condition growing attention. It can be classified into intra and extra oral due to its origin [4]. Most studies agree that the 80-90% of reported cases must be elicited by intra-oral causes and they are generally correlated to periodontal disease, bacterial coating of the tongue and some types of food [4, 5]. Anaerobic bacteria present in tongue coatings are usually the primary cause of this condition [6]. The tongue dorsum and its papillary structure retain a consistent amount of food debris and are often colonized by Gram-negative anaerobes, *i.e. Porphyromonas gingivalis, Prevotella intermedia, Fusobacterium nucleatum, Bacteroides forsythus, and Treponema denticola* [3, 6]. Metabolites of these bacteria, when released in the exhaled air are perceived as offensive odors. They include Volatile Sulfur Compounds (VSC), such as hydrogen sulfide and methyl mercaptan, diamines, like cadaverine and putrescine, and short chain fatty acids, like butyric, valeric and

* Address correspondence to this author at Dental School, University of Siena, Piazza Pietro Leopoldo 8 Firenze I-50134, Italy; Tel: +393356262040; Fax: +39055482861; E-mail: piercateadsl@libero.it

propionic acid [2, 7]. Various foods, such as garlic, onion, cabbage, Brussels sprouts, cauliflower or radish, spices such as curries, licorice, as well as habits such as tobacco smoking or drinking alcohol can be the reason of bad breath [2]. Oral malodor is also common on awakening and is usually a consequence of low salivary flow and stagnation during sleep [7]. A small number of cases may be classified as extra oral halitosis; in these conditions, a proper investigation or systemic causes and management of underlying conditions is of great importance. Starvation, acute/chronic respiratory diseases, severe renal or liver failure, drugs that cause a dry mouth are the most important extra-oral causes for oral malodor [4]. The traditional assessment of the presence and of the degree of oral malodor was performed by smelling the exhaled air (organoleptic method) [2, 8]. Years ago, the use of a portable VSC detector (Halimeter) was introduced and widely used to investigate bad breath, but this device could not distinguish among the different types of sulfides [8]. Today, gas chromatography is generally considered the best method to analyze, separate and quantify the volatile sulfur compounds [1, 5, 8, 9]. Moreover, the microbial testing of tongue coatings was proposed as another possible method for the assessment of oral malodor. The oral flora of the tongue, linked to the bad breath, can be assessed by using the BANA Strip Test (benzoyl-arginine-naphthyl-amide), which exploits an enzyme found in *Treponema denticola, Porphyromonas gingivalis, and Bacteroides forsythus*, three anaerobic bacteria highly associated with periodontitis and malodor [10]. There is no specific treatment for the oral malodor, rather a workout of potential advice and measures to be performed by the patient and the clinician. The first step must be a correct diagnosis and the treatment of dental and oral diseases or any medical and systemic cause. Patients must be informed to take regular meals and to avoid smoking and odiferous foods. Moreover, oral hygiene by tooth brushing, flossing and tongue cleaning is compulsory [1, 2, 4]. Currently, oral antiseptics containing chlorhexidine, cetylpyridinium chloride or zinc acetate are used and proved to be effective in reducing malodor [11]. Zinc salts are reported to have a prolonged effect over three hours when administered in mouthrinses, while shorter effects have been reported with zinc containing lozenges or chewing gums [11 - 16]. It is assumed that zinc ions form stable and non-volatile mercaptides with precursors of VSC or with the VSC directly since zinc has an affinity for sulfur [17]. Zinc containing chewing gum was tested in a trial on 11 volunteers and it reduced oral VSC by 45% after 5 minutes of mastication, similarly to a zinc-containing mouthrinse, otherwise a placebo chewing gum was ineffective [13]. In a previous research "*in vivo*," a chewing gum containing zinc acetate and magnolia bark extract showed a significant reduction of oral VSC levels for over one hour and, moreover, it reduced oral VSC significantly more than a control chewing gum [16]. Recently, it has been published a trial on the effect of zinc lactate and magnolia bark extract added tablets on VSC. Authors reported that the mean of percentage reductions from baselines at the end of tablets sucking was 39% in the control group (p <0.001) and 62% in the test group (p <0.001), one hour later it was 6% in the control group and 30% in the test group (p <0.001), and two hours later it was 2% in the control group and 18% in the test group (p <0.001). The comparison between the two groups after baseline adjustment showed a statistic significant difference for reductions of VSC between the test and the control tablets at the end of the sucking period (p <0.005), after one hour (p <0.001) and after two hours (p <0.05), therefore they concluded that tablets containing zinc lactate and magnolia bark extract could significantly reduce the oral VSC levels for over two hours [18]. VSC are subjected to great variation among different hours of the day [10] however, a score for total VSC of more than 75 ppb is recognized by literature as clearly detected clinical malodor [1, 8]. This controlled clinical study was designed and conducted to evaluate the efficacy over time of sugar free tablets, added with zinc lactate, on oral VSC levels versus placebo tablets.

