The Effect of *Salvadora persica* L. Sticks on Bacterial Counts and Oral Odor: A Crossover Clinical Study

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Abstract: The study aimed to determine the effects of *Salvadora persica* L. sticks on oral bacteria and odor in comparison to traditional mouth disinfection with mouth rinses. Thirty-five healthy fasting volunteers took part in a crossover clinical trial involving the application of Listerine® Cool Mint® mouth rinse by the traditional panoral rinsing method or a site-specific method targeting the subgingival and supragingival plaque with *Salvadora persica* L. sticks. The viable anaerobic and aerobic bacterial counts, volatile sulfur compounds (VSCs) levels, organoleptic assessment of oral odor and the tongue-coating index were compared at baseline, as well as 1, 5 and 9 hours after the treatment. *Salvadora persica* L. sticks reduced the VSCs and anaerobic bacterial loads while keeping the aerobic bacterial numbers higher than the traditional panoral disinfection method with Listerine® Cool Mint® rinse. Therefore, *Salvadora persica* L. sticks maintain a healthy oral cavity more effectively by predominantly disinfecting the niches of anaerobic bacteria within the oral cavity.
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1. Introduction

Many surfaces, fissures and sulci harboring bacterial biofilms exist inside the human mouth (Aas et al., 2005; Preza et al., 2009). Oral infections, such as periodontitis, dental caries, oral thrush and oral malodor, are common complications of poor oral hygiene, caused by the growth of certain bacterial communities (Bornstein et al., 2009; Kamppi et al., 2013; Liu et al., 2006). Cases of oral malodor, known as halitosis, originating from the oral cavity represent 85-90% of all halitosis conditions (Scully and Greenman, 2008). *Fusobacterium, Prophyromonas*, *Actinobacillus, Prevotella* and other Gram-negative anaerobic bacteria are responsible for producing foul volatile sulfur compounds (VSCs), leading to malodor and halitosis (Krespi et al., 2006). In contrast, an elevated aerobic Gram-positive bacterial count acts as a defensive mechanism against halitosis (McNamara et al., 1972). Currently, oral hygiene is maintained by regular tooth brushing and rinsing with an antiseptic mouthwash. In many eastern countries, however, *Salvadora persica* L. (miswak) is used to brush the tooth surfaces, and was found effective in removing sub- and supra-gingival plaque (Uddin and Kanatas, 2014). Empirical evidence indicates that 50% *Salvadora persica* L. extracts and 0.2% chlorhexidine have similar effect on dentin (Almas, 2002), as they contain tooth-strengthening fluoride (Aslani et al., 2013) and possess antimicrobial properties (Talha et al., 2013). However, no clinical comparison of the clinical effect of *Salvadora persica* L. sticks on oral odor, bacterial counts and VSCs to that of other conventional methods, like mouth rinses, has been performed to date.

Different oral surfaces, fissures, spaces and sulci harbor different microorganisms (Aas et al., 2005; Preza et al., 2009). That is, there are different communities located at different location inside the human mouth (Allaker et al., 2008). The offensive malodor-producing Gram-negative anaerobic bacteria colonize the sub- and supra-gingival plaque material (Tezal et al., 2006). The otherwise harmless commensal bacteria (Preza et al., 2009) and beneficial bacteria (Burton et al., 2005) colonize the anteroapical surface of the tongue and the medial aspects of the cheeks (Burton et al., 2005). Accordingly, sensitive molecular techniques failed to detect the anti-halitosis beneficial *Streptococcus salivarius* strain k12 on tooth surfaces and sulci (Horz et al., 2007). *Salvadora persica* L. sticks can be used in the maintenance of oral hygiene, whereby the tooth surfaces are brushed and rubbed, enabling the removal of sub- and supra-gingival plaque (Uddin and Kanatas, 2014). In contrast, the mouth rinses are applied indiscriminately to the entire oral cavity.

Extant literature contains many reports on the effects of mouth rinses (Shinada et al., 2008) and chewing gums (Keller et al., 2012) on oral malodor reduction. However, these methods are known to have only a temporary effect (Tezal et al., 2006). Thus, this study aims to compare the treatment with *Salvadora persica* L. sticks to that using Listerine® Cool Mint® mouth rinse by analyzing the bacterial composition and counts, the tongue-coating index, and volatile sulfur compounds (VSCs) levels, in addition to conducting the organoleptic (olfactory perception by a trained examiner of oral odor) assessment.

