Effect of Honey and Green Tea Solutions on *Streptococcus mutans*

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***Objectives**: The aim of this cross-sectional in vivo study was to assess the effect of green tea and honey solutions on the level of salivary Streptococcus mutans. **Study design:** A convenient sample of 30 Saudi boys aged 7-10 years were randomly assigned into 2 groups of 15 each. Saliva sample was collected for analysis of level of S. mutans before rinsing. Commercial honey and green tea were prepared for use and each child was asked to rinse for two minutes using 10 mL of the prepared honey or green tea solutions according to their group. Saliva samples were collected again after rinsing. The collected saliva samples were prepared and colony forming unit (CFU) of S. mutans per mL of saliva was calculated. **Results:** The mean number of S. mutans before and after rinsing with honey and green tea solutions were $2.28* 10^8(2.622*10^8)$, $5.64 *10^7(1.03*10^8)$, $1.17*10^9(2.012*10^9)$ and $2.59*10^8$ $(3.668*10^8)$ respectively. A statistically significant reduction in the average number of S. mutans at baseline and post intervention in the children who were assigned to the honey (P=0.001) and green tea (P=0.001) groups was found. **Conclusions**: A single time mouth rinsing with honey and green tea solutions for two minutes effectively reduced the number of salivary S. mutans of 7-10 years old boys.*

Key words: *Streptococcus mutans, Antibacterial Agents, Inhibition, Honey, Green Tea*

INTRODUCTION

Caries is mainly caused by *S. mutans* and *S. sobrinus* bacterial factors.[1-3] Additional etiological factors are *Lactobacillus* and *Actinomyces*.[1-4] Other contributing factors include vulnerabilities to demineralization, oral cleanliness and hygiene as well as dietary habits for instance frequency of eating.[2,3,5] Considerable research has focused on *S. mutans* and its involvement in the caries process.[1,6-8] Prevention of dental caries is founded on the eradication of at least one of the producing factors.[9]

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Honey is super saturated nectar collected by bees from a wide variety of plants.[10] The composition of the honey depends on the composition of the nectar, from which it originates.[11,12] The natural antioxidants and flavonoids of honey show an extensive range of biological effects such as antibacterial, anti-thrombotic, anti-inflammatory, antiallergic and vasodilator action.[11,12] There is scientific evidence about honey in several experimental and clinical conditions including treatment of gastrointestinal disease through gastric protection against gastric lesions, healing of wounds and burns and it is use as antimicrobial agent including *S. mutans*.[13-17] Manuka honey have been used for years in United Kingdom, Australia, New Zealand, Canada and recently the Food and Drug Administration approved it is use in burn and wound treatment in the United States.[18] A recent study showed that manuka honey was effective in reducing salivary *S. mutans* counts in children after 10 and 21 days.[19] Several studies have demonstrated that many bacterial and fungal pathogens that are sensitive to honey.[20-22]

Currently some foods and components of food are identified as possible anticaries agents.[9] For example, the biological properties of polyphenolic compounds found in plant foods include antioxidant[23-25] and anti-inflammatory effects.[26] The polyphenols are present in plants such as coffee, tea, fruit and cereals.[25] Research has revealed that extracts from green tea, red grape and unfermented cocoa have a bacteriostatic influence on *S. mutans* and diminish its adherence to glass.[27] Extracts of polyphenol and green tea mixtures prevent production of insoluble glucan.[28] The efficiency of green tea polyphenols in decreasing intensities of *S. mutans* and *Lactobacilli* in the saliva has been reported.[25,29]

Currently a global trend has been witnessed for the use of natural products due to their demonstrated pharmacological influence on

the oral environment as efficient caries preventive agents. As some of the aforementioned studies on honey and green tea have shown the antibacterial activity and inhibitory effect on *S. mutans*; which consider as a foundation for using it for prevention of dental caries. Although *in vitro* properties of green tea and honey have been studied extensively, only few *in vivo* studies were completed.[19-31] Hence clinical studies are the way to confirm the real contributions of green tea and honey to caries reduction in children. Therefore, the aim of this cross-sectional population-based *in vivo* study was to assess the effect of green tea and honey solutions on the level of salivary *S. mutans* and the null hypothesis tested in this study was there is no effect of honey and green tea solutions on the level of salivary *S. mutans* on the children.