2. MATERIALS AND METHODS

This was a single-center, double-blind, clinical trial in adult volunteers. To enter the trial, they were required to have at least 24 of their teeth, no report of oral and systemic diseases and no removable dentures. All eligible participants were required to avoid any professional oral hygiene, to refrain from taking medicines for two weeks before the test, and to be not menstruating. The following restrictions were given for the six-hour period before the visit and during the test: they did not have to brush their teeth and tongue, to smoke, to drink alcohol, coffee or tea and to eat onion, garlic, cabbage, Brussels sprouts, cauliflower, radish, curries, licorice. They received a plain toothpaste containing only sodium monofluorophosphate (Elmex®, Gaba, Colgate-Palmolive, PL-58-100 Świdnica) to use for three days before the study. At the beginning of the trial, they had to show a basal oral VSC score of ≥75 ppb in order to join the assessment. Each eligible subject was randomly allocated to the test or the control group. The test tablet (0.7g) contained zinc lactate (delivering 0.255 mg of zinc per tablet) and no additional agents reputed to be active against oral VSC; the control tablet was identical in taste, shape, weight, color, and packaging but without active ingredients to blind the operator and the participants each other. All tablets investigated were sugarless, available on the market labeled as common food, and provided by the manufacturer (Perfetti Van Melle S.p.A., Lainate, MI, Italy). The OralChroma2© device was utilized following the manufacturer's instructions to evaluate the total oral VSC. This newly developed portable gas chromatograph (OralChromaTM, Abilit Corp., Osaka, Japan) does not use a special carrier gas (using air instead) and is highly sensitive, yet relatively low cost, compared with a standard gas chromatograph [8, 9]. Sterile single-use syringes without needle (1ml capacity) were used to collect oral breath.

2.1. Protocol

Each participant joined in a random table one of the two groups. Subjects had their oral total VSC (H_2S, CH_3S, $(CH_3)_2S$) measured before sucking tablets (baseline), at the end of the two tablets sucking (one followed by another), after 1 hour and after 2 hours. They were requested to have a deep breath, to close their mouth setting inside a thin sterile syringe, to fix it by their lips and to wait for 30 seconds. They had to avoid contact between syringe and saliva. At the end of the time, the operator pulled the syringe's piston up and down and then up

again to sample 1ml of oral air. After this procedure, the operator injected the air into the OralChroma2© device. The analysis time was four minutes allowing the follow up of the oral VSC content at the fixed time points. This sequence was repeated at baseline, at the end of sucking that generally took about 10 minutes and, then, after 1 hour and 2 hours.

2.2. Statistical Analysis

In literature, there is only one trial with tablets added with similar ingredients (zinc lactate and magnolia bark extract) [18]. Authors of this trial experienced a drop out of 22% [18]. Therefore, based on the data from this research, to set the power at 80% and a significance of α=0.05, for the test after 2 hours, the minimum number of participants resulted in 85 subjects for each group which grows to 109 for drop-out adjustment. The raw data were analyzed by an independent statistical consultant blinded to the objective of the trial. All data were analyzed with SPSS software (IBM, Armonk, NY, USA) for mean, SD, variance, the Levene test was performed to assume the homogeneity of variances. The comparisons within the groups were performed using paired T-test. The differences of VSCs at the end of sucking, after 1 hour and after 2 hours between the test and the control group were compared after baselines adjustment with ANOVA and Tukey post-hoc test. All statistical tests of hypotheses were two sided and employed a level of significance of α=0.05.

