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Recibido: 27/09/13  
Revisado: 16/10/13  
Aceptado: 29/10/13  
Online: 29/10/13

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## Anticariogenic properties and effects on periodontal structures of *Stevia rebaudiana Bertoni*. Narrative review.

Contreras S. Anticariogenic properties and effects on periodontal structures of *Stevia rebaudiana Bertoni*. Narrative review. J Oral Res 2013; 2(3): 158-166.

**Abstract:** Introduction: *Stevia rebaudiana Bertoni* is a natural non-caloric sweetener, with more sweetness than sucrose, without adverse effects, which has demonstrated to have multiple benefits to the systemic health and recently to the oral health. This review's objective is to describe anti-cariogenic and anti-periodontopathic properties of its extracts. Results: Stevioside and rebaudioside A are the most important glycosides of the Stevia and none is cariogenic. *In vitro* researches have shown that Stevia extracts have anti-bacterial activity on *Streptococcus mutans*, *Streptococcus sobrinus* and *Lactobacillus acidophilus* organisms that are closely related to the production and development of tooth decay. *In vivo* and *in vitro* it has been observed that the production of bacterial acids decrease attributing it a low acidogenic potential and a lesser effect of the demineralization of the enamel in comparison with others sweeteners. Furthermore, *in vivo* it has been proved an anti-plaque effect mainly due to a decrease in the production of bacterial insoluble polymers. These characteristics in combination with anti-inflammatory properties could result potentially effective in the treatment of periodontal diseases in significant numbers, as it has been observed in studies conducted in animals. Conclusion: Stevia presents properties that potentially are anti-caries and anti-periodontal-diseases. However, *in vivo* studies are necessary to confirm these assumptions and provide a greater understanding of the mechanisms of action of this plant and the components involved. Notwithstanding, with the existing background, this sweetener can be postulated as a potential therapeutic complement in the odontological care, especially in patients that present base conditions such as obesity, diabetes and high blood pressure. Keywords: "Stevia" [MeSH], "Sweetening agents" [MeSH], "Tooth decay" [MeSH], "Periodontal disease" [MeSH].

## Propiedades anticariogénicas y efectos a nivel periodontal de *Stevia rebaudiana Bertoni*. Revisión narrativa.

**Resumen:** Introducción: *Stevia rebaudiana bertoni* es un edulcorante natural no calórico, con mayor dulzor que la sacarosa, sin efectos adversos, que ha demostrado tener múltiples beneficios para la salud sistémica y recientemente para la salud oral. El objetivo de esta revisión es describir propiedades anticariogénicas y anti-periodontopáticas de sus extractos. Resultados: Estevósido y rebaudiósido A son los glicósidos más importantes de Stevia y ninguno es cariogénico. Estudios *in vitro* han demostrado que extractos de Stevia presentan actividad antibacteriana sobre *Streptococcus mutans*, *Streptococcus sobrinus* y *Lactobacillus acidophilus* organismos estrechamente relacionados en la producción y desarrollo de caries. *In vivo* *in vitro* se ha observado que disminuye la producción de ácidos bacterianos atribuyéndosele un bajo potencial acidogénico y un menor efecto de desmineralización del esmalte en comparación con otros edulcorantes. Además, *in vivo* se ha comprobado un efecto anti-placa principalmente debido a una disminución en la producción de polímeros insolubles bacterianos. Estas características, más sus propiedades antiinflamatorias y cicatrizantes, podrían resultar potencialmente efectivas en el tratamiento de enfermedades periodontales en cifras significativas, como se ha observado en estudios desarrollados en animales. Conclusión: Stevia presenta propiedades potencialmente anti-caries y anti-enfermedades periodontales. Sin embargo, son necesarios estudios *in vivo* que confirmen estos postulados y proporcionen una mayor comprensión de sus mecanismos de acción y de los componentes que intervienen. No obstante, con los antecedentes existentes, se puede postular a este edulcorante como un potencial complemento terapéutico en la atención odontológica, sobre todo en pacientes que presentan condiciones de base como obesidad, diabetes e hipertensión. Palabras clave: Stevia, agentes endulzantes, caries dental, periodontitis.

