Is Whitening Pre-Brush Rinse a Double Edged Weapon? Evaluation of Listerine Effect on Enamel Microhardness and Surface Morphology

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Abstract: ***Background:*** In a craze for getting that flash Hollywood style smile, the majority of people rush into teeth bleaching without realizing if there are repercussions. Whitening mouth rinses appeared recently in the market and manufacturers advertised that they could prevent stains and fight plaque build-up. Generally a low concentration of hydrogen peroxide (1.5%) can be used in the formulation and it may protect the teeth surface from new stains. Listerine is one of the most common pre-brush rinses in market. Although generally positive results have been reported concerning its whitening ability, concerns still remain as its effect on dental tissues. ***Aim of the Study:*** The purpose of this investigation was to evaluate the effect of this product on enamel surface morphology using SEM and measuring its micro-hardness by Vickers hardness testing. ***Methodology:*** Thirty sound premolars were hemsectioned and divided into 3 groups. Group I served as control group where specimens were immersed in artificial saliva, group II treated with single daily application of Listerine and group III treated with double daily applications of Listerine ***.Results:*** SEM examination revealed minor surface alterations of group II when compared with group III that appeared pitted and eroded. Vickers hardness numbers of same groups were significantly lower than those of control group. ***Conclusions:*** Listerine had a potential harmful effect on enamel surface and caution should be warranted during and after its whitening procedure.

[Radwa Hegazy and Rabab Mubarak. **Is whitening pre-brush rinse a double edged weapon? Evaluation of Listerine effect on enamel microhardness and surface morphology.** Journal of American Science 2012; 8(3):126-132]. (ISSN: 1545-1003). http://www.americanscience.org. 14

Keywords: Whitening mouth rinse: Microhardness; enamel surface morphology.

1. Introduction

Mouth rinses have been used for centuries for medical and cosmetic purposes. Recently, the rationale behind the use of the mouth rinse ingredients has been subjected to scientific research and clinical trials (***Adams and Addy 1994***). The use of mouth rinses to deliver chemotherapeutic agents is well accepted by the public, both by self administration and under supervision (***Finn et al., 1975***). Mouth rinses formulations are generally simpler than dentifrices, and compatibility problems are not as large an issue as they are with dentifrice products (***Driscoll et al., 1982***).

Discoloration of teeth is a common aesthetic problem. Color changes of teeth are usually classified as intrinsic, extrinsic and internalized stains (***Watts and Addy, 2001***). Professionally supervised at-home vital tooth bleaching has become a popular method used to treat tooth discoloration. The popularity of this method is related to its quick esthetic improvement, low incidence of side effects and ease of technique with reduced chair time (***Brunton et al., 2004***). Today, bleaching products including gels, rinses, gums, dentifrices, whitening strips or paint on films are freely available at pharmacies, supermarkets and over the internet (***Auscilli et al., 2005)***

Listerine whitening pre-brush rinse is one of the most popular pre-brush rinses known on the market. It is recommended to use as a complement to more powerful primary tooth whitening solutions in response to the demand in esthetic dentistry.

According to the free encyclopedia,Wikipedia (http://en.wikipedia.org/wiki/listerine): Listerine is a brand of antiseptic mouth wash and it is one of the most popular mouth washes sold in the United States. The product is marketed under the slogan "kills germs that cause bad breath". It was named after “Joseph Lister” who promoted the idea of sterile surgery by sterilizing instruments in the 19th century. Listerine's whitening pre-brush rinse is a product to help whiten teeth prior to brushing and this will help killing germs and will add a foaming agent to get in between teeth. For the clean mint flavor of pre-brush rinse, there are water, flavor and eight other ingredients:

Alcohol: The clean mint formula has 8 percent alcohol in the pre-brush rinse. Alcohol can be drying but it also can help clean and be used as a detergent. It is used to kill germs in the mouth.

Hydrogen peroxide: it provides the foaming properties, aids in killing micro-organisms as it is antiseptic. It will oxygenate stains or blemishes on the surface and whitens the teeth.

Sodium phosphate: It is recognized as "generally recognized as safe" or GRAS, by the Food and Drug Administration. It is a buffering agent that cleanses the surface area of the teeth.

Poloxamer 407: It is used to create emulsions by helping ingredients dissolve into each other, they allow water and oil to mix with items that need to be cleansed and washed away.