MATERIALS AND METHOD

The study and informed consent were approved by the Ethical Committee of Human Studies, College of Dentistry Research Center, King Saud University. The power of the sample size when number of participants' equal or more than 15 was calculated. At $\alpha = 0.05$, SD = 1 and maximum difference 1, at power ≈ 0.8, the sample size was ≥ 15. A pilot study was carried out before the start of the main study to test the honey and green tea solutions and be familiar with all procedures, data collection forms, methodology, outcome measures and statistical analysis. Training and calibration exercises were undertaken during the pilot study. Intra-examiner reproducibility of the investigator who performed the clinical examination was performed during the pilot study.

A convenient sample of 30 Saudi boys aged 7-10 years were included in this study and randomly assigned into 2 groups of 15 each. Group 1 was assigned to use commercial honey (Langnese; Langnese Honig GmbH & Co. KG; Bargteheide, Germany - Patch # 4023300850102) and group 2 was assigned to use green tea (Rabea; A.M.S Baeshen & Co.; Jeddah, Saudi Arabia - Patch # 6281013151048). The inclusion criteria were healthy Saudi children, not using any medication, have no active periodontal disease, not under active orthodontic treatment and with dmft and DMFT equal or less than 4.2. Demographic information and dental as well as medical history were obtained from parents.

Green Tea and Honey Preparations

A modification of the methods described by Subramaniam *et al* (2012)[8] and Motamayel *et al* (2013)[32] were used to prepare green tea and honey solutions respectively. A 100 mL of boiling distilled water to which 10 grams of green tea was added and further boiled for 30 minutes. The obtained solution was reduced to 10 mL to obtain 100% weight/volume (w/v) concentration. Ten grams of commercial honey was added to 100 mL of boiling distilled water and further boiled for 30 minutes. The solution obtained was reduced to 10 mL to obtain 100% (w/v) concentration. The solutions of honey and green tea were cooled to the room temperature and stored in sterile individual containers prior to use. A pilot study was completed in four children to test the prepared solutions before the main study. Children participated in the pilot study were not included in the main study.

Clinical examinations were carried out for dental caries and oral hygiene status. For caries assessment, presence of decayed, missed and filled surfaces was recorded in the primary (dmfs) as well as in the permanent (DMFS) dentition according to World Health Organization.[33] Oral hygiene was assessed using DI-S score, which describes the extent of soft deposits and is one of the 2 components of the simplified oral hygiene index (OHI-S) developed by Green and Vermillion.[34-36]

Collection of Saliva Samples

Participating children did not consumed food for 60 minutes before collection of saliva. Each child rinsed twice with 10 mL of water for 30 seconds. Then after 2 minutes, each child was asked to spit normal (unstimulated) saliva (2 mL) into a sterilized disposable 5 ml tube (before rinsing with honey or green tea solutions) to use for baseline count of *S. mutans*. Each child was inquired to rinse with 10 mL of honey or green tea solutions for 2 minutes according to their assigned group. Saliva samples were collected again after rinsing with honey or green tea solutions using the same procedures for the baseline. Each tube was then labeled with a unique identifier. The key of the unique identifier was known to the dentist who collected the samples but not to the laboratory personal. Samples were transferred within two hours to Microbiology Laboratory, Department of Pharmaceutics, College of Pharmacy, King Saud University for preparation and analysis of salivary levels of *S. mutans*.