### Introduction.

Since ancient times, humanity has shown a marked preference for sweet food<sup>1</sup>. The most widely used sweetener historically is sucrose, which provides a high-quality sweet taste, has a texture and acceptable shape, and due to this, it has maintained as the most popular

sweetener<sup>2</sup>. However, with the passage of the years, several researches have proven that sucrose has led to various nutritional and medical problems<sup>3</sup>. Its persistent consumption is highly related to the increase of weight, percentage of body fat, risk of development of cardiovascular disease, type 2 diabetes at adult age<sup>4,6</sup>, breast<sup>7-9</sup>,

pancreas<sup>10</sup> and colon cancer<sup>11</sup>, risk of developing ulcerative colitis, Crohn disease<sup>12</sup> and inflammatory bowel disease<sup>13</sup>.

At oral level, sucrose is an important factor that contributes the formation and development of the bacterial plaque. This is easily metabolized by oral bacteria<sup>14</sup> forming glucan, which promotes bacterial adhesion to the teeth<sup>15</sup>. The metabolic activity of the microbiota in the mouth not only is involved in cariogenic and periodontopathic phenomena, but also in the development of tumors in the mouth<sup>16</sup>. In addition, sucrose exhibits the greatest promotion of adhesion to *Candida albicans* and *Candida tropicalis*<sup>17</sup>, helping to the emergence of the candidiasis.

Most part of the diseases in the mouth begins with the formation of dental plaque, therefore both tooth decay and periodontal diseases could be controlled if the formation of dental plaque is reduced<sup>14</sup>. However, eating habits are hard to change, especially when it is associated with the consumption of sweet products<sup>14</sup>. That's why, that the increase in the availability of safe and effective substitutes for the sucrose in the diet during critical periods of tooth development is vital for public health at long term<sup>18</sup>. In this way is how substitutes of sucrose have arisen, additives-food which is able to simulate the presence of sugar<sup>1</sup>.

The sweeteners that are used in the food industry are divided into two groups: Carbohydrate sweeteners or nutritious and no-carbohydrate sweeteners or not nutritious<sup>1</sup>.

The first group is composed by sucrose, several oligosaccharides (palatinosa, fructo-oligosaccharides, galacto-oligosaccharides, lacto-oligosaccharides and xylo-oligosaccharides), starch sugars (glucose, starch syrup, fructose, sugar, maltose, invert sugar, and fructose) and sugar alcohols (erythritol, sorbitol, mannitol, xylitol, maltitol, lactitol, Palatinit<sup>TM</sup>, and reducing starch syrup)<sup>2</sup>. Most of them are neither calorie-free, nor beneficial dietary components for those who suffer from alteration in the metabolism for carbohydrates and other conditions<sup>18</sup>. Besides, sugar alcohols generally trigger side effects such as abdominal discomfort, flatulence, softened stools and diarrhea when they are consumed in excess<sup>2</sup>.

The members of the second group are divided into chemically synthesized sweeteners and those obtained from plants. The first include saccharin, aspartame and sucralose<sup>2</sup>, which are associated with a greater probability of increased caloric intake<sup>18</sup>, this is because they present a low power of satiety<sup>19</sup>, interfering in the energy balance<sup>19, 20</sup>, with the consequent incapacity to achieve or maintain a healthy body weight<sup>18</sup>. In addition, they don't offer health benefits<sup>18</sup>. Those obtained from plants include, stevioside (Stevia-glucoside), thaumatins, and monellin<sup>2</sup>.

With the increase of the incidence of diabetes and obesity and also because of the growing concern for the safety of some chemical sweeteners such as aspartame, alitame, cyclamate, saccharin, sucralose, among others, the need of natural non-caloric sweeteners with acceptable taste and relatively safe, is exigent<sup>2</sup>. *Stevia rebaudiana bertoni* is a natural sweetener that has shown, in several publications<sup>2, 14, 18, 21-25</sup>, respond to these needs and also submit beneficial properties for general and oral health.