2.3. Ethical Issue

This study was conducted in accordance with ethical principles that have their origin in the Declaration of Helsinki and approximate Good Clinical Practice guidelines [19] and all subjects signed a written informed consent form clearly explaining the trial procedure and the foods administrated. The tablets used in the trial were supplied by the manufacturer. They are and were available on the market under the Clean Breath 2 hours brand (Perfetti Van Melle Group B.V. 1117 CE Schiphol - Oost Amsterdam (The Netherlands)). Both tablets are a common human food and therefore they are constituted by common food ingredients and authorized food additives, moreover, they were administrated in a common dosage. The dentifrice used in the trial is available over the counter and participants were requested to maintain their regular oral hygiene procedures. As a matter of fact, the protocol of this study could reflect the normal conduct of everyday life and therefore all Ethical Issues were respected.

3. RESULTS

218 subjects entered the trial, but 32 were lost due to personal reasons or to their lower basal level of oral VSC (drop off 4.7%), therefore, 186 volunteers completed the protocol, 93 in the test group and 93 in the control group. In the test group, they were healthy adults between the age of 18 and 58 (mean 37±12 years), 51 women and 42 men. In the control group, they were healthy adults between the age of 18 and 56 (mean 36±11 years), 46 women and 47 men. None reported problems referred to zinc lactate. At baseline, the VSC mean was 145±68 ppb in the control group and 143±63 ppb in the test group; after the tablets sucking, it was 84±42 ppb in the control group and 47±37 ppb in the test group, after 1 hour it was 137±61 ppb in the control group and 108±55 ppb in the test gro-up, after 2 hours it was 140±61 ppb in the control group and 126±58 ppb in the test group. Results are summarized in Table **1**.

Table 1. VSC at different times within the groups.

Time	Control Group (ppb)		Test Group (ppb)	
	Mean	SD	Mean	SD
Baseline	145[a]	68	143[a]	63
After Ts*	84[b]	42	47[b]	37
After 1 hour	137[a]	61	108[c]	55
After 2 hours	140[a]	61	126[d]	58

*after ten minutes of tablets sucking.
Different letters mean statistical significance (p <0.001) intra-groups (paired T-test)

The mean of percentage reduction from baselines at the end of tablets sucking was 43% in the control group (p <0.001) and 67% in the test group (p <0.001), after 1 hour it was 6% in the control group (p=NS) and 25% in the test group (p <0.001) and after 2 hours it was 3% in the control group (p=NS) and 12% in the test group (p <0.001). The analysis of variance indicated no statistical difference between the baseline scores of the two groups. The comparisons between the test group and the control group were analyzed after the baseline adjustment as covariate. ANOVA and Tukey post-hoc test showed a statistically significant difference for reductions of VSC between the test and the control tablets at the end of the sucking period (p <0.001), after 1 hour (p <0.001) and after 2 hours (p <0.05) showing a better activity of the tablets added with zinc lactate in decreasing and maintaining a lower score of total oral VSC over all the time. The acceptance of both tablets was high, and no subjects reported problems related to them.

4. DISCUSSION

In a previous study [18], we showed the efficacy of tablets containing the association of zinc lactate and magnolia bark extract to decrease oral malodor, the present study was planned with sugar free tablets containing only zinc lactate, however at a higher dose than previously tested.

The tablets employed in the present study were sugar free food products and, because they are not intended to cure or prevent any disease, they were tested after only one consumption episode, to determine the reduction of the offen-sive oral malodor immediately after consumption and up to two hours. Tablet assumption itself may have a mechanical role in reducing bacteria or food debris by increasing saliva flow. Therefore, in this research, to clearly evaluate this factor on the effect over VSC reduction, a tablet without active ingredients was used as control. The organic salt was contained at a concentration lower than those reported in past trials with mouthrinses [11, 15], and quite like other researches made with chewing gums [12 - 14, 18]. Previous research reported the superior efficacy of 2 mg zinc acetate containing chewing gum over placebo gum in diminishing oral VSC after use [13]. Placebo gum showed a measurable effect, probably due to the production of stimulated saliva and the partial removal of plaque and food debris and decreased oral VSC better than the negative control (water rinse) [13]. Lately, a placebo and a zinc citrate chewing gum were reported to decrease volatile sulfur compounds over time, but, without clear differentiation bet-

