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Comparison the effect of stevia extract with glucose and fructose on dental enamel caries formation

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ABSTRACT

Introduction: According to the sweeting effect and antibacterial activity of Stevia extracts against cariogenic bacteria, the purpose of this *in-vitro* study is to compare the experimental effect of Stevia extract with Glucose and Fructose on enamel demineralization.

Materials and Methods: In this laboratory study, 72 premolar teeth were divided into 6 experimental groups as experimental study as Acetone, Ethanol, Methanol and Aqueous extracts 20% and Glucose Fructose solution 20%. Each group of teeth was located in a bottle of cariogenic material for 28 days. After 28 days, a section of buccal surface of each tooth was separated and evaluated in terms of the caries depth. Data analysis was applied using SPSS version 21, analysis of variance (ANOVA) and post hoc tukey tests were used at the significant level of 0.05.

Results: The mean and standard deviation of the caries depth induced by the glucose, fructose, methanol, and water extracts were 237.08± 26.48, 216.08±26.57, 88±16.69, and 170.66±35/53, respectively. Acetone and Ethanol extracts did not cause caries. The groups were statistically different in mean revealed by ANOVA test (p-value<0.05). The demineralization depth induced by methanol, ethanol, acetone, and water extracts differed one from another, however, there was not observed any difference between glucose and fructose in this regard.

Conclusion: According to the results of present study, the enamel caries depth of stevia extracts, especially acetone and ethanol ones, were less than conventional sweeteners such as glucose and fructose.

KEY WORDS: Stevia, Glucose, Fructose, Tooth caries.

1. INTRODUCTION

Using appropriate medicines is a necessity to succeed in the treatment of diseases. Herbs and plants are always regarded as a source of therapeutic substances which can be used in their natural form or as a raw material for making advanced medicines (who, 1992). Over the years, medicinal plants have been utilized for making various medicines for treating microbial infectious diseases (Jebashree, 2011). These useful types of plants can be a reliable alternative of many medicines. Nowadays, Chinese and Japanese scientists pay huge attention to these plants and their usages, moreover, world health organization (WHO, 1992) has provided a comprehensive list of plants with such uses (WHO, 1992, Evans, 2009). Considering the fact that plant resources are abundant, available, and healthy, the way is always open to design and conduct various studies on various aspects of them. Such studies are very helpful, because there always are possibilities of developing new medicines from them (Huang, 1998).

Stevia Rebaudiana Bertoni is a plant which has attracted many attentions in recent years. The plant is a member of the Asteraceae family and normally grows in the South America (Dweck, 1996; Kuntal, 2013). It is a natural organic sweetener, with a good stability and no calories. Extracts obtained from the dried leaves of this plant contain glycosides which is 200 to 300 times sweeter than sugar (Dweck, 1996; Kinghorn, 2003; Kuntal, 2013). A considerable amount of research has been conducted to investigate the leaves of this plant. The findings of these studies have shown that the leaves contain such compounds as diterpenoid steviol-glycosides, sesquiterpenes, bis-nor-diterpene, sterols, and flavonoids which have many therapeutic properties such as anti-inflammatory, anti-diabetic, anti-hypertensive, anti-tumor, anti-cancer, anti-diarrhea, diuretic, and immunomodulatory (Kuntal, 2013; Carbonell-Capella, 2013). More importantly, these compounds are non-toxic without any known adverse health side effect (Dweck, 1996; Kinghorn, 2003). Recently in America, the products of this plant are approved by FDA as a healthy dietary supplement, and also there is a great desire in Europe to use such products, as well (Carbonell-Capella, 2013). Contrary to sucrose, Stevia not only does not cause blood sugar levels to rise, but also is commonly used to adjust the blood sugar level in diabetic patients (Dweck, 1996). Besides, Stevia prevents dental caries by reducing the acid production and inhibiting bacterial adhesion to salivary (Suwannawong, 2004; Singh, 2005).