This literature review seeks to describe this natural sweetener giving emphasis to its anti-periodontopathic and anti-cariogenic properties, putting forward possible uses in dental care.

### Origins and composition.

Its scientific name is *Stevia rebaudiana Bertoni*, commonly is known as sweet grass or paraguayian sweet-grass; but the native people call it kaa hee, caa ehe, kaa-je<sup>26</sup>. The Stevia plant is a perennial grass and belongs to the *Asteraceae family*<sup>27</sup>. It comes from certain parts of South America, mostly in Paraguay and Brazil<sup>28</sup>. It was used for centuries by the Guarani natives as a sweetener to counteract the bitter taste of medicines based on different plants and drinks, and for medicinal purposes that include the regulation of glycemia (blood sugar levels) and hypertension<sup>29</sup>.

In 1887 a South American naturalist scientist, Dr. Moisés Santiago Bertoni, director of the College of agriculture in Asunción<sup>30</sup>, described it for the first time. In 1900, the Paraguayan chemist Ovidio Rebaudi, managed to isolate the active ingredients responsible for sweetness<sup>31</sup>.

It is estimated that there are more than 80 species of Stevia that grow in the wild in the American continent; of these species, only *Stevia rebaudiana Bertoni* and another species already extinct seem to possess the natural sweetness which differentiates them<sup>30</sup>. Its sweeteners, mostly concentrated in the leaves, are synthesized, at least in the initial stages, using the same route as the gibberellic acid from the mevalonate<sup>32, 33</sup>.

Stevia has two main glycosides that are stevioside (110-270 times sweeter than sugar) and rebaudioside A (180-400 times sweeter than sugar), the last one with higher commercial valuable<sup>34</sup>, because it shows a nice flavor profile<sup>35</sup>, unlike artificial sweeteners that have metallic taste<sup>25</sup> or the same stevioside that has a subtle bitter flavor<sup>36</sup>. The difference between these glycosides lies only in the presence of a glucose<sup>37, 38</sup> and its fraction of weight in the tissues of the plant is 5-10% for stevioside and 2-4% for rebaudioside A<sup>39-41</sup>. These have a sweetness of higher quality than the sugar in terms of smoothness and freshness<sup>42</sup> and tend to produce an instantaneous sweet taste lower than sucrose, but

long term<sup>42</sup>.

The full chemical composition of the Stevia is not available yet<sup>24</sup>, but several of its components have been described. The fresh leaves contain a high amount of water (80 to 85%). Aside from the mentioned components (glycosides), the leaves contain ascorbic acid,  $\beta$ -carotene, chromium, cobalt, magnesium, iron, potassium, phosphorus, riboflavin, thiamin, tin, zinc, among others. Among the chemicals products found are apigenin, austroinilina, avicularin,  $\beta$ -sitosterol, caffeic acid, campesterol, caryophyllene, centaureidin, chlorogenic acid, chlorophyll, kaempferol, luteolin, quercetin, stigmasterol, among others<sup>43</sup>.

It is considered a "noble molecule", because it is 100% natural, has no calories, the leaves can be used in their natural state and only small quantities are required<sup>44</sup>. About a quarter of a teaspoon of leaves is equivalent to one sugar tea spoon<sup>25</sup>.

The dispersion of the Stevia's phenomenon is huge: It started being cultivated in Japan<sup>30</sup> and today it is widely used in China, Russia, Korea, Indonesia, Malaysia, Australia, New Zealand, Singapore, Taiwan, Thailand, United States, USA, Canada, Europe and South America<sup>45</sup> among others. The entry into the Chilean market is recent, in 2009<sup>46</sup>.

