Effect of *Sanguinaria canadensis* Tincture Associated to a Chewing Gum on the Bacterial Biofilm

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Abstract: The aim of this study was to evaluate the effect of the *Sanguinaria canadensis* tincture associated to a chewing gum regarding to dental plaque score (O'Leary) and *Streptococcus* CUFs. Forty eight students of the University José do Rosário Vellano – Brazil took part in this double blind, placebo control study, with ages ranging from 18 to 25 years old, patterned into three groups: placebo group; *Sanguinaria canadensis* 2.1 mg/day, and *Sanguinaria canadensis* 4.2 mg/day. Chewing gums were used three times a day for ten days. During the first phase of the study, the chewing gum action was evaluated on dental plaque already installed and in the second phase its action was analyzed on the forming plaque. The results showed statistically significant differences among group I in the first and second phases of the study ($p < 0.05$ Anova One Way – Tukey Test, t student). The results related to the number of *Streptococcus* sp. showed statistically significant differences among groups I, II and III, with 0, 5, 15 and 30 minutes after using the chewing gums ($p < 0.01$ t Student test), These values suggest that *Sanguinaria canadensis* associated to a chewing gum decreased significantly dental plaque scores and number of *Streptococcus* sp. when compared to placebo chewing gums.

Keywords: *Sanguinaria canadensis*, chewing gum, dental plaque.

INTRODUCTION

Sanguinaria extract is a mixture of benzophenanthridine alkaloids derived from *Sanguinaria canadensis* L. (bloodroot). This mixture of alkaloids has a long history of use in tinctures and expectorants in pharmaceutical products. The chemistry and biochemistry of these alkaloids, including the dynamic equilibrium between acid and base forms, and pharmacokinetics of Sanguinaria extract shall be presented when this extract is incorporated into a dentifrice or oral rinse formulation [1].

Sanguinarine is a benzophenanthridine alkaloid derived from rhizomes of *Sanguinaria canadensis* L. (bloodroot). It is a cationic molecule which converts from an iminium ion form at pH less than 6 to an alkanolamine form at pH greater than 7. Sanguinaria extract is composed of sanguinarine and five other closely related alkaloids. The safety profile of both sanguinarine and sanguinaria extract provide a broad margin for their safe use in oral health products. Sanguinarine has broad antimicrobial activity as well as anti-inflammatory properties. In vitro studies indicate that the anti-plaque action of sanguinaria is due to its ability to inhibit bacterial adherence to newly formed pellicle, its retention in plaque being 10-100 times its saliva concentration, and due to its antimicrobic properties. The MIC of sanguinarine ranges from 1 to 32 micrograms/mL for most species of plaque bacteria. Long term use of sanguinaria-containing toothpaste and oral rinse products does not predispose users to detrimental shifts in oral flora. Electron microscopic studies of bacteria exposed to sanguinarine demonstrate that bacteria aggregate and become morphologically irregular. Sanguinarine-containing slow release polymer systems are currently being developed for use in periodontitis treatment applications [2].

Prevention and control of formation of the bacterial biofilm is the most important measure for maintenance of gingival and dental health, being toothbrushing and flossing the safest and most effective methods when regularly performed. However, for achievement of a good oral health, application and motivation of the patient are fundamental for maintenance of a constant preventive program, since in the absence of education and motivation the oral hygiene is reasonably worsened, returning to the initial condition. After acknowledgement of the limitations of mechanical oral hygiene methods, investigations with chemical agents have been conducted with a view to find an effective coadjutant for control of bacterial biofilm.

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1876-391X/09

Antiseptics have been available in dentifrices, mouthrinses and irrigation solutions, gels and chewing gums. For a long time, investigators have been reporting that sugarless chewing gums reduced the accumulation and formation of dental plaque, besides increasing the oral pH to make it alkaline even after consumption of sucrose, avoiding damage to the tooth structure secondary to acidity.

Considering the good results achieved with chewing gums on the oral pH and plaque formation and the wide acceptance of its consumption by the population and longer period of contact with the tooth structure, the association of antiseptics to the chewing gum may be a good coadjutant to oral hygiene, for prevention of gingival pathologies and dental caries.