Although the general health has been promoted in many societies, dental caries is still a prevalent problem (Kinghorn, 2013; Featherstone, 2000). Dental caries are a multifactorial disease which normally is a result of complex interactions between cariogenic oral floras, especially *Streptococcus mutans*, and fermentable

carbohydrates on the tooth surfaces. The metabolisms of these bacteria produce acid, which gradually destroys the dental enamel (Featherstone, 2000). Consequently, many efforts have been made by scientists to explore new ways to deal with such bacteria using herbal plants (Kinghorn, 2003, Featherstone, 2000).

In this regard, Mohammadi-Sichani (2012) carried out a study to assess the effect of Stevia extracts on *Streptococcus mutans*. They concluded that acetone and methanol extracts of the plant had the highest inhibitory effect on the *Streptococcus* growth. In the same vein, another study conducted by Brambilla (2014) explained that, in contrast to sucrose, Stevia extracts have no effect on the production of acidic compounds. Moreover, a study carried out by Giacaman (2013) on several types of sweeteners demonstrated that compounds containing Stevia have anti-bacterial effects and are able to interfere with the metabolism of bacteria.

By reviewing the literature published in this area, one can conclude that although extensive research has been carried out on this topic, further studies are still required to prove the usefulness of such plants. Therefore, the aim of this study was to evaluate and compare the effects of glucose, fructose, and stevia extract on the enamel demineralization depth.

2. MATERIALS AND METHODS

Preparing the teeth samples: In the present study, seventy two premolars newly extracted from young humans were selected as the sample of the study. All these teeth were healthy without caries and previous restorations and their enamel surfaces were intact without any crack. The teeth were extracted for orthodontic or periodontal treatment in the last three months before the beginning of the study and were immediately placed in the normal saline. In order to prepare the teeth for the study, the following procedure was implemented; firstly, in order for disinfecting the teeth, they placed in formalin solution for 48 hours. In the second step, the residual soft tissues were separated from them and they were cleaned by a fluoride-free pumice and rubber cap brush (Ahmadi-Motamayel, 2013). Finally, the teeth surfaces other than buccal region were covered by two layers of nail rubbish and mounted in an acrylic resin medium up to 1mm below the CEJ line. The teeth were randomly categorized into in six groups. Each group were placed in a separate bottle and sterilized by autoclaving at 121^0 C and 15 psi for 15 min (Rezaei-Soufi, 2013).

Preparing the extracts: 1200 gr dried leaves of *Stevia* was obtained from the north of Iran, Rasht. The leaves were powdered by a hammer mill and then divided into four equal portions. These four portions were transferred to four Erlenmeyer flasks containing four various solvents, namely, ethanol, methanol, acetone, and water. The flasks were covered with aluminum cap, and for further mixing, they were placed on the Heater stiller, as a mixer, for 48 hours in the room temperature. In the next step, in order to separate the waste part of the plant, the solution was filtered through a 20 cm-diameter Buchner funnel. In order for separating the solvent from the extracts, the solution obtained from the previous step was placed in a vacuum rotary evaporator. What remained from this step were ethanol, methanol, acetone, and water extracts of Stevia (Mohammadi-Sichani, 2012; Aghaie, 2011). These extracts were stored in sterilized vials in the room temperature for the following experiments (Aghaie, 2011).

Assessing the anti-caries properties of Stevia extracts: In order for assessing the anti-caries properties of Stevia extracts and comparing their effectiveness by common sugars including glucose and fructose, the six groups of teeth were exposed to these materials in six test tubes;

The tube number 1: 12 teeth alongside the water extract of *Stevia* containing BHI-Broth medium (20%),
The tube number 2: 12 teeth alongside the methanol extract of *Stevia* containing BHI-Broth medium (20%),
The tube number 3: 12 teeth alongside the ethanol extract of *Stevia* containing BHI-Broth medium (20%),
The tube number 4: 12 teeth alongside the acetone extract of *Stevia* containing BHI-Broth medium (20%),
The tube number 5: 12 teeth alongside the glucose extract of *Stevia* containing BHI-Broth medium (20%),
The tube number 6: 12 teeth alongside the fructose extract of *Stevia* containing BHI-Broth medium (20%).