#### Properties.

There is a global consensus of Stevia's advantages to the human health<sup>47</sup>. In addition to its sweetener properties, Stevia has important health effects<sup>31</sup>, many of them detected in animals and *in vitro* studies. Among them have been described:

**Antihypertensive:** Stevia can be considered as an alternative or complementary therapy for patients with hypertension<sup>23</sup>. Significant diminishments in systolic and diastolic pressure have been reported in subject with mild hypertension<sup>48</sup>. It has been described that the hypotensor effect of stevioside is due to the inhibition of the influence of calcium from the extracellular fluid<sup>49</sup>. Plus, it has been shown that stevioside and steviol induce diuresis and natriuresis, without a significant change in glomerular filtration rate or renal plasma flow<sup>50</sup>. All this, without any adverse effect on cardiac frequency or the levels of catecholamine in serum<sup>23</sup>.

**Antioxidant:** Its use has been associated with an improvement of the antioxidant defense in the adipose tissue and the vascular wall, which leads to the inhibition of the development of the atherosclerotic plaque and stabilization of the induction plaque<sup>51</sup>.

**Anti-tumor y anti-carcinogenic:** It has been demonstrated that there are inhibitory effects of extracts of Stevia's leaf and its polyphenolic constituents, on promotion and initiation of tumors<sup>52</sup>. This is because

the stevioside has a similar activity to many triterpenoids in the promotion of 12-O-tetradecanoyl-phorbol-13-acetate (TPA tumors)<sup>53</sup>. At an experimental level, it was observed an inhibition of inflammation induced by TPA in a dose-dependent way<sup>52</sup>, with the significant inhibition of the mouse skin's carcinogenesis<sup>54</sup>.

**Anti-inflammatory and immunomodulator:** It has been demonstrated that stevioside significantly decreases the production of TNF-R and IL-1a and slightly decreases the production of NO in stimulated cells with LPS and THP-1. It is presented as an immunomodulator agent acting through the stimulation of humoral immunity, phagocytic function and cellular immunity<sup>55</sup>.

**Anti-diarrheal:** It has demonstrated to feature anti-inflammatory effects on epithelial cells from the colon<sup>45</sup>. Stevioside inhibits the contraction and stimulation of intestinal smooth muscle, which are linked to the hypermobility-associated diarrhea<sup>56</sup>. In addition, the dihydroisosteviol reduces intestinal fluid secretion<sup>23</sup>. Dihydroisosteviol and similar have been postulated as a new kind of CFTR inhibitors that may be useful for the development of antidiarrheal agents<sup>23</sup>.

**At brain and psychological level:** It keeps the feel of vitality and wellbeing as well as decreases the desire of eat sweets and fatty food<sup>25</sup>. Others indicate that its consumption reduces the desire of tobacco and alcoholic drinks<sup>25</sup>. It has been observed recently that stevioside has anti-amnesia properties declaring a preservative memory effect in cognitive deficits of rats<sup>57</sup>.

**Cicatrizant:** Reports indicate that when a few drops are applied in places where there are cuts and scrapes, they heal quickly<sup>58</sup>. It is used as a topical wound dressing<sup>59</sup>, elasticizing of the skin, and to eliminate blemishes and acne<sup>58</sup>.

**Anti-diabetic:** Steviol glycosides do not induce a glycemic response when it is ingested, which makes them attractive as zero or low-calories natural sweeteners to diabetics and other people with carbohydrate-controlled diets<sup>23</sup>. Study's results demonstrated that the treatment with Stevia increased the tolerance to glucose and decreased concentrations of plasmatic glucose<sup>60</sup>. The evidence suggests that stevioside improves secretion and sensitivity to insulin<sup>18</sup>. Besides, it generates the concomitant suppression of glucagon's secretion<sup>18</sup> and the decline in the renal tubular reabsorption of glucose<sup>50</sup>.

**Anti-viral:** Stevia has an anti human-rotavirus effect (HRV)<sup>61</sup> because it inhibits the binding of the antibody monoclonal anti-VP7 to MA104 cells infected with HRV<sup>61</sup>.