The aim of this study was to evaluate the effect of *Sanguinaria canadensis* tincture combined with a chewing gum on the oral hygiene index and number of colony forming units (CFU) of *Streptococcus* sp.

MATERIAL AND METHODS

Before onset of this study, it was approved by the Institutional Review Board, so that the volunteers could be included in the study. Written consent was individually achieved from the participants concerning their voluntary participation, with the understanding that they might leave the study at any moment and for any reason.

Obtaining of the *Sanguinaria canadensis* Tincture

The *Sanguinaria canadensis* tincture was obtained from Ely Martins Co. (Control 99020 - MS 0119), located at the city of Ribeirão Preto, São Paulo State, Brazil. It showed average of total alkaloids 6% and 2,6% of sanguinarine.

CLINICAL ANALYSIS

This double-blind study comprised a longitudinal comparison performed in two stages, of three parallel groups of volunteers aged 18 to 25 years old, Dental Students at Unifenas, who received chewing gums with different concentrations of *Sanguinaria canadensis* tincture. Each volunteer received three gums per day to chew for seven days; in Group I, the gums added up to 4.2 mg/day of *Sanguinaria* tincture, Group II 2.1 mg/day of the tincture, and Group III received gums without the active substance (placebo). The gums in the different groups had the same pattern of color, taste, weight, size and package.

Two examiners were strictly trained to evaluate the bacterial plaque index [3], after staining with 0.75% fluorescein in glycerol and observation with aid of blue light of light curing units. During the entire study period, the examiner was the same for each volunteer.

After initial examination as to the bacterial biofilm, the volunteers were separated into three groups, and a mean was calculated between them. After onset of the tests, the volunteers were submitted to examinations for quantification of dental plaque and were asked not to change their oral hygiene habits and chew each gum for twenty minutes, being one at morning, one in the afternoon and one at night for ten days, returning for re-evaluation of dental plaque at the 8th day. This was stained and observed as previously described, and participants received professional prophylaxis for complete plaque removal (completion of the first stage). The volunteers were asked to continue chewing the gums as before, yet they should not perform any oral hygiene procedure for 48h and should return for re-evaluation of bacterial biofilm. At completion of the second stage, all participants received professional prophylaxis for ethical reasons.

LABORATORY ANALYSIS

Nearly 5mL of saliva were collected from five volunteers of each group at period zero, i.e. before they chewed the gums in their respective groups. Following, they chewed the gum for 20 minutes and after 5 minutes the saliva was once again collected. These procedures were also performed at 15, 30 and 60 minutes.

These saliva samples were submitted to counting of the number of colony forming units (CFU) of *Streptococcus* sp, as they were collected and immediately processed. For determination of the number of CFUs of *Streptococcus* sp, saliva was initially collected in sterile Petri dishes, transferred to test tubes under laminar flow and homogenized in ultrasound. Then, 0.5mL of saliva was diluted in 4.5mL of sterile PBS (phosphated buffered saline solution), for achievement of a 10-1 dilution, from which the 10-2 dilution was achieved by transference of 0.5mL for the second tube containing 4.5mL of PBS. Similarly, successive transference of this dilution to the next tube, containing 4.5mL of PBS, provided dilutions 10-3, 10-4, 10-5. Before preparation, the first tube received addition of 0.5mL of tannin solution to inhibit the activity of *Sanguinaria* tincture beyond the period required for the tests.

The culture medium employed as agar mitis salivarius. A volume of 0.05mL of saliva dilutions was pipetted on the center of the dishes, from the most diluted saliva (10-5) to the dish with the most concentrated saliva (10-3). Spreading on the Petri dishes was performed in duplicate. These procedures were conducted in an aseptic chamber, and after completion the dishes were stored in inverted position in anaerobiosis jars (Permution – Brazil). These jars were incubated at 37^{o}C for 24 hours. The counting of CFUs of *Streptococcus* sp was performed with aid of a colony counter, and the numbers were recorded in forms.