2. Materials and Methods

Subjects: Thirty-five healthy males (aged 19-44 years) adhering to the Islamic ritual of fasting participated in this clinical crossover study during the month of Ramadan in the lunar Hajri year 1433 (July - August 2012) in Albaha city, Saudi Arabia. All participants' salivary flow rates were normal (>1 ml/min) and none reported using antibiotics or *Salvadora persica* L. sticks at any point during the 3 months preceding the study. Moreover, none of the participants obtained any form of dental care (scaling, root planning, or dental surgery) or medical advice in the year prior to the study. All participants provided written informed consents. Ethical approval was obtained from the Institutional Research Committee, Faculty of Medical Sciences, Albaha University.

Treatment Protocol: The study commenced on the third day of Ramadan in the lunar Hajri year 1433. All participants practiced the ritual of fasting during the entire course of the study and brushed their teeth with toothpaste twice daily. They abstained from eating and drinking (and avoided any other ingestible substances) from dawn (4 am) to dusk (7 pm) while eating and drinking during dark hours only. The study was conducted in two phases, each lasting 13 days, and involved the use of Listerine® Cool Mint® mouth rinse and *Salvadora persica* L. sticks. Thus, in order to facilitate the comparison between these two treatment modes, the participants were randomly allocated into two groups. The first group (n = 18) used the mouth rinse in the first phase of the study, after which they used the *Salvadora persica* L. sticks. The second group (n = 17) used the *Salvadora persica* in the first study phase, and the mouth rinse in the second. The mouth rinse was used per the manufacturer's instructions (Johnson & Johnson, Skillman, New Jersey, USA), i.e., by swirling 20 ml in the mouth for 20 seconds. *Salvadora persica* L. sticks were used by rubbing and brushing all tooth surfaces and the gum line for 20 seconds per tooth area. As noted above, both groups were exposed to each treatment type (in reversed order), which lasted for 13 consecutive days, on the 6th hour of their fast. Every day, saliva samples, organoleptic assessment of mouth air, Winkel tongue-coating index (WTCI) and VSCs measurements were obtained for each participant, at baseline (the 6th hour of the fast) and 1, 5 and 9 hours later.

Oral Malodor Assessment: The process commenced by a trained examiner, located at a distance of 10 cm from the participant's mouth, rating the odor using a 0-5 scale after mouth closure for 1 min (Rosenberg et al., 1991). This was followed by the assessment of the coating of the tongue by the WTCI, whereby the tongue was divided into six parts and each was scored from 0 to 2 (Winkel et al., 2003). Mouth levels of VSCs were determined, in parts per billion (ppb), by means of a portable detector, Halimeter® (Interscan Corporation, Chatsworth, CA, USA), 1 min following mouth closure. Two successive measurements were carried out and the mean value used.

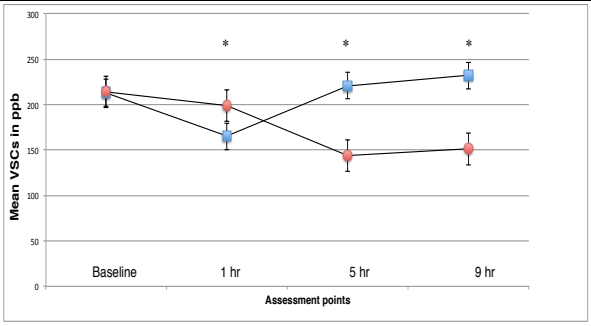
Saliva Specimens: Saliva samples were collected by asking the participants to spit inside a sterile test tube without stimulation. Each sample was immediately processed by vortexing for 30 seconds, before serially diluting the sample in normal saline and inoculating 100 µl of the resulting dilutions in two series of 5% sheep blood agar plates (Oxoid, Basingstoke, England). These series were incubated aerobically at 37 °C for 24 hours and anaerobically for 48 hours, respectively. Finally, the plates showing 30-300 colonies were used to calculate the number of colony-forming units (CFU) per ml of saliva. The CFU/ml results were averaged and log transformed.