**Anti-bacterial and anti-fungal:** It has been found that inhibitory activity *in vitro* of extracts from leaves of Stevia in solvents like water, methanol, ethyl acetate and hexane against four gram-positive cells (*B. subtilis*

*S. aureus*, *M. luteus*, *B. megaterium*), four gram-negative cells (*S. marcescens*, *P. aeruginosa*, *E. coli*, *P. vulgaris*) and fungus such as *R. oligosporus* and *A. niger*<sup>80</sup>.

This last property in conjunction with the anti-inflammatory and cicatrizing capacity will give the starting point to the oral benefits that produces. In this way, Stevia could become a contributing element in the treatment of tooth decay and periodontal disease, both conditions are considered as the most prevalent diseases generated throughout the stomatognathic territory.

#### Anti-cariogenic properties.

Dental caries are considered to be a localized disease which results from the metabolic processes of the biomass in contact with the tooth surface, and the diet provides nutritional requirements and therefore the energy to the microorganisms of the oral microbiota<sup>30</sup>.

It is believed that the use of substitutes for sucrose in candy have contributed in part to the decrease in the prevalence of tooth decay in industrialized countries<sup>2</sup>. However, researches about the effect on teeth of the commercial sweeteners currently available, is rather insufficient<sup>92</sup>. It is important to highlight that, most of the available research of an anti-caries effect or not presumptive cariogenic of the sweetener comes from the pure chemical compound. Information about the cariogenicity of sweeteners when they are combined with filling carbohydrates is more limited and may be important in enamel and dentin caries<sup>92</sup>.

Being the Stevia a sweetener added and investigated recently, it has been observed that it's not cariogenic<sup>17, 45, 63</sup>. Das *et al.* did a *in vivo* study about rats to assess the cariogenic potential of stevioside and rebaudioside A with prolonged use. The rats were fed a diet containing 0.5% of stevioside or 0.5% rebaudioside A for 5 weeks. Neither of these compounds showed the potential to increase the risk of the development of dental caries<sup>63</sup>.

Since the first signs of its anti-cariogenic properties until today, several studies have been done. These studies confirm this property and describes the mechanisms of how this happens. In general, it is possible to group these mechanisms into three groups:

1° Antibacterial effect on microorganisms associated to the production of teeth decay: The complexity of the bacterial community found on the surfaces of the teeth makes difficult to associate specific groups of bacteria as the cause of teeth decay. However, *Streptococcus mutans* and *Lactobacillus acidophilus* are found in almost all tooth decay lesions, and their proportion in the plaque and saliva is positively related to the frequency and activity of tooth decay<sup>64</sup>. It is also known that the *Streptococcus sobrinus* is involved in the development of

tooth decay<sup>65</sup>.

The *in vitro* effect was observed in extracts of Stevia in different solvents on *Streptococcus mutans* using tetracycline 1% as a positive control. The extracts of Stevia in acetone, ethanol and methanol had a dose-dependent anti-bacterial activity, being the two first the ones that present the largest inhibition zone against that bacterium reaching values of 28.7 mm (acetone) and 27.0 mm (ethanol), both in a 100 mg/ml concentration, compared to the positive control which had an inhibition zone of 10 mm<sup>28</sup>.

Buitrago *et al.* evaluated the antimicrobial effect of Stevia extracts in methanol and concluded that concentrations starting from 200 mg/ml lead to an inhibitory effect on *Streptococcus mutans*<sup>86</sup>.

In experiments of tooth decay in rats, significant differences were found in the count of sulcal caries and *Streptococcus sobrinus* between the group of sucrose and the group of Stevia sweeteners. There were no significant differences between stevioside and rebaudioside A. The study came to the conclusion that neither of them is cariogenic<sup>63</sup>.

Noting the *in vitro* effect of the extracts of Stevia to 20% on *Streptococcus sobrinus* it registered an inhibition in the growth rate (50% inhibition) and a diminishment in the production rate of the acid of the bacteria. It was concluded that Stevia extract had an inhibitory effect on the caries-producing properties of *Streptococcus sobrinus*<sup>87</sup>.

