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Evaluation of the Antibacterial Effect of Cinnamon Extract on Streptococcus Mutans

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ABSTRACT

Purpose: To compare the effect of cinnamon extract mouthwash with chlorhexidine mouthwash on Streptococcus mutans counts in saliva of some children. **Materials and methods:** A total of 40 children from both sex were involved in the present study. Age of Children was between 6 and 12 years in a good physical condition. Children were distributed into 2 groups (A&B), each one consists of 20 children. Group A (Experimental group**):** each participant was given a new bottle of specific effective amount of cinnamon extract mouthwash (150ml) to be used. Group B (Control group): each participant was given a new bottle of specific effective amount of (150 ml) chlorhexidine mouthwash (0.12 %) to be used. Unstimulated salivary samples were collected and examined. **Results:** The average number of S.mutans was reduced significantly in cinnamon extract group and chlorhexidine group as well. **Conclusion:** Cinnamon extract mouthwash was successful as an antimicrobial agent and it can be used as a natural alternative to chlorhexidine.

KEYWORDS

Cinnamon extract,
Chlorhexidine,
Streptococcus mutans.

INTRODUCTION

Dental care is a base for being a healthy person. All over the world, it was proved that more than half of children and almost all adults have dental caries. Many types of bacteria found in our mouth have the ability to cause dental decay [(1)]. Researches have shown that Streptococcus mutans (S.mutans) forming about thirty percent of bacteria existing in dental cavities due to caries. The participation of S.mutans in dental

- Paper extracted from Master thesis titled "Evaluation of the Antibacterial Effect of Cinnamon Extract on Streptococcus Mutans "

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caries is very important in detecting its liability to antibacterial means. Because antibiotics are unsafe to be used daily as an anti-caries agent, mouthwashes are considered as an effective alternative [2].

Among types of antimicrobial means, chlorhexidine is the most often prescribed antibacterial mouthwash because it has bactericidal effect on many types of bacteria. But unfortunately, it has a lot of unwanted side effects, as taste changes, alteration of teeth, restorations and tongue color, in addition to formation of calculus [3]. Usually, only around half of teeth surfaces could be reached using toothbrushes and other mechanical aids, so mouthrinses should be used beside mechanical cleaning techniques to achieve the optimum effect [4].

Therefore, researchers begin to be interested in traditional medications to discover alternative materials that can fight bacteria. Plants were accepted to be an origin of novel antibacterial means; so, studies have devoted to find out antimicrobial medicaments from plants [5]. A lot of plants used in medical field can reduce adhesion forces between bacteria and enamel, therefore, it aids in inhibition of plaque deposition, cinnamon is one of these plants [6].

Cinnamon is an old plant that consists of volatile oils and chemical components as transcinnamaldehyde, eugenol, and cinnamyl acetate. Ancients were usually using cinnamon in treating tooth pain and to overcome unwanted mouth odor. Cinnamon bark contains essential oils which have been used medically due to its antimicrobial, antioxidant, and anti-inflammatory effects [7]. Because of cinnamon antimicrobial activity, it was assumed that rinsing with cinnamon-containing mouthwash could probably lead to a reduction in dental plaque and decay. Thus, the current study aimed to clarify the validity of an herbal mouthwash containing the extract of cinnamon on streptococcus mutans bacteria in subjects after rinsing for two weeks. Additionally, we compared it with chlorhexidine mouthwash [8].

MATERIAL AND METHODS

Selection of patients: 40 children were visited the Pedodontic and Oral Health Department, Faculty of Dental Medicine for Girls Al-Azhar University, Egypt. Full detailed treatment plan was explained to the children' guardian and informed consent was assigned by guardians. Also verbal consent from the children was obtained. Inclusion criteria [9]: Systemically healthy patients, no history of oral prophylaxis done for at least 3 months before the study, and children with low caries index (DMF&def: ≤ 4), Exclusion criteria [9]: History of recent antibiotic administration (previous 2 weeks), history of using antimicrobial mouth rinse (previous 12 hs.), or history of fluoride treatment in the past 2 weeks.