Statistical Analysis: Bacterial colony-forming units were log transformed and presented as averages, which were subjected to the analysis of variance (ANOVA test) in order to estimate whether differences are statistically significant. The changes between the baseline and post-treatment in the two groups were measured using paired *t-test*, and all statistical analyses were performed using SPSS (Version 14; 2005 SPSS Inc. USA) software package. All comparisons were declared significant at $p < 0.05$.

3. Results

At baseline, the organoleptic and VSCs values ranged from 1.4 to 1.9 (standard deviation, SD = 0.5–0.8) and 190 to 230 ppb, respectively, for both groups. The baseline WTCI scores were more heterogeneous, ranging from 2 to 10. Nonetheless, at baseline, lower WTCI scores were obtained for the group following *Salvadora persica* L. protocol (4.7, SD = 1.4), whereas in the mouth rinse protocol, the mean WTCI (6.2, SD = 1.4) was higher. While these mean values were significantly different (p = 0.05), no correlation was found between the WTCI and VSCs. However, VSCs correlated with organoleptic scores (r = 0.45, p = 0.05). The average VSCs values pertaining to the *Salvadora persica* L. protocol significantly decreased from 214 ppb at baseline to 151 ppb, 9 hours post-treatment compared to the values obtained for the mouth rinse protocol, which increased from 213 to 232 ppb ($p < 0.001$). The VSCs values obtained at baseline and at the 1-hour assessment point were almost identical for the two protocols (213 and 214 ppb). However, the measurements performed 5 and 9 hours post-treatment revealed significant differences between the two protocols, whereby lower VSCs values were obtained for using *Salvadora persica* L. sticks (Figure 1). The decline in the VSCs achieved by the mouth rinse protocol during the first hour was

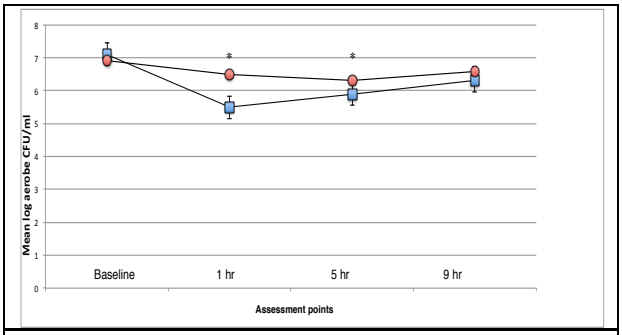
significantly ($p = 0.05$) more pronounced (from 213 to 165 ppb) compared to the reduction under the *Salvadora persica* L. protocol (214 to 198.7 ppb). However, the assessments performed 5 and 9 hours later revealed that the VSCs pertaining to the mouth rinse group subsequently increased (Figure 1). In contrast, the *Salvadora persica* L. protocol caused a gradual reduction in the first hour, faster reduction between the first hour and fifth and a slight increase between the fifths and ninth hour (Figure 1). That is, following a gradual reduction, the *Salvadora persica* L. protocol had a more long-lasting effect (Figure 1).



Legend: ●, *Salvadora persica* L. protocol; ■, mouth rinse protocol; *, statistically significant difference.

Figure 1. Mean VSCs levels for each group at every assessment point.

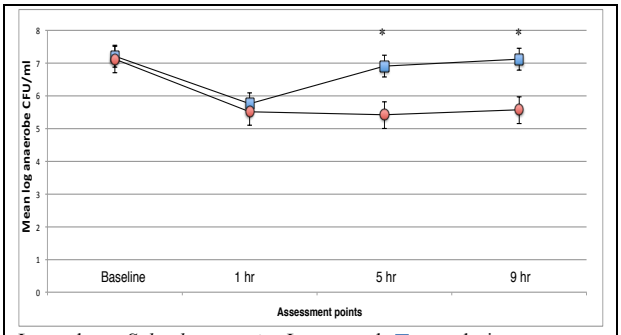
At baseline, the aerobic bacteria counts, depicted in Figure 2 as average log-CFU/ml, between the two protocols were similar. At subsequent measurements, those pertaining to the *Salvadora persica* L. protocol were more stable, whereby the log-CFU/ml of aerobic bacteria ranged between 6.9 and 6.49 during the entire assessment period (Figure 2). The average log-CFU/ml of aerobic bacteria declined from 7.1 to 5.49 within 1 hour for the mouth rinse protocol, and remained lower than the *Salvadora persica* L. protocol values during the entire assessment period ($p = 0.045$).