Sodium laurylsulfate: It is a surfactant that creates a smooth surface area that allows a product to glide easily over it. It helps dirt cling to water and oil and get washed away.

Sodium citrate: It controls the pH of the item, making sure that it is as acidic or alkaline as it needs to be, it may also function as a preservative to keep the product from spoiling.

Sodium saccharin: It is a flavoring or sweetening agent by making a product more palatable by making it sweeter. It is around 300 times the sweetness of natural sugar.

Sucralose: Another sweetening agent. It is 600 times sweeter than natural sugar. It is low calorie and is used to keep the flavor of a product from getting too tart.

Contemporary tooth whitening (tooth bleaching) systems are based primarily on hydrogen peroxide (H_2O_2) or one of its precursors (carbamide peroxide). These bleach the chromogens within the dentine or enamel. Such agents can be applied externally to the teeth (vital bleaching) or internally within the pulp chamber (non-vital bleaching) (***Tredwin et al., 2006).***

Hydrogen peroxide is a reactive oxygen species, along with super oxide (O2-), hydroxyl (HO), peroxyl (ROO) and alkoxyl (RO) ***(Walsh 2000).*** In human tissue, intrinsic sources of H_2O_2 are organelles (especially mitochondria), salivary cells, micro-organisms and the lungs ***(Marshall et al., 2001).*** Besides, it is a colorless liquid with a bitter taste and is highly soluble in water to give acidic solution, thus it is an oxidizing agent with a wide number of industrial applications ***(Boyd 1989).*** Hydrogen peroxide production can be followed by the liberation of highly reactive oxygen species in the body via enzymatic and spontaneous redox reactions that often involve interaction with transitional metals such as iron or copper. Enzymes such as catalase, glutathione peroxidase and super oxide dismutase catalyse the decomposition of H_2O_2 into water and oxygen ***(Desesso et al., 2000).***

Tombes and Galluci (1993) reported some objective and subjective adverse effects of H_2O_2 mouth rinses, including mouth irritation, discomfort, dryness and loss of taste. Thus concerns have been expressed over the potential adverse effects of the use of H_2O_2 tooth whitening agents. Therefore, the aim of the present study was to evaluate the effect of Listerine pre brush mouth rinse on enamel surface morphology using SEM and measuring its micro-hardness by Vickers hardness testing.

2. Materials and Methods:

Thirty sound premolars extracted for orthodontic reasons were collected from the outpatient clinic of Pedodontic department, Faculty of Oral and Dental Medicine, Cairo University. Teeth were cleaned of gross debris and stored in artificial saliva (Trade name Glandoson, Glandoson Synthetic Carmellose (Saliva Spray by Fresenius Krabi Company) which is a carboxymethyl cellulose salivary substitute ***(Meyer et al., 2010).*** In our study, artificial saliva was used for simulation of the conditions given by natural human saliva.

The teeth were divided into 3 groups, ten teeth each as following:

- **Group I:** teeth were kept in 5 ml artificial saliva at 37^{o}C for 12 weeks.
- **Group II:** teeth were immersed or exposed to one daily application of 10 ml of Listerine mouth wash **(Fig.1)** for 60 seconds under constant stirring. After each session, teeth were thoroughly rinsed with deionized water for 10 seconds and then stored in artificial saliva until the next treatment.
- **Group III:** teeth were immersed or exposed to two daily applications, once in the morning and once in the night, of 10 ml of Listerine following same steps of group II.

The experiment lasted for 12 weeks according to manufacturer's instructions and following their extreme recommended situations (Johnson and Johnson health care products. MCNEIL PPC. Inc. 2007. USA. Made in Canada). All the teeth were hemi-sectioned longitudinally in mesio-distal direction with a low speed air and water cooled diamond disc to obtain sixty specimens (halves). The lingual specimens of all groups were tested for micro-hardness to find out the changes in enamel microhardness, while the buccal specimens were examined by scanning electron microscope to detect the ultra-structural changes of enamel of different groups.

Scanning electron microscopy:

Buccal halves were prepared for SEM examination as follows: mounting on the metal stub by their cut surfaces using double sided adhesive tape**,** then enamel surfaces were coated under vacuum with gold by a sputter coater (**Fig.2)** (to be coated with an electrically conductive film) to prevent image distorting electrical charges from building up on the surfaces and for better achievement of secondary electron emission which is important for the formation of an image ***(Heide and Jams, 2007).*** Coated halves were then examined by the scanning electron microscope for the detection of any possible changes in the enamel of the experimental groups when compared to the controls.