Ten tubes which contain 4.5 mL sterile 0.9% (w/v) sodium chloride were used for each specimen. The tubes were marked from one to ten. A 0.5 mL of saliva was added to the first tube to make tenfold dilution. The solution was mixed vigorously by vortex mixer (VWR International Global Exports, Arlington Heights, IL, USA). A 0.5 mL from the first tube was transferred into the second tube and mixed vigorously by vortex mixer. The procedure was repeated down to the tenth tube. A 100µL volume from each dilution was plated and evenly distributed into 10% sheep blood agar containing 20% sucrose using the glass rod spreader (Thomas Scientific, Swedesboro, NJ, USA). Each dilution was done in duplicate. The plates were incubated aerobically (Memmert 854; Memmert GmbH + Co.KG; Schwabach, Germany) for 24 hours at 37°C. After incubation period, the colonies on the plates were counted manually and colony forming unit (CFU) of *S. mutans* per mL of saliva was calculated.

Statistical Analysis

Data was analyzed using SPSS version 20.0 statistical software (SPSS Inc., Chicago, Ill). The comparison of *S. mutans* count between baseline and post intervention (after rinsing) values of each of the two groups (honey and green tea) was carried out using non-parametric Wilcoxon sign rank test in which the positive and negative ranks were compared, based on the difference between the baseline and post intervention values. All statistical analyses were set at a significance level of $p<0.05$.

RESULTS

For intra-examiner reliability, Kappa was 0.91 which indicates very good agreement. The mean ($\pm$ SD) of dmfs and DMFS in the honey group were 5.20 ± 4.69 and 0.73 ± 1.34 respectively. The mean ($\pm$ SD) of dmfs and DMFS in the green tea group were 3.67 ± 3.04 and 0.87 ± 1.46 respectively. The mean ($\pm$ SD) of Di-S in the honey group was 0.84 ± 0.27 and in the green tea group was 0.86 ± 0.31. None of the children was plaque free.

The mean and standard deviation of number of *S. mutans* at baseline and after rinsing with honey and green tea are shown in Table 1. There was statistically significant decrease in the mean number of *S. mutans* at baseline and post intervention in the children who were assigned to the honey (P=0.001) and green tea (P=0.001) groups.

Table 1. Mean and standard deviation of number of *S. mutans* at baseline and after (post intervention) rinsing with honey and green tea solutions

Groups	Mean (SD)		p-value
	Baseline	Post Intervention	
Honey	2.28* 10^8(2.622*10^8)	5.64 *10^7(1.03*10^8)	0.001*
Green Tea	1.17*10^9(2.012*$10^{9)}$	2.59*10^8(3.668*10^8)	0.001*

* Significant - Wilcoxon sign rank test

There was statistically significant difference between the positive and negative ranks of the difference between baseline and post intervention number of *S. mutans* for the children who were assigned to the honey and green tea groups (Figs. 1 & 2). The data also showed no significant difference in the mean ranks of number of *S. mutans* between honey and green tea groups both at baseline (p=0.19) and post intervention (P=0.051). Figs. 4-7 showing culture results at baseline and post intervention in the honey and green tea groups.

Figure 1. Comparison of the positive and negative ranks of the difference in the baseline and post intervention of the number of *S. mutans* in the honey and green tea groups

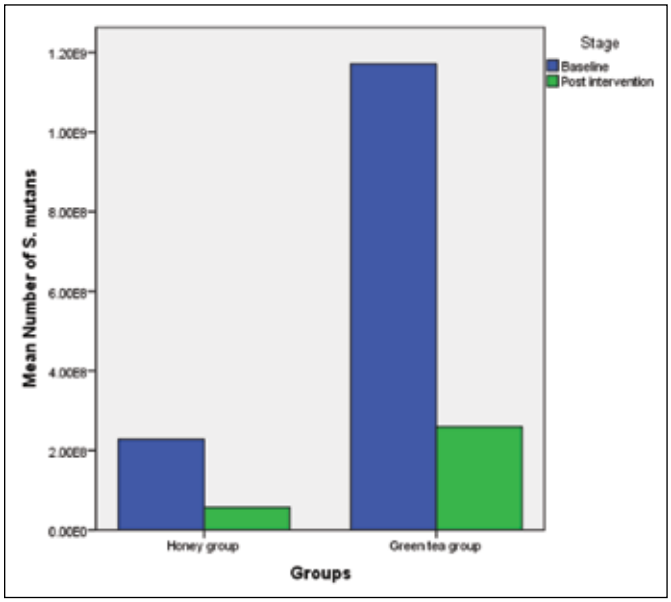


Figure 2. Comparison of the difference between baseline and post intervention of the mean logarithmic number of *S. mutans* in the honey and green tea groups