STATISTICS

Data were expressed as the percentage of dental aspects and statistical analysis was carried out using Tukey's test. The results were statistically treated with standard deviations. Results with $p < 0.05$ were considered significantly statistically.

RESULTS

The results achieved revealed that oral exposure to a daily concentration of 4.2 mg of *Sanguinaria*, provided by utilization three times a day, promoted a significant reduction both in the plaque index, when compared to Groups II and III, and in the number of CFUs of *Streptococcus* sp, as presented in the Table **1**.

The regression equation for the effect of chewing gums on the bacterial biofilm, during the study period, in relation to the dosages of *Sanguinaria canadensis* tincture, are displayed in Fig. (**1**). This presents a linear nature and indicates

Table 1. Means According to Groups and Days for the Percentage of Dental Aspects Stained During Consumption of Chewing Gums Three Times a Day

Days	GROUPS		
	I	II	III
1º	58.65a	49.92a	54.27a
8º	31.85b	38.84a	44.52a
10º	37.45b	51.17ab	65.03a

At the 1st day of the study, i.e. before onset of utilization of chewing gums. At the 8th day of the study, considering that the volunteers used chewing gums and performed regular tooth brushing from the 1st to the 8th day. At the 10th day of the study, after analysis of the tooth aspects stained on the 8th day, this was totally removed and the volunteers continued to use the chewing gums, yet did not perform oral hygiene for 48 hours (10th day).
The numbers represents the percentage of stained tooth aspects. The means followed by similar lower case letters are not statistically different from each other according to the Tukey test ($p<0.05$).

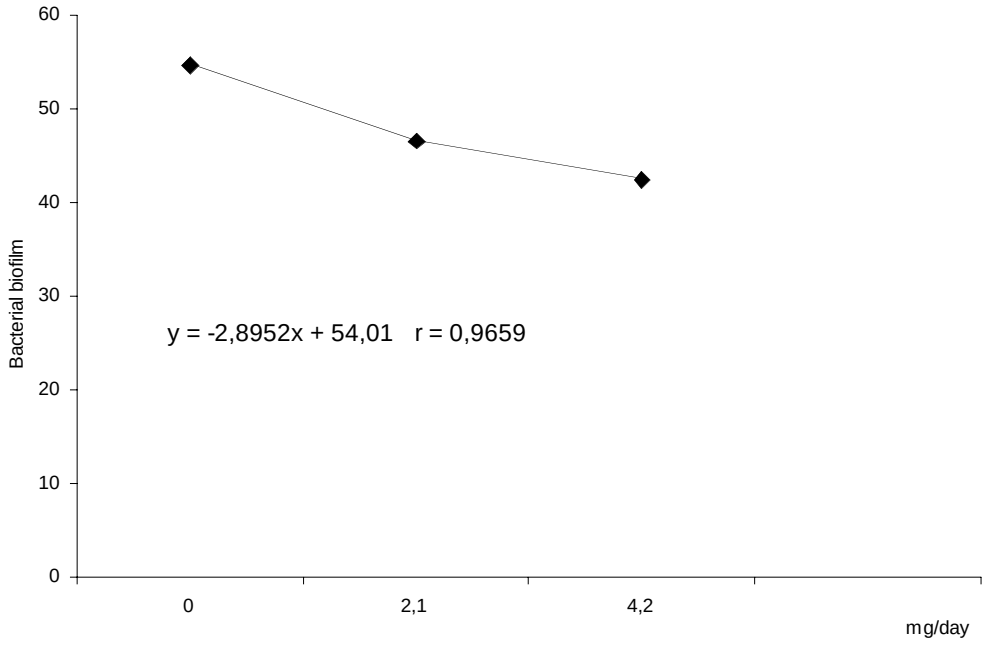

Fig. (1). Regression equation for the effect of chewing gums (4.2 mg/day; 2.1mg/day and without active substance) on the bacterial biofilm. y = -2.8952 + 54.01 and $r^2 = 0.9659$, in which n = 14.

that, at each increase of 2.1mg/day of this tincture, a decrease of 50% in the percentage of tooth aspects with presence of plaque.