SEM examination was done in the electron microscopic department of National Research Center, Cairo, Egypt.

Microhardness analysis:

Assessment of micro-hardness was done for enamel of the lingual halves of the different groups of teeth. Micro-hardness testing was measured by the Vickers Hardness tester (Shimadzu Micro-hardness tester HMV-2 Series, China) in the department of Solid State Physics at the National Research Center, Cairo, Egypt.

The Vickers hardness test is often easier to use than other hardness tests since the required calculations are independent of the size of the indenter, and the indenter can be used for all materials irrespective of their hardness. The basic principle is to observe the questioned material's ability to resist plastic deformation from a standard source and this test is suitable for determining hardness of tooth structure ***(Lysaght and DeBellis, 1969).***

The Vickers indenter is a square based diamond pyramid that creates a clear measurable indentation in the field as diagonals with two arms approximately equal in length.

A load of 100 grams was found suitable for this test for a loading period of 15 seconds and a loading speed of 0.017 mm/second. The Vickers hardness number was calculated using the following formula:

$VHN\ (kg/mm^2) = 185.4xP/d^2$

VHN = micro-hardness for Vickers

P = testing load in grams.

d = length of the diagonal line across the indent in microns.

The hardness could be obtained directly from special conversion tables giving the value of Hardness which corresponds to each d value obtained under this load. Readings were taken from several areas of the cut enamel surface ***(Bashir, 2000).*** Collected data of micro-hardness results were tabulated and subjected to statistical analysis.

3. Results:

Scanning electron microscopic results:

The ultra-structure examination of group I (control group) at low magnifications revealed intact, smooth enamel surface with fine scratches **(Fig. 3).** The structural arrangement was found to be characteristic of the normal enamel surface with no morphological irregularities. It showed numerous characteristic minute depressions representing enamel rod ends **(Fig. 4).** At higher magnification numerous bands of small depressions representing enamel rod ends alternating with parallel lines representing perikymata (external manifestation of incremental lines of Retzius on enamel surface) (**Fig.5)**. While specimens of group II showed minor alternations of enamel surface manifested as numerous scratching with few micropores in between the ends of enamel prisms (**Fig. 6).** Pitting, porosity as well as craters with elevated peripheries were detected **(Figs.7, 8).** Scanning electron examination of enamel surface of group III revealed more pronounced alternations and severe destruction of the surface. These were manifested as increased number of pores and pittings of various sizes and depths (**Fig. 9).** The prolonged period of treatment with two daily applications of Listerine in group III specimens showed Partial removal of aprismatic surface layer of enamel in certain areas resulting in indiscriminate erosions, dissolution of prismatic substance and heavy surface roughness (**Fig. 10).**

Microhardness Results:

Statistical analysis of Vickers hardness numbers of the enamel surface of group I (control group) and post-immersion measurements of groups II and III using paired student t test are shown in **Tables (1 & 2) and Graph (1).** Enamel microhardness decreased significantly (P<0.001) after immersion of specimens in Listerine.

Table 1: Showing difference in mean Vickers microhardness numbers between control group and experimental group II using Paired Student's t-Test.

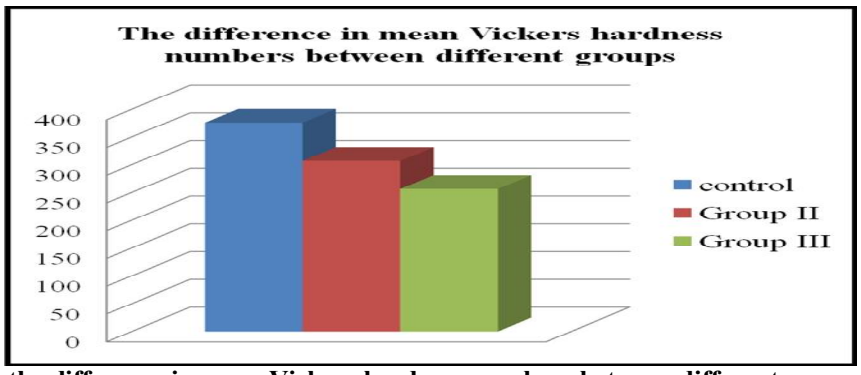
Group	Mean Vickers microhardness		
	Ms±SD	t-Value	p-Value
Control	375.860± 6.822	25.9855	0.0001**
Experimental Group II	306.900± 11.318		

****High Significant difference (p<0.001).**

Table 2: Showing difference in mean Vickers microhardness numbers between control group and experimental group III using Paired Student's t-Test.