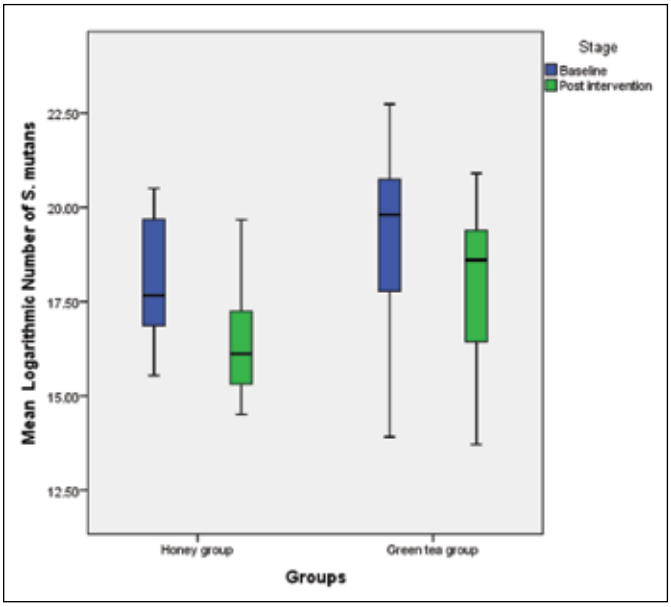


Figure 3. Comparison of the difference between baseline and post intervention of the mean logarithmic number of *S. mutans* in the honey and green tea groups