It is worth to mention that the extracts with growth medium were added to the test tubes using a 0.2 micron membrane syringe filter which prevents bacteria from passing through it (Aghaie, 2011). The solution was added to each test tube until the teeth were totally submerged in the solution. Moreover, in order to ensure that there were no bacteria in the tubes, they were incubated for 24 hours at 37^0C, after that, several samples were collected from the tubes and cultivated on the Blood agar medium. Fortunately, all tubes were cleaned and there was not observed any infection in this regard. In the next step, 1.5×10^8 *streptococcus* cells (0.5 McFarland) were added to each tube (Rezaei-Soufi, 2013).

In order for monitoring the bacterial growth, every day between 9-10 am several samples were collected from the tubes and cultivated on the blood agar medium. Unfortunately, the tube number six, which contained fructose solution, was infected by a fungus, so we repeated the study for this tube from the beginning. In the cases that the growth of *streptococcus* had been observed (glucose, fructose, water extract and methanol extract), every two days 10 mm of the solution were removed and replaced by the same but fresh ones in order for providing the necessary nutrients for the organisms (Rezaei-Soufi, 2013). Considering the fact that at least twenty eight days are needed for initiating the process of tooth decay, after this period, the teeth were brought out of the tubes. Then, they were washed by distilled water for one minute. In the next step, they were cut in two equal pieces from the CEJ

region using a nonstop cutting device (Featherstone, 2000). The crowns were mounted in the two end sealed ducts by epoxy resins. After 24 hours, the teeth were cut so that a segment of the buccal surface with the thickness 1mm was obtained. The thickness of these segments was reduced to 0.2-0.3 mm using an abrasive sandpaper grade 600. The thickness of the segments was measurable using a Stereo microscope. The residual debris remaining on the surface of teeth segments were removed using the ultrasonic device.

Finally, the demineralization depth of enamel in coronal, median, and cervical region was measured by a Stereo microscope at the ×20 magnification and the average of them was regarded as the demineralization depth final score (Figure 1).

Moreover, the data were assessed using SPSS software package version 20. All the tests were performed at the 0.05 level of significance.

3. RESULTS

The results of the present study demonstrated that the average demineralization depth of enamels exposed to glucose solution was the highest one and equal to 237.08 µm. Moreover, the lowest demineralization depth was observed among teeth which submerged in the methanol extract of Stevia. The average demineralization depth of these samples was equal to 88 µm. interestingly, there was not observed any tooth caries among teeth which were exposed to the ethanol and acetone extracts. These results are presented in Table 1. The Kolmogorov-Smirnov test indicated that all data obtained from the six test tubes had the normal distribution. One-way ANOVA test was applied to assess the differences among six groups of the study. Accordingly, it was found that there was a significant difference between the six groups in this regard. These data also are presented in Table 2.

Table.1. Average depth of demineralization, minimum and maximum values of the teeth exposed to various solution (All values are based on micrometer)

group	Number of teeth	Average depth of demineralization (std error)	Min	max
20 percent solution of glucose	12	237.08 (26.47)	198	281
20 percent solution of fructose	12	216.08 (26.57)	173	251
20 percent solution of water extract of stevia	12	170.66 (35.53)	119	211
20 percent solution of methanol extract of stevia	12	88 (16.69)	64	121

Table.2. The results of one way ANOVA performed to assess differences between the groups

Source effect	Sum of squares	Degree of freedom	Fisher statistics	p-value
Between groups	157139.41	3	71.066	<0.0001
Within groups	32430.5	44		
Total	189569.917	47		

DISCUSSION

The teeth caries are a prevalent health problem all around the world which imposes a huge cost on the economy of families (Rezaei-Soufi, 2013; Park, 2015; Roberson, 2006). It is a multifactorial disease which mainly stems from the microorganisms living in the mouth, including *Streptococcus mutans* and *Streptococcus sobrinus* (Park, 2015; Gupta, 2014; Struzycka, 2014).