Group	Mean Vickers microhardness		
	Ms±SD	t-Value	p-Value
Control	375.860± 6.822	138.018	0.0001**
Experimental Group III	256.540± 6.542		

****High Significant difference (p<0.001).**



Graph (1) showing the difference in mean Vickers hardness numbers between different groups

Fig. (1): Photograph showing the product of Listerine.
Fig. (2): Photograph showing buccal half of premolar after gold coating.

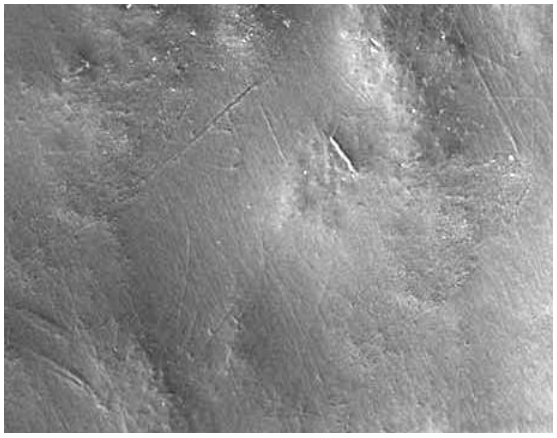


Fig. (3): SE micrograph of group I (control group) showing relatively smooth, intact enamel surface with fine scratches (x 1000).

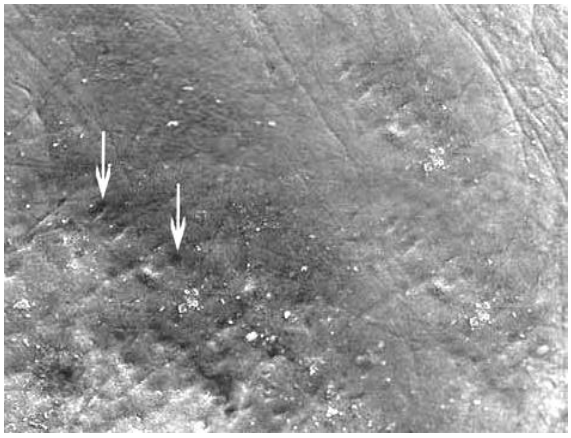


Fig. (4): SE micrograph of control group showing characteristic minute depressions on enamel surface representing rod ends (arrows) (x 2000).

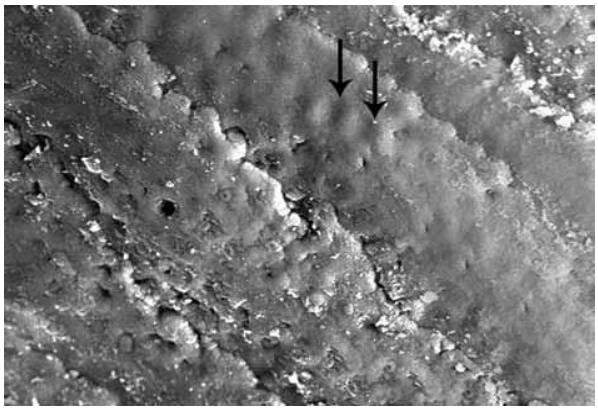


Fig. (5): SE micrograph of control group showing bands of small depressions representing enamel rod ends (arrows) alternating with parallel lines representing perikymata (x 3000).

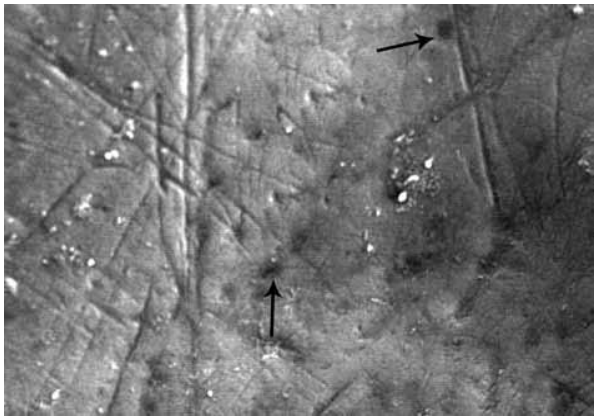


Fig. (6): SE micrograph of group II showing few micropores (arrows) and numerous scratching of enamel surface (x 2000).