Legend: ●, *Salvadora persica* L. protocol; ■, mouth rinse protocol; *, statistically significant difference.

Figure 2. Mean log CFU/ml of aerobic bacteria for each group at every assessment point.

The quantities of anaerobic bacteria in saliva for the two mouth hygiene protocols, depicted as log-CFU/ml of anaerobic bacteria in saliva in Figure 3, were almost identical at baseline (7.2 and 7.1 for the mouth rinse and *Salvadora persica* L. protocol, respectively). Similarly, both protocols showed comparable reduction in the anaerobic bacteria levels in saliva at the 1 hour assessment point (Figure 3). However, while the anaerobic bacteria log-CFU/ml values corresponding to the mouth rinse protocol subsequently returned to their baseline level, those pertaining to the *Salvadora persica* L. protocol remained below the baseline ($p < 0.05$).



Legend: ●, *Salvadora persica* L. protocol; ■, mouth rinse protocol; *, statistically significant difference.

Figure 3. Mean log CFU/ml of anaerobic bacteria for each group at every assessment point.

The volunteers favored the *Salvadora persica* L. protocol, stating that it provided them with a better feeling and greater comfort than the mouth rinse protocol did. They also indicated that they would be keen to continue using *Salvadora persica* L. sticks in the future. Finally, the ratio of aerobic/anaerobic bacterial count levels remained above parity (> 1) in the *Salvadora persica* L. protocol, whereas it remained below parity (< 1) in the mouth rinse protocol throughout the 9-hour assessment period.

4. Discussion

Oral diseases, such as halitosis, dental caries and periodontitis are common bacterial infections and alternative treatments to conventional methods are now being sought (Bassiouny and Gazaerly, 2014; Ibrahim and Shaker, 2012; Yousef et al., 2012). Current treatment and management protocols are not very successful (Tezal et al., 2006). This study aimed to compare the effectiveness of *Salvadora persica* L. sticks to that of the traditional mouth rinsing. The investigation followed a crossover design, whereby the reduction in malodor-related outcomes, anaerobic and aerobic bacteria and VSCs were compared. Although the study participants were healthy, the results were sufficiently informative for drawing

conclusions and performing comparisons with the findings of similar studies in similar settings examining the efficacy of different mouth rinses when used by healthy participants (Roldan et al., 2004). The obtained results indicated that *Salvadora persica* L. sticks were more effective in reducing VSCs and anaerobic bacterial counts compared to the traditional mouth rinse method. In addition, the *Salvadora persica* L. sticks did not reduce the aerobic bacterial counts, while the traditional mouth rinse protocol caused a significant reduction of this bacterial group. Malodor producing bacteria—*Fusobacterium, Prophyromonas*, *Actinobacillus, Prevotella* and other Gram-negative anaerobic bacteria—are associated with poor oral hygiene (Krespi et al., 2006). In contrast, an elevated aerobic Gram-positive bacterial count is considered defensive against halitosis (McNamara et al., 1972).

Almost one third of patients endure halitosis with its social and psychological implications (Bornstein et al., 2009; Liu et al., 2006). Thus, it is likely that the traditional mouth rinsing practices indiscriminately kill both aerobic and anaerobic bacteria. Killing aerobic Gram-positive bacteria, which are believed to be promoting oral health (Burton et al., 2005), exposes a possible weakness in the antimicrobial mouth rinse protocols. That is, owing to their unspecific antimicrobial nature, they act against not only Gram-negative anaerobic bacteria, but also Gram-positive aerobic oral bacteria, which include beneficial probiotic strains (Burton et al., 2005). These aerobic bacteria include strains that have been shown to be beneficial in the medical management of tooth decay, plaque formation, streptococcal pharyngitis and oral malodor (Burton et al., 2006a; Burton et al., 2005; Burton et al., 2006b; Pradeep et al., 2014). This can explain the observed short-term effect of mouth rinses on VSCs and aerobic/anaerobic ratios of bacteria described in this study and elsewhere for the traditional mouth rinses used as part of oral hygiene (Roldan et al., 2004). Moreover, the current mouth rinsing protocol may be more effective in killing the aerobic bacteria located in areas more exposed to air (on bare surfaces), than the anaerobic bacteria sheltered within crypts, fissures and in the sub- and supra-gingival plaque. The palate, cheek mucosae and the anterior dorsum of the tongue are neither proliferation nor accumulation sites for anaerobic bacteria, as these surfaces are usually free from crypts, fissures and accumulated debris. The anaerobic bacteria are noticeably more commonly identified within areas characterized by lower ventilation and higher debris accumulation. That is, the sub- and supra-gingival plaque material (Tezal et al., 2006) and the coating of the crypts and fissures of the posterior tongue dorsum (Donaldson et al., 2005). Therefore, it is plausible that the superior effect of *Salvadora persica* L. protocol is due its effectiveness in eliminating the anaerobic Gram-negative bacteria while avoiding any unwanted effects on the health-promoting aerobic bacteria.