It was found that *Streptococcus mutans* experiments higher growth suppression when it is grown in mediums that contain stevioside, than when it's grown in mediums with sucrose, glucose or fructose<sup>68</sup>.

In an *in vitro* study, Vitery *et al.* compared the effect of different concentrations of Stevia extracts, in water, methanol, ethanol, hexane and ethyl acetate, on strains of *Streptococcus mutans* and *Lactobacillus acidophilus* using vancomycin as positive control. The Stevia extract that showed the best results in the inhibition of growth, both for *Streptococcus mutans* and *Lactobacillus acidophilus* was the hexanoic, in which after 48 hours, inhibition halos were formed with an average of 14.5 mm with *Streptococcus mutans* and 15.5 mm of *Lactobacillus acidophilus* at a concentration of 50 mg/ml. The other solvents also show activity against the studied bacteria, it is clear that to achieve this, it was necessary to increase the concentrations. This study verifies the antibacterial activity of Stevia, against *Streptococcus mutans* and *Lactobacillus acidophilus*<sup>80</sup>.

This study matches the one made by Gamboa *et al.* where it evaluated the antibacterial effect of extracts from leaves of Stevia in hexane, methanol, ethanol, ethyl acetate and chloroform on 12 strains of streptococcus (including *Streptococcus mutans*) and 4 strains of

lactobacillus (including *Lactobacillus acidophilus*), using vancomycin as positive control (180 µg/ml) and azithromycin (150 µg/ml) which managed inhibition zones with values between 18 mm and 25 mm. The zones of inhibition produced by the 5 extracts in the minimum inhibitory concentration (MIC) for the 16 strains were variable, with figures ranging from 9 mm to 17.3 mm. The best performance was found for the hexanoic extract whose MIC was 30 mg/ml, which had similar values to the one achieved with ethanol and methanol (MIC = 120 mg/ml)<sup>69</sup>.

Giacaman *et al.* with an experimental work with commercial sweeteners and its cariogenic potential, observed that Stevia, sucralose and saccharin leave significantly less viable cells (*Streptococcus mutans*) in biofilms if they are compared with other sweeteners with similar counts than the negative control (NaCl). Plus, Stevia and sucralose have tendency to induce less biomass<sup>62</sup>.

2° Low acidogen potential: Sucrose is easily metabolized by oral bacteria, that are found in the dental plaque and the result is the release of acids. These acids are responsible for the demineralization of dental tissues in the dynamic process of caries<sup>70</sup>.

In an *in vivo* study was evaluated the cariogenic potential of rebaudioside A. In which It was compared the effect on the pH of a solution of rebaudioside A and sucrose, both with 4.7%. The rinse with Rebaudioside A showed a minimum pH of 6.92, which was significantly higher than the sucrose 5.62, verifying a low acidogenic potential and complying with the criteria established by the FDA for a not cariogenic sweetener<sup>71</sup>.

Giacaman *et al.* also noted that saccharin, Stevia and sucralose induced a significantly lower acidogenicity throughout the entire experiment. Regarding enamel demineralization, all the tested sweeteners, including Stevia, showed a statistically significant lower percentage of loss of surface hardness compared to the positive control, sucrose.

It has seen that *Streptococcus mutans* generates a lower production of acid when are grown on medium that contain Stevioside in comparison with the ones that are grown in sucrose, glucose or fructose<sup>68</sup>.

3° Anti-plaque effect: Most of the diseases in the mouth begin with the formation of dental plaque<sup>14</sup>, which are complex structures that are associated to similar microorganisms and different bacterial species<sup>72</sup>.

In an *in vivo* research it was measured the accumulation of dental plaque after mouthwashes with a solution of sucrose and one of Stevia for 5 days. The accumulation of plaque after the mouthwashes with Stevia was 57.82% less than with the sucrose mouth-

washes when it was measured with the Silness - Lo index and 10.40% less when it was measured with the O'Leary index<sup>14</sup>.