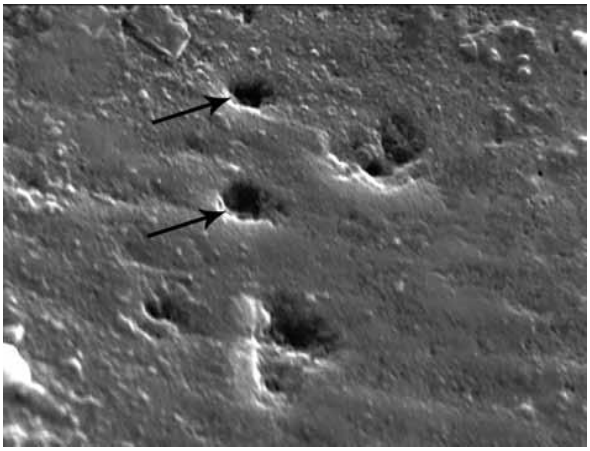


Fig. (7): SE micrograph of enamel surface of group II showing pitting and some porosity (arrows (x 3000).

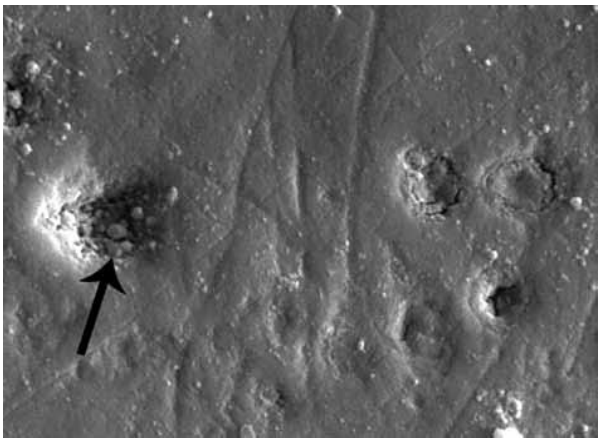


Fig. (8): SE micrograph of group II showing craters (arrow) with elevated peripheries and small micro pores at their floor (x 4000).

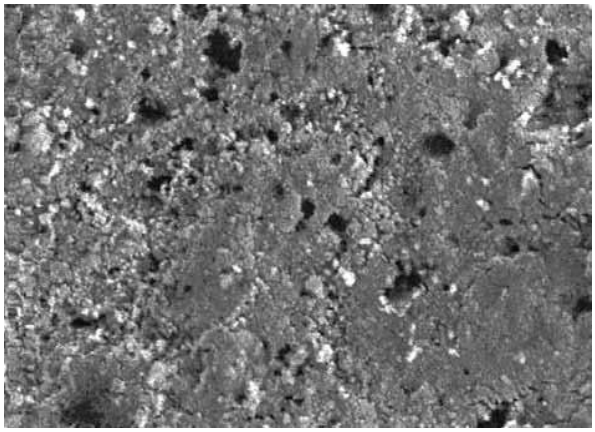


Fig. (9): SE micrograph of group III showing severe destruction of enamel surface having numerous pores with different sizes and depths (x 2000).

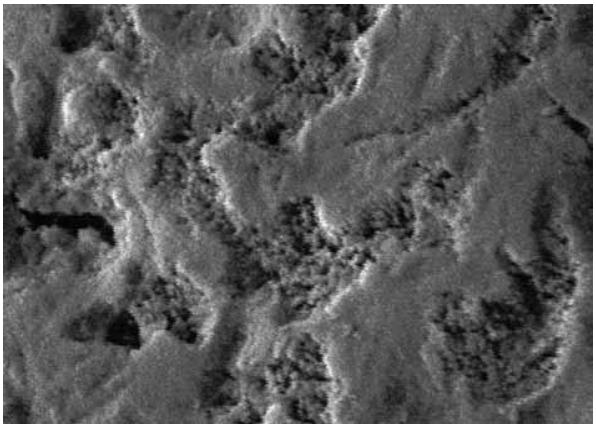


Fig. (10): SE micrograph of group III showing indiscriminate erosions of enamel surface, prismatic structure dissolution and surface roughness (x 4000).