While *Salvadora persica* L. sticks did not alter the Winkel tongue-coating index scores, as high variability was detected among the study participants, the values obtained in this study were in agreement with those reported for other alternative and traditional mouth hygiene protocols (Roldan et al., 2004; Winkel et al., 2003).

5. Conclusion

The bacterial counts revealed that the *Salvadora persica* L. sticks could more selectively target the anaerobic Gram-negative bacteria, while preserving the aerobic Gram-positive bacteria. In contrast, the mouth rinsing protocol reduced the numbers of both aerobic and anaerobic bacteria.

The comparative analyses of the two protocols demonstrated that the mouth rinsing protocol was effective in achieving initial reduction in VSCs. However, its values reverted to high levels after 5 hours. On the other hand, the *Salvadora persica* L. protocol was more effective in reducing the VSCs levels and anaerobic bacteria throughout the 9-hour assessment period.

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References

1. Aas, J.A., Paster, B.J., Stokes, L.N., Olsen, I., Dewhirst, F.E., 2005. Defining the normal bacterial flora of the oral cavity. J Clin Microbiol. 43(11): 5721-5732.
2. Allaker, R.P., Waite, R.D., Hickling, J., North, M., McNab, R., Bosma, M.P., Hughes, F.J., 2008. Topographic distribution of bacteria associated with oral malodour on the tongue. Arch Oral Biol. 53(Suppl 1): S8-S12.
3. Almas, K., 2002. The effect of *Salvadora persica* extract (miswak) and chlorhexidine gluconate on human dentin: a SEM study. J Contemp Dent Pract. 3(3): 27-35.
4. Aslani, B., Ghannadi, A., Mortazavi, S., Torabi, M., 2013. Design, Formulation and Evaluation of