In two *in vitro* experiments which aimed to register the inhibitory effect of Stevia extracts to 10% and 20% of *Streptococcus sobrinus* it was noted a decrease in the hydrophobicity of the surface, the inhibition of the extracellular polysaccharides production and the adhesion of bacteria to the plaques coated with saliva<sup>67, 73</sup>.

Giacaman *et al.* also revealed a decrease in the production of intra-cellular polysaccharides (IPS) and extra-cellular polysaccharides (EPS) in statistically significant numbers, by *Streptococcus mutans*.

The EPS are responsible for 40% of the composition of the dental biofilm, and are one of the main virulence factors of the bacterial consortium, as they enable the adhesion of the bacterial cell to the acquired film, they are used as scaffolding for the maturation of biofilm and increase the porosity of the structure allowing the diffusion of sugar within the biofilm<sup>74</sup>.

Possible explanations for these properties are based on its contents. This plant is rich in flavonoids and terpenes. The phytochemicals present in Stevia are austroinullin, β-carotene, dulcoside, niacin, oxides rebaudi, riboflavin, steviol, stevioside and thiamine<sup>75</sup>. These nutritious substances affect the microbial flora of the mouth<sup>76</sup>, and also, the content of tannin, xanthines (theobromine and caffeine) and flavonoids have anti-plaque<sup>70</sup> activity. In addition, the leaf extracts of Stevia and its major polyphenolic constituents, stevioside and rebaudioside A, are not cariogenics<sup>18</sup>. Stevioside has a slight effect on the enzymes that are responsible for the decomposition of sugars, a discreet inactivation of the dextran-sucrase and a light static-effect on the cariogenics bacteria<sup>77</sup>.

Findings on Stevia rebaudiana bertoni utility at periodontal level.

The chronic marginal periodontitis and gingivitis are diseases that affect the periodontium of protection and/or insertion. The presence of bacterial plaque is relevant within its etiology. These oral pathologies acquire significance at level of general health and vice versa. In recent years, it is known that the condition of oral hygiene is associated with chronic diseases: the periodontopathies are considered as a risk factor for heart diseases<sup>78</sup>. At the same time, a high amount of evidence suggests that diabetes is associated with an increase in prevalence, extension, and severity of gingivitis and periodontitis<sup>79</sup>. There is a constant feedback between the mouth and the rest of the body.

Gingivitis is the inflammation of the gums as a result of the activity of bacteria located at the height of the gingival margin and is considered to be the most common form of periodontal disease. Clinical signs of inflammation, including changes in the outline, color and consistency of the gum, are associated with a tissue of stable inclusion level<sup>80</sup>.

Stevia ability to inhibit the growth of certain bacteria helps to explain the traditional use in the treatment of wounds, sores and gum disease<sup>81</sup>, besides this contributes its anti-inflammatory effect and antiplaque<sup>82</sup>.

De Slavutzky in his study of the effect about the formation of bacterial plaque, owing to sucrose and Stevia, managed to observe a significant reduction of biofilm in patients who used a mouthwash with Stevia. In this study, they conclude that this natural sweetener can act as an anti-cariogenic and anti-gingivitis product<sup>14</sup>.

Chronic marginal periodontitis is an infectious disease that leads to a slow and progressive loss of the union of the teeth. Within the signs and characteristic symptoms of the disease we can find clinical insertion loss, loss of alveolar bone, periodontal pockets and gingival inflammation<sup>80</sup>.

Within the many benefits that describe the Stevia there is also healing property, to complement the existing procedures of scraping, smoothing and periodontal surgery, to improve the oral health of people with periodontal disease<sup>58</sup>.

The effects of extracts of Stevia in periodontal disease were observed in an experimental study on dogs. It showed a significant reduction in the rate of gingival hemorrhage that initially ranged from 65% to 80% and after the treatment was reduced from 12% to 10%. It was reduced significantly reduced the depth of the periodontal pockets reaching differences of 4 mm. It decreased the gingival inflammation of 66% to 33%. At histological level significant improvements were shown where chronic inflammation is confined to the basal lamina and there is presence of squamous epithelium stratified with hyperplasia in postsurgical biopsy. In control cases there were no significant improvements<sup>58</sup>.