ween the two chewing gums [12]. In a recent study with a chewing gum containing zinc acetate and magnolia bark extract, authors reported a VSC reduction of 50.9% (p <0.05) from baseline at the end of 10 minutes of test chewing gum mastication and of 27.6% (p <0.05) after one hour [16]. Since any proposed odor reducing mean should work when needed, in this study the efficacy of this zinc lactate tablet was not tested in all subjects at the same time (*e.g.* morning), but around the day, only when the volunteers showed a clinically measurable malodor (VSC > 75 ppb). It is supposed that Zinc lactate decreases the concentration of oral VSC because as the tablet is dissolved it comes in contact with oral fluids releasing Zn^{2+} ions, which can form nonvolatile and stable mercaptides with VSC and their precursors due to its affinity for sulphur [15, 20]. Recently, we published a trial on the effect of zinc lactate and magnolia bark extract added tablets on VSC and we reported that the mean of percentage reductions from baselines at the end of tablets sucking was 62% (p <0.001), one hour later 30% (p <0.001), and two hours later 18% (p <0.001), therefore, we concluded that tablets containing zinc lactate and magnolia bark extract could significantly reduce the oral VSC levels for over two hours [18]. The results of this trial are similar to those shown in this previous study suggesting that an increased dose of zinc could substitute the enhancing effect due to the magnolia bark extract [21]. However, additional research is needed to clearly assess the interaction between zinc lactate assumption and oral VSC reduction over time.

CONCLUSION

Tablets are used as a good vehicle for delivering active ingredients as common food. Zinc lactate added tablets can significantly reduce the oral VSC levels after sucking and for over 2 hours. Moreover, the test tablets reduce oral VSC significantly more than the control tablets at any time, showing a better activity of the tablets added with zinc lactate in decreasing and maintaining a lower score of total oral VSC over time.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The ethical approval was not obtained due to the peculiar matter of the study.

HUMAN AND ANIMAL RIGHTS

This study was conducted in accordance with ethical-principles that have their origin in the Declaration of Helsinkiand approximate Good Clinical Practice guidelines.

CONSENT FOR PUBLICATION

The participants provided written informed consent to be involved in this study.

AVAILABILITY OF DATA AND MATERIALS

The data sets analyzed during the current study are available from the corresponding author (P.F.Porciani) upon reasonable request.

FUNDING

None.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

ACKNOWLEDGEMENTS

Perfetti Van Melle S.p.A., Lainate MI, Italy supported this trial with materials and funds.

REFERENCES

[1] Rösing CK, Loesche W. Halitosis: An overview of epidemiology, etiology and clinical management. Braz Oral Res 2011; 25(5): 466-71. [http://dx.doi.org/10.1590/S1806-83242011000500015] [PMID: 22031062]

[2] Tangerman A. Halitosis in medicine: A review. Int Dent J 2002; 52(52)(Suppl. 3): 201-6. [http://dx.doi.org/10.1002/j.1875-595X.2002.tb00925.x] [PMID: 12090453]

[3] Loesche WJ, Kazor C. Microbiology and treatment of halitosis. Periodontol 2000 2002; 28: 256-79. [http://dx.doi.org/10.1034/j.1600-0757.2002.280111.x] [PMID: 12013345]

[4] Tangerman A, Winkel EG. Intra- and extra-oral halitosis: Finding of a new form of extra-oral blood-borne halitosis caused by dimethyl sulphide. J Clin Periodontol 2007; 34(9): 748-55. [http://dx.doi.org/10.1111/j.1600-051X.2007.01116.x] [PMID: 17716310]

[5] Nalçaci R, Sönmez IS. Evaluation of oral malodor in children. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2008; 106(3): 384-8. [http://dx.doi.org/10.1016/j.tripleo.2008.03.001] [PMID: 18602293]

[6] De Boever EH, Loesche WJ. Assessing the contribution of anaerobic microflora of the tongue to oral malodor. J Am Dent Assoc 1995; 126(10): 1384-93. [http://dx.doi.org/10.14219/jada.archive.1995.0049] [PMID: 7594010]