Methods:

Preparation of cinnamon extract mouthwash: Cinnamon was bought from the marketplace, ground into fine powder in an electrical mixer. 100gm of cinnamon powder was added to 1 litre of sterile water and maintained in a water bath at 60°C for 5 hours, then filtered using sterile filter paper (Whatman, UK). After that, cinnamon extract was allowed for drying at 40°C in hot air oven [10]. (Fig.1)

Figure (1): Cinnamon powder.

Various concentrations of cinnamon extract mouthwash were studied in vitro. These concentrations are 10%, 20%, and 50% of cinnamon extract

mouthwash. Trials cleared the most effective concentration which was studied on a group of children. The highest concentration (50%) showed the greatest inhibition zone diameter and it was the concentration of choice in the current study.

Children randomly were distributed into two groups A&B, 20 children each. The two groups followed the same oral hygiene instructions, and differed only in allocated mouth rinses [8].

Group A (Experimental group): each participant was given a new bottle of specific effective amount of cinnamon extract mouthwash (150ml) to be used.

Group B (Control group): each participant was given a new bottle of specific effective amount of (150 ml) chlorhexidine mouthwash (0.12 %) to be used.

Children of both groups were told some instructions as: rinsing with 5 ml of the mouthwash, stop eating or drinking for at least one hour after rinsing, use tooth brush without any dental toothpaste after rinsing, and rinse twice a day, each rinse for about 1 minute.

Collection of saliva samples:

Non stimulated samples of saliva were taken from children by requesting from them to spit in a labeled, sterilized, plastic container (3 ml on the average).

Possible fluctuations in saliva microbial counts were controlled by sampling saliva at least 1 hour after meal and immediately transmitted to microbiological laboratory (The Regional Centre for Mycology and Biotechnology – Al-Azhar University).

Baseline sample (S1): The initial sample was taken before using the mouthwash.

Second sample (S2): The second sample was taken 30 minutes after using specific mouthwash.

Third sample (S3): The third sample was taken after using specific mouthwash for two weeks.

Preparation of the media: [11]

The selective medium Mitis Salivarius Bacitracin (MSB) was prepared as following:

90 grams of dehydrated mitis salivarius agar were solved in one litre of sanitized water. Heating of the medium was done for dissolving its constituents and it was allowed to enter the autoclave at 121° C for 15 minute. Then it was allowed for cooling to 55° C and 1 ml of 200 units sterilized bacitracin was added. Sterilization of bacitracin was performed by filtration. Each plate was filled with approximately 20 ml of MSB and left 24 hours for dryness at room temperature under 5-10% CO_2 tension (incubator).

Microbial cultivation: [12]

One millilitre of the salivary sample was transmitted to standardised centrifuging tubes holding 4 ml of Brain Heat Infusion broth (BHI). Then, the saliva and BHI broth were mixed by a vortex-mixer. With the aid of automated pipette, 10 micro liter of the mixed and diluted saliva was flown on MSB agar in laminar flow. Incubation time of the plates was 48 hours and temperature was 37° C in 5% of CO_2 in nitrogen. Streptococcus mutans colonies were examined.

Statistical methods:

Statistics were manipulated using SPSS program, version 25 (IBM Corp., Armonk, NY, USA). Comparisons between groups were done using non-parametric Mann-Whitney test. The non-parametric Friedman test was used to compare successive readings for every child. Significance level was set at ≤ 0.05.

RESULTS

Greater mean percent reduction in colony forming unit (CFU) of streptococcus mutans was recorded in chlorhexidine group, which was 99.98% after 30 minutes and, 100% after 2 weeks, in comparison to cinnamon extract group which was 96.93% after 30 minutes and, 99.99% after 2 weeks. Mann-Whitney test showed that the difference was statistically significant ($p<0.001$). (Table.1), (Fig.2).