#### Metabolism and toxicity.

Recent studies have shown that a portion of stevioside is absorbed and degraded to steviol, which seems to undergo an additional metabolism<sup>83</sup>. Other studies indicate that none of the digestive enzymes in the gastrointestinal tract of different animals and the man are able to degrading stevioside in steviol<sup>84, 85</sup>. It is established that eventually is excreted by renal and biliary system<sup>86</sup>.

There are no reports in the medical literature of any adverse effects by using Stevia, because it is recog-

nized that it has a wide margin of safety<sup>30</sup>. One of the most obvious indicators of safety from Stevia is that there is no adverse effect reported in more than 1500 years of continuous use by the Paraguayans<sup>87</sup>. About 1000 tons of Stevia extract are consumed in Japan, and at the moment there haven't been reported toxic effects by the Japanese Food and Drug Safety Center. This fact has taken on vital importance due to the comments arised from the use of other synthetic sweeteners such as aspartame, which initially would not have the same safety margin<sup>88</sup>.

In September, 1995 the U.S. Food & Drug Administration (FDA) approved Stevia as a dietary complement<sup>30</sup>. It was recently approved for its commercial use by Joint Food and Agriculture Organization (World Health Organization Expert Committee on Food Additives, 2005) and more recently the approval of generally recognized as safe (GRAS) of the Food & Drug Administration<sup>89</sup>.

The Joint FAO/WHO Expert Committee on Food Additives (JECFA) at their 68<sup>th</sup> and 69<sup>th</sup> meetings in 2008, established an admissible daily intake (IDA) for steviol glycosides of 0-4 mg per kg of body weight per day, expressed as steviol<sup>89</sup>, additionally available data support the conclusion that the daily human intake of 5 mg to 6 mg of leaf of Stevia's extract as a dietary sweetener per kg of body weight is safe<sup>90</sup>.

The 70% of world production is used for processing crystals of stevioside, while 30% is earmarked for herbal uses<sup>91</sup>. Glycosides are sold in its natural form as dry leaves (6-15% of glycosides), and processed, in the form of extracts (liquid or powder) and combinations in tablets, whose concentrations of glycosides vary according to the manufacturer in a range that goes from 7%<sup>92</sup> to 97% glycoside content<sup>91</sup>.

#### Future of Stevia in Dentistry.

The application of Stevia in the dental treatment is a barely explored field. In order to materialize their contribution to this area, further studies are needed on the isolation, characterization and identification of substances present in the extracts<sup>69</sup>. It has to be found the solvent that achieve the best use of the active components of this plant and make it biocompatible; the concentration has to be selected to suits the standards of acceptable daily intake and make it effective at the same time, and has to be found a mean of administration considering the time spent at the site of action so that the active compound will achieve the desired effect.

The massification of Stevia in the food industry and as a sweetener, enhances the importance of investigating its effectiveness in combination with other substances (other sweeteners and filler items), which

is the form that is delivered to the public in the majority of commercial shapes.

### Conclusion.

The research about Stevia have left in manifest a potential anti-cariogenic and anti-periodontopathic role, result of its ability to reduce the bacterial load, the formation of biofilm, avoiding high pH decreases, and acting as an anti-inflammatory and healing agent at the periodontium level.

The evidence regarding a possible anti-cariogenic role is used as a base to support future research,

especially randomized controlled clinical trials of which there is no constancy. Studies about a possible anti-periodontopathic role are insufficient to assert benefits in humans, but the research lines are promising and thus, *in vivo* and *in vitro* studies become necessary to continue investigating about this property.

If deeper studies are done regarding this subject, *Stevia rebaudiana* Bertoni could become a complement to oral care used in the form of mouthwashes, toothpastes, chewing gum, artificial saliva, chewable tablets, among others, being especially beneficial in patients with obesity, diabetes and hypertension.

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