4. Discussion:

Most of the recent innovations in oral care products have been directed towards cosmetic marketing claims (***Zero 2006).*** Although generally positive results have been reported concerning the whitening ability of these agents, concerns still remain as to their side effects on dental tissues (***Varginha et al., 2003).*** In our study, the hardness values found for sound enamel of control group (VHN 368-375) are in agreement with earlier published data by ***Gaspersic (1995)*** and with results of ***Salazar and Gasga (2003)*** (VHN 270-360). ***Featherstone et al., (1983)*** reported a direct relationship of enamel hardness values with mineral content of the tissue in a weight basis. ***Attin et al., (1997)*** found a significant correlation between initial enamel hardness and abrasion degree. Moreover, the hardness can influence the caries susceptibility because of the exposition of enamel to environmental oral factors. In our study there was a significant influence on the micro- hardness of enamel specimens of groups II and III by Listerine pre-brush rinse which is a whitening agent. Statistical analysis showed that hardness values were significantly reduced in these groups (P-value < 0.05).This is consistent with ***Zantner et al., (2007)*** who evaluated the influence of different home bleaching procedures on surface micro-hardness of human enamel. But these results are different from those of ***Ameri et al., (2011)*** which revealed non-significant influence of vital home bleaching procedures on surface toughness of bovine enamel. This indifference may be due to the different time intervals of the bleaching procedures, the composition of the applied products and their pH values.

Manufacturers have introduced different concentrations of carbamide peroxide (5% to 22%) (***Li et al., 2004)*** or hydrogen peroxide (3% to 14%) for at home bleaching ***(Auschill et al., 2005).*** Few controlled clinical trials have observed the improved efficacy of at home whitening when increasing concentration of the bleaching agent. Additionally an increase in side-effects has been detected ***(Braun et al., 2007).***

Significant surface alterations in enamel topography were detected in our SEM evaluation of specimens following Listerine application. The double daily application during the prolonged period of Listerine treatment (12 weeks) produced sever destruction of enamel surface integrity, but less so than phosphoric acid etch ***(Ernst et al., 1996).*** Scanning electron microscope results of ***Varginha et al., (2003),*** revealed regional variation in tooth morphology surface with higher concentrations of H_2O_2 (up to 35%) that had tendency to promote an increase in density of pits and pores.

As a result of this increased surface roughness and irreversible changes it is possible that teeth may be more susceptible to extrinsic discoloration after bleaching or rinsing with whitening rinses. Minor surface alterations were noted on specimens of group II with single daily application. While specimens of group I, were stored in artificial saliva, they revealed no changes in Vickers micro-hardness or surface morphology of enamel and this is in agreement with ***Cavalli et al., (2004).*** Another study evaluated the efficacy and safety of a whitening mouth rinse (2% hydrogen peroxide) that was used daily during one week, the results showed very mild tooth color improvement but authors recommended to be careful with self-applied whitening products that contain peroxide since they have potential to produce oral irritation and tooth hypersensitivity ***(Demarco et al., 2009).***

As mentioned before, hydrogen peroxide gives an acidic solution with water thus rendering the pre-brush mouth rinse more acidic with low pH ranging from 3.0 to 3.8. ***Ponterfract et al., (2002)*** measured enamel erosion by low pH mouth rinses, and reported that low pH mouth rinses should not be considered for long term or continuous use and never as pre-brush rinses. Although, ***Pretty et al., (2003)*** monitored the erosive effect of several mouth washes including Listerine and found that it is the only one that caused any erosion compared to the negative control, but this was only significant after 14h of continuous use.

Conclusion

Whitening with a pre-brush whitening mouth rinse is a superficial stain removing agent and not a bleaching agent per se. Independent, long term clinical trials should be performed to evaluate the effectiveness and side effects of various types of mouth washes that must be used following the directions of a reputable company with no over use.

Concerns have appeared due to the potential abusive use of these self-medication agents, especially in young patients, with potential harmful results. Thus dentists should be acquainted with these kinds of products to be able to inform their patients of their adverse side effects.

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