In the present study, analysis of the action of *Sanguinaria canadensis* on *Streptococcus* sp by collection of saliva revealed statistically significant outcomes in reduction of CFUs for Groups I and II, when compared to the control (Group III), as observed in Fig. (**2**). The results related to utilization of the chewing gums in Groups I and II demonstrated significant changes in relation to the number of CFUs of *Streptococcus* sp at 5, 15 and 30 minutes after utilization of the gums. At 60 minutes, the number of CFUs returned to the initial condition. The control group did not present a significant reduction in the number of CFUs of *Streptococcus* sp from the onset to completion of the tests.

DISCUSSION

The high occurrence of gingivitis indicates that the current methods for patient education and motivation in utilization of the mechanical oral hygiene means have not been achieving the expected success [4]. Based on these results, different media for administration of synthetic and natural chemical substances have been evaluated. In the present study, the *Sanguinaria canadensis* tincture was combined with a chewing gum. The concentration of *Sanguinaria* tincture in the chewing gum followed strict data evaluated and established by a group of specialists with the objective to evaluate the safety of utilization of *Sanguinaria* tincture in oral hygiene products [5]. According to the present data, the minimum dose of *Sanguinaria* is 0.060 mg/kg/day, with possibility of increase of this dosage in up to 250 times, without occurrence of adverse effects. In the present study, the established dosage was 0.060 mg/kg/day (Group I) and 0.030 mg/kg/day (Group II), corresponding to 4.2 mg/day and 2.1 mg/day, respectively (considering a mean weight of 70 kg per individual).

The group using chewing gums with 4.2mg of *Sanguinaria* per day demonstrated a larger reduction in the plaque index, when compared to the groups using gums with 2.1mg

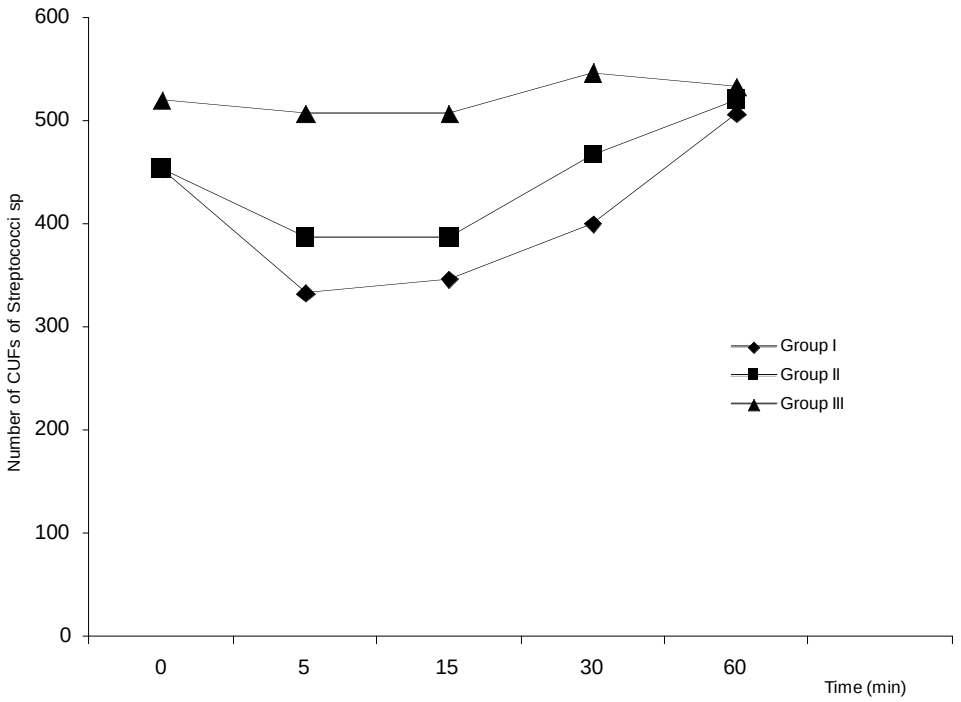

Fig. (2). Number of colony forming units (CFU) of *Streptococci* sp after 0, 5, 15, 30 and 60 minutes of utilization of chewing gums with *Sanguinaria*. Group I, the gums added up to 4.2 mg/day of *Sanguinaria* tincture, Group II 2.1 mg/day of the tincture, and Group III received gums without the active substance (placebo).

of *Sanguinaria* per day, leading to the assumption that efficacy of this substance is related with the dosage [6,7]. A similar effect was observed in the treatment of skin cancer [8].