[7] Rosenberg M. Clinical assessment of bad breath: current concepts. J Am Dent Assoc 1996; 127(4): 475-82. [http://dx.doi.org/10.14219/jada.archive.1996.0239] [PMID: 8655868]

[8] van den Broek AM, Feenstra L, de Baat C. A review of the current literature on aetiology and measurement methods of halitosis. J Dent 2007; 35(8): 627-35. [http://dx.doi.org/10.1016/j.jdent.2007.04.009] [PMID: 17555859]

[9] Murata T, Rahardjo A, Fujiyama Y, *et al.* Development of a compact and simple gas chromatography for oral malodor measurement. J Periodontol 2006; 77(7): 1142-7. [http://dx.doi.org/10.1902/jop.2006.050388] [PMID: 16805675]

[10] De Boever EH, De Uzeda M, Loesche WJ. Relationship between volatile sulfur compounds, BANA-hydrolyzing bacteria and gingival health in patients with and without complaints of oral malodor. J Clin Dent 1994; 4(4): 114-9. [PMID: 8031479]

[11] Young A, Jonski G, Rölla G. Inhibition of orally produced volatile sulfur compounds by zinc, chlorhexidine or cetylpyridinium chloride--effect of concentration. Eur J Oral Sci 2003; 111(5): 400-4. [http://dx.doi.org/10.1034/j.1600-0722.2003.00063.x] [PMID: 12974683]

[12] Rösing CK, Gomes SC, Bassani DG, Oppermann RV. Effect of chewing gums on the production of Volatile Sulfur Compounds (VSC) *in vivo*. Acta Odontol Latinoam 2009; 22(1): 11-4. [PMID: 19601490]

[13] Wåler SM. The effect of zinc-containing chewing gum on volatile sulfur-containing compounds in the oral cavity. Acta Odontol Scand 1997; 55(3): 198-200. [http://dx.doi.org/10.3109/00016359709115416] [PMID: 9226432]

[14] Reingewirtz Y, Girault O, Reingewirtz N, Senger B, Tenenbaum H. Mechanical effects and volatile sulfur compound-reducing effects of chewing gums: Comparison between test and base gums and a control group. Quintessence Int 1999; 30(5): 319-23. [PMID: 10635286]

[15] Young A, Jonski G, Rölla G. The oral anti-volatile sulphur compound effects of zinc salts and their stability constants. Eur J Oral Sci 2002; 110(1): 31-4. [http://dx.doi.org/10.1034/j.1600-0722.2002.00157.x] [PMID: 11878757]

[16] Porciani PF, Grandini S. The effect of zinc acetate and magnolia bark

extract added to chewing gum on volatile sulfur-containing compounds in the oral cavity. J Clin Dent 2012; 23(3): 76-9. [PMID: 23210417]

[17] Ng W, Tonzetich J. Effect of hydrogen sulfide and methyl mercaptan on the permeability of oral mucosa. J Dent Res 1984; 63(7): 994-7. [http://dx.doi.org/10.1177/00220345840630071701] [PMID: 6588090]

[18] Porciani PF, Grandini S, Chazine M. The effect of zinc lactate and magnolia bark extract added tablets on volatile sulfur-containing compounds in the oral cavity. J Clin Dent 2014; 25(3): 53-6. [PMID: 26054178]

[19] Shaw D, McMahon A. Ethicovigilance in clinical trials. Bioethics 2013; 27(9): 508-13. [http://dx.doi.org/10.1111/j.1467-8519.2012.01967.x] [PMID: 22506737]

[20] Campus G, Cagetti MG, Cocco F, *et al.* Effect of a sugar-free chewing gum containing magnolia bark extract on different variables related to caries and gingivitis: A randomized controlled intervention trial. Caries Res 2011; 45(4): 393-9. [http://dx.doi.org/10.1159/000330234] [PMID: 21822018]

[21] Tian M, Dodds MWJ, Greenberg MJ, Estrich C. Interaction of MBE with oral bacteria and salivary protein J Dent Res 2010; 89(Spec Iss B): 3302..