Table (1): *Percent change in CFU of streptococcus mutans in cinnamon extract and chlorhexidine groups post-operatively, and significance of difference between groups.*

Colony forming unit of Streptococcus moutons	Cinnamon extract group					Chlorohexidine group					P value
	Mean	SD	Median	Minimum	Maximum	Mean	SD	Median	Minimum	Maximum	
% change after 30 min	-96.93	4.57	-98.94	-99.93	-80.00	-99.98	0.02	-99.99	-99.99	-99.95	< 0.001*
% change after two weeks	-99.99	0.01	-100.00	-100.00	-99.95	-100.00	0.00	-100.00	-100.00	-100.00	< 0.001*
P value	<0.001*					<0.001*					

*Significance level p<0.05, *significant*

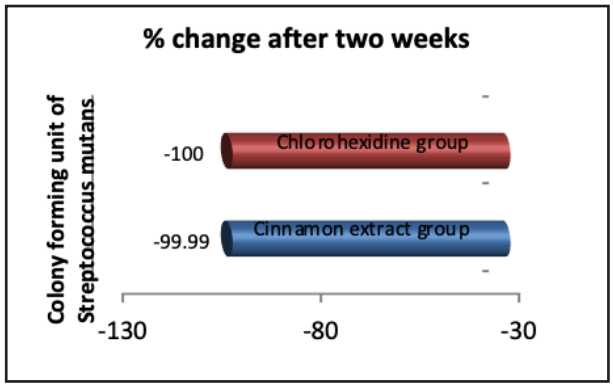

Figure (2): Bar chart showing mean percent change in CFU of streptococcus mutans in cinnamon extract and chlorhexidine groups after 2 weeks of treatment.

DISCUSSION

Untreated dental caries is associated with a negative impact on general health, development and productivity [13]. Therefore, applications of antimicrobial agents (such as dentifrices and mouthwashes) reduce and control microorganisms in plaque biofilm [14]. Chlorhexidine-containing mouthwash is one of the most widely used one because of its bactericidal effect. On the other hand, rinsing with chlorhexidine products for long time may cause several side effects [15]. Herbal mouthwash contains natural ingredients called phytochemicals that contains desired anti-microbial and anti-inflammatory effect. Herbal mouthwash performs its effect without alcoholic compounds, chemical additives, or coloring agents. So, in the present study, cinnamon mouthwash, which is safe, available, and easy to prepare, was the one of choice [16].

In the present study, aqueous cinnamon extract was prepared. Which has no side effects during rinsing or even if child swallow it accidentally, this was in accordance with a recent study [17]. On contrast, in previous studies, ethanol was used as a preservative and solvent [18, 19]. The study of the levels of salivary S.mutans is a common way for detecting high caries risk patients. In the current study, non-stimulated saliva samples were preferred because it is easier and it reflects accurately the caries experience and the risk in every individual this was in accordance with another study [20]. On contrast, a recent study used paraffin wax stimulated saliva [21].

Mitis Salivarius bacitracin media was assigned for detection and counting of colonies of S.mutans because it is the most selective for such colonies. The addition of bacitracin to the media allows S.mutans to grow and form colonies and prevent most other types of bacteria from growth [22]. In this

present study, the age of the selected children was (6-12) years because they can avoid swallowing reflex during using the mouthwash. This agree with another study in which authors assumed that children aged less than 6 years should not use mouthwash [23].

In this study, the average of decayed and filled teeth (DMF) and (def) in these children were ≤ 4, this indicates that the selected children are of low caries index, this was in agreement with a previous study [24]. In the current study, the concentration of chlorhexidine mouthwash was 0.12% which is in a low concentration; this is in agreement with other study. Low concentration of chlorhexidine is as effective as high concentration but has fewer side effects [25].

In the current study, mean percent reduction in colony forming unit (CFU) of S.mutans in cinnamon extract group was 96.93% after 30 minutes and, 99.99% after 2 weeks of treatment. Thus, cinnamon extract, if used as a mouthwash, has an excellent antimicrobial action on eradicating streptococcus mutans in vivo. Similarly, our study also in agreement with another study which compared the efficiency of cinnamon extracts, chlorhexidine and distilled water [10].

Also, the current study is in accordance with another study which confirmed the antibacterial effect of Cinnamon verum extract. Therefore, it can be suggested that using Cinnamon as a natural remedy is effective on several oral diseases, including dental caries, and can be used as a substitute for other existing chemicals [26].

CONCLUSION

Cinnamon extract mouthwash was successful as an antimicrobial agent. It significantly reduced the total bacterial count in the saliva of children when compared with a potent antiseptic like chlorhexidine.

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