It is postulated that the Stevia plant has the capability to reduce dental caries in several ways, including; reducing the amount of the acid produced in the mouth as a result of food decomposition by the microorganisms, decreasing the cell surface hydrophobicity, inducing the cell aggregation, inhibiting the production of extracellular polysaccharide, and finally by preventing bacteria from adhering to salvia (Suwannawong, 2004; Singh, 2005).

In the study conducted by Mousumi (2008) the antimicrobial properties of chloroform and methanol extract of Stevia were assessed and compared. They found that the antimicrobial effects of such materials were reliant on their concentration, however Stevia extracts were observed to be more efficient in this regard. Jayaraman (2008) was also investigated the antimicrobial properties of extracts obtained from the Stevia leaves on microorganisms, including *Escherichia coli, Bacillus subtilis, Staphylococcus aureus, Salmonella typhi*, and *Vibrio cholera*. The results of that study demonstrated that the acetone extract of Stevia had a stronger antimicrobial than that of other extracts, moreover, its effect on the gram-positive bacteria were more favorable than its effect on the gram-negative ones. In contrast to the present study, Fazal (2011) reported that the antimicrobial effect of the methanol extract on the *streptococci* was lower than the effects of other extracts. The same results were obtained by Tadhani (2007) they found that the hexane extract of Stevia had a higher antimicrobial effect in comparison with that of methanol extract. Moreover, it was reported by that study that the water extract of Stevia had no antimicrobial effect. Ajagannanavar (2014) also outlined that the alcohol extract of Stevia, in comparison with the water extract, had a stronger capability to inhibit the growth of *streptococcus mutans* and *lactobacillus acidophilus*.

The results of the current study indicated that all extracts obtained from the Stevia plant had a lower decay-inducing effect than those of glucose and fructose.

It was demonstrated by Ahmadi-Motamayel (2013) that a 20 percent solution of glucose and fructose caused 245.98 and 195.98 µm carries on the enamel surface, respectively. The results are comparable by those observed in the present study. In that study, the exposure time was twenty one days and the depth of demineralization was evaluated using the polarized light microscopy, while in the present study, the exposure time was twenty eight days and evaluation was performed by a stereo microscope.

Moreover, in the present study, the average depth of demineralization on the surface of enamels exposed to Stevia extracts was observed to be 170.66 µm, much lower than those induced by glucose and fructose. Stevia extracts have several effects which can be used to explain such findings; they reduce the amount of acid produced by oral microorganisms, the production of extracellular polysaccharide, the cell surface hydrophobicity, and the induction of cell aggregation. Moreover, they inhibit *streptococcus* bacteria adhesion and reduce dental plaque (Suwannawong, 2004; Triratana, 2006; Singh, 2005).

Gamboa and Chaves (2011) investigated the antimicrobial activity of Stevia extracts against sixteen groups of *streptococcus* and *lactobacillus*. They reported the Minimum Inhibitory Concentration (MIC) of methanol and ethanol extracts as 120 gr/ml which differs from the findings presented here (we found the MIC of these extracts equal to 20 gr/ml). These differences can be explained in part by the different bacterial groups investigated by the two studies. However, the results for the methanol extract are the same, and similar to that study, we also found the MIC of methanol extract equal to 20 gm/ml.

In the study performed by Aghaie (2011), it was demonstrated that the MIC of ethanol and methanol extracts of Stevia against *streptococcus* bacteria was equal to 50 and 100 gr/ml, respectively. In addition, it was reported by that study that cubic sugar had no inhibitory effect on the growth of *streptococcus mutans*. In contrast, in the present study, we found that the 20 percent solution of ethanol extract did not induce any carries on the surface of enamels. This discrepancy in results can be attributed to the different types of *streptococcus mutans* used by these two studies. However, the results for methanol and water extracts were similar to each other.

4. CONCLUSION

The results of the present study indicated that ethanol and acetone extracts of stevia did not cause any dental caries in the laboratory experiments. Moreover, it was demonstrated that the stevia extracts caused less demineralization in enamel than did fructose and glucose solutions. However, despite these promising results, further research needs to be undertaken in this regard.

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