Medicinal Chewing Gum by the Extract of *Salvadora persica* L. Life Sci J. 10(4s): 47-55.
5. Bassiouny, G., Gazaerly, H.A., 2014. Effect of subgingival application of phytotherapeutic agents using thymus vulgaris essential oil gel along with scaling and root planing on clinical parameters and gingival tissue level of VEGF and CD34 in patients with chronic periodontitis. Life Sci J. 11(3): 346-355.
6. Bornstein, M.M., Kislig, K., Hoti, B.B., Seemann, R., Lussi, A., 2009. Prevalence of halitosis in the population of the city of Bern, Switzerland: a study comparing self-reported and clinical data. Eur J Oral Sci. 117(3): 261-267.
7. Burton, J.P., Chilcott, C.N., Tagg, J.R., 2005. The rationale and potential for the reduction of oral malodour using *Streptococcus salivarius* probiotics. Oral Dis. 11(Suppl 1): 29-31.
8. Burton, J.P., Chilcott, C.N., Moore, C.J., Speiser, G., Tagg, J.R., 2006a. A preliminary study of the effect of probiotic *Streptococcus salivarius* K12 on oral malodour parameters. J Appl Microbiol. 100(4): 754-764.
9. Burton, J.P., Wescombe, P.A., Moore, C.J., Chilcott, C.N., Tagg, J.R., 2006b. Safety assessment of the oral cavity probiotic *Streptococcus salivarius* K12. Appl Environ Microbiol. 72(4): 3050-3053.
10. Donaldson, A.C., McKenzie, D., Riggio, M.P., Hodge, P.J., Rolph, H., Flanagan, A., Bagg, J., 2005. Microbiological culture analysis of the tongue anaerobic microflora in subjects with and without halitosis. Oral Dis. 11(Suppl 1): 61-63.
11. Horz, H.P., Meinelt, A., Houben, B., Conrads, G., 2007. Distribution and persistence of probiotic *Streptococcus salivarius* K12 in the human oral cavity as determined by real-time quantitative polymerase chain reaction. Oral Microbiol Immunol. 22(2): 126-130.
12. Ibrahim, R.O., Shaker, O., 2012. The effectiveness of adjunctive systemic antibiotics to non surgical therapy in chronic periodontitis patients. J Am Sci. 8(12): 374-383.
13. Kamppi, A., Tanner, T., Pakkila, J., Patinen, P., Jarvelin, M.R., Tjaderhane, L., Anttonen, V., 2013. Geographical distribution of dental caries prevalence and associated factors in young adults in Finland. Caries Res. 47(4): 346-354.
14. Keller, M.K., Bardow, A., Jensdottir, T., Lykkeaa, J., Twetman, S., 2012. Effect of chewing gums containing the probiotic bacterium *Lactobacillus reuteri* on oral malodour. Acta Odontol Scand. 70(3): 246-250.
15. Krespi, Y.P., Shrime, M.G., Kacker, A., 2006. The relationship between oral malodor and volatile sulfur compound-producing bacteria. Otolaryngol Head Neck Surg. 135(5): 671-676.
16. Liu, X.N., Shinada, K., Chen, X.C., Zhang, B.X., Yaegaki, K., Kawaguchi, Y., 2006. Oral malodor-related parameters in the Chinese general population. J Clin Periodontol. 33(1): 31-36.
17. McNamara, T.F., Alexander, J.F., Lee, M., 1972. The role of microorganisms in the production of oral malodor. Oral Surg Oral Med Oral Pathol. 34(1): 41-48.
18. Pradeep, K., Kuttappa, M.A., Prasana, K.R., 2014. Probiotics and oral health: an update. SADJ. 69(1): 20-24.
19. Preza, D., Olsen, I., Willumsen, T., Grinde, B., Paster, B.J., 2009. Diversity and site-specificity of the oral microflora in the elderly. Eur J Clin Microbiol Infect Dis. 28(9): 1033-1040.
20. Roldan, S., Herrera, D., Santa-Cruz, I., O'Connor, A., Gonzalez, I., Sanz, M., 2004. Comparative effects of different chlorhexidine mouth-rinse formulations on volatile sulphur compounds and salivary bacterial counts. J Clin Periodontol. 31(12): 1128-1134.
21. Rosenberg, M., Septon, I., Eli, I., Bar-Ness, R., Gelernter, I., Brenner, S., Gabbay, J., 1991. Halitosis measurement by an industrial sulphide monitor. J Periodontol. 62(8): 487-489.
22. Scully, C., Greenman, J., 2008. Halitosis (breath odor). Periodontol. 48: 66-75.
23. Shinada, K., Ueno, M., Konishi, C., Takehara, S., Yokoyama, S., Kawaguchi, Y., 2008. A randomized double blind crossover placebo-controlled clinical trial to assess the effects of a mouthwash containing chlorine dioxide on oral malodor. Trials. 12(11):14.
24. Talha, W.K.M., Elsaid, M., Omar, O.M., Eissa, S.A., 2013. The effect of miswak and fluoride toothpastes on dental plaque, a comparative clinical and microbiological study. Nat Sci. 11(9): 1-7.
25. Tezal, M., Scannapieco, F.A., Wactawski-Wende, J., Grossi, S.G., Genco, R.J., 2006. Supragingival plaque may modify the effects of subgingival bacteria on attachment loss. J Periodontol. 77(5): 808-813.
26. Uddin, I., Kanatas, A., 2014. Oral health: *Salvadora persica*. Br Dent J. 216(3): 98.
27. Winkel, E.G., Roldan, S., Van Winkelhoff, A.J., Herrera, D., Sanz, M., 2003. Clinical effects of a new mouthrinse containing chlorhexidine, cetylpyridinium chloride and zinc-lactate on oral halitosis. A dual-center, double-blind placebo-controlled study. J Clin Periodontol. 30(4): 300-306.
28. Yousef, M., Shoukheba, M., Ahmed, M.H., 2012. Histological evaluation of the effect of melatonin gel in the treatment of acute one-wall intrabony defect in dogs. J Am Sci. 8(12): 929-938.

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