The saturation, as to concentration of *Sanguinaria* in the mouthrinses, does not alter its retention in the bacterial biofilm, i.e. this substance is not retained in the biofilm even at lower concentrations [9]. The results of this study concerning the efficacy of *Sanguinaria* are in agreement with other findings of studies on dentifrices and/or mouthrinses containing *Sanguinaria* tincture [2, 10-14].

Sanguinaria is absorbed and maintained in the bacterial biofilm for at least 3 hours after utilization, at superior levels of minimum inhibitory concentration, MIC (20µg/g), often with minimum bactericidal concentration, MBC, for most oral bacteria. Salivary concentrations of *Sanguinaria* were evaluated because they are relatively low (<1.5µg/g) [6,10], and thus the results of the present study in saliva may be extrapolated to the bacterial biofilm, since, if in this microbiological test in saliva, the results were statistically significant as to reduction of CFUs of *Streptococcus* sp, and analyzing that retention of *Sanguinaria* in the biofilm is 10 to 100 times higher [9], the results on the biofilm may have their values increased, with consequent reduction of the existing biofilm and inhibition of its formation.

The positive results obtained in the present study may result from some factors, including the longer period of permanence of *Sanguinaria* in the oral cavity, by utilization of the chewing gum, than with utilization of dentifrices and mouthrinses, corroborating the findings of Smith *et al*. [6], 1996 in studies related to chlorhexidine. The fluoride administered in chewing gums remains in the oral cavity for 30 minutes to 1 hour [15], with minimum risk of systemic effect [16].

Salivary stimulation occurs during chewing. This increase in saliva may aid in the process of neutralization of acids produced by bacteria in the biofilm [13,17-20]. Chewing gums increase the salivary flow, increasing the pH [13,19,21-27], which leads to inhibition of formation of biofilm and its cariogenic potential [18,23-26], besides aiding in dental remineralization [28-30].

Reduction in gingivitis and increased amount of bacterial biofilm (without mechanical cleaning) were reported with utilization of *Sanguinaria mexicana* solution, yet this substance provided formation of a disorganized biofilm, similar to food debris and easily removed [3]. In the present study, even in the absence of oral hygiene, the gum with *Sanguinaria canadensis* provided smaller formation of a new bacterial biofilm, probably because of the glycolysis yielded by this substance, associated to the potential of mechanical cleaning provided by the gum.

An association of utilization of Viadent® products with leukoplakia revealed questions that should be answered by further studies: what percentage of patients using the Viadent products developed leukoplakias? Does the utilization of tobacco and/or alcohol influence the development of these leukoplakias? [31] In the present study, no alteration was diagnosed on the mucosa and tongue, what may be related to the low concentration of *Sanguinaria* in the chewing gum, which was much lower than in the aforementioned products, or to the study period. Since indication of these chewing gums should be careful, i.e. for patients with temporary dif-

ficulty in oral hygiene, its utilization should be indicated in short term, which probably will not give rise to problems as to its utilization.

CONCLUSIONS

Chewing gums with *Sanguinaria canadensis* displayed a significant reduction in the dental plaque index with habitual toothbrushing and smaller formation of biofilm (new biofilm) after professional prophylaxis.

The reduction in the number of CFUs of *Streptococcus* sp was larger in Group I, followed by Group II, being that Group III did not reveal any statistically significant difference. This reduction was more effective during 30 minutes after consumption of gums with *Sanguinaria canadensis*.

ACKNOWLEDGEMENTS

Our thanks are due to Prof. Dr. Sérgio Bruzadelli Macedo for his generous assistance.

The chewing gums were kindly provided by the Pró-Aroma Indústria e Comércio Ltda, São Paulo – Brazil.

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Received: April 11, 2009 Revised: July 22, 2009 Accepted: July 31, 2009