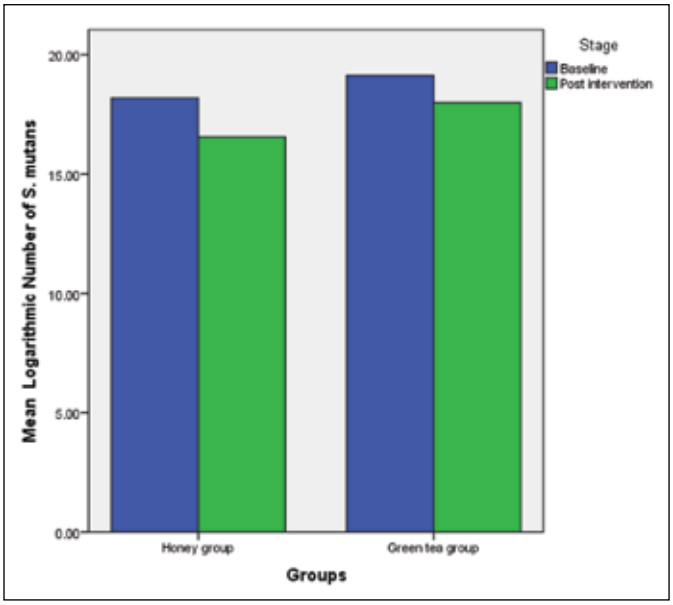


Figure 4. Culture showing results at baseline in group 1 before using honey

Figure 5. Culture showing results post intervention in group 1 after using honey

Figure 6. Culture showing results at baseline in group 2 before using green tea

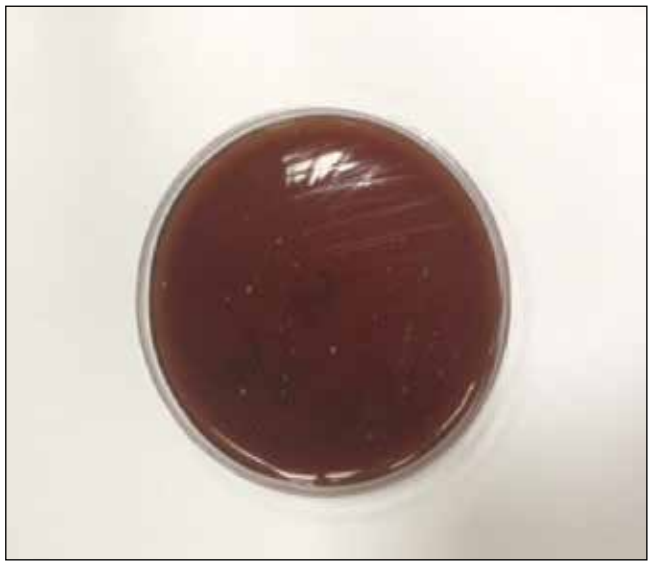
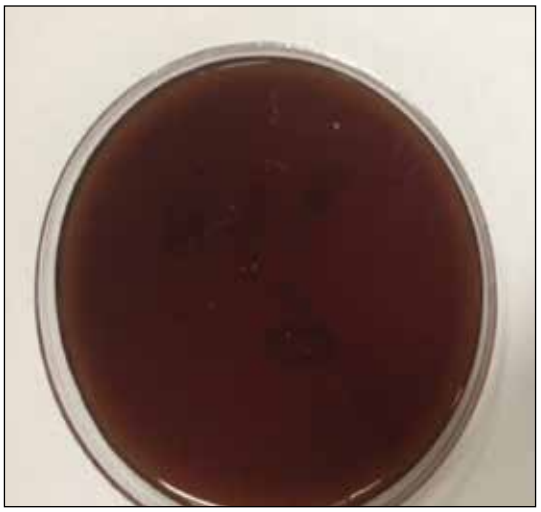


Figure 7. Culture showing results post intervention in group 2 after using green tea



DISCUSSION

The null hypothesis test in this study was rejected as the findings showed the efficacy of honey and green tea solutions on reduction of the level of salivary *S. mutans*.

Honey has antimicrobial activity against anaerobic, aerobic, gram negative and gram positive bacteria, molds and yeasts with unique properties because of its bacteriostatic and bactericidal effect.[37] In the present study we found that honey has the antibacterial properties that are responsible for decreasing the quantity of *S. mutans*. Our results were in agreement with the finding of another study which reported that the antibacterial activity of honey is enhanced when the honey was diluted.[38] However, another *in vitro* study used solutions containing different concentrations ranging from 0% to 100% (w/v) of natural Hamadan honey reported substantial antibacterial action for honey on *S mutans* in concentrations more than 20% and on *Lactobacillus* in 100% concentration.[32] Also, another study used manuka honey reported good antibacterial activity at very low concentration of 1.8%.[39] The difference of results between different studies may be due to difference in methodology between the investigations such as the method used for agar dilution, the type of honey used, water content of the honey, composition of the honey and honey sources.[11,12,22] Because the antibacterial activity of honey varies with the source and processing, results of one study showed that natural honey had an antibacterial effect on cariogenic bacteria (*S. mutans* and *Lactobacillus*).[32] While in the present study we used commercial honey. Another study reported effectiveness of antibacterial action against different pathogenic bacteria for different Egyptian and Saudi honeys.[40] Although the data obtained from the present study did not investigate the effective components of the honey which results in reduction of *S. mutans* and could not fully represent the profile of the antibacterial activity of the honey used, it does confirm that honey solution used had *in vivo* antibacterial activity and honey might be useful for the prevention of dental caries. An *in vitro* study reported that Slovenian honeys are effective antibacterial and antifungals due to its peroxide action.[41] In addition, among the possible mechanisms of honey is the presence of inhibitory factors such as flavonoids,[42] hydrogen peroxide[43-46] and low pH as well as high osmolality due to its sugar

concentration.[39] It was also demonstrated that hydrogen peroxide in the honey exerted bacteriostatic and DNA degrading activities to bacterial cells.[46] However, the specific antimicrobial mechanism of honey still unclear and need more research.[31,47,48]

In the present study, similar to honey we found that green tea solution used has the antibacterial properties that are responsible for decreasing the quantity of *S. mutans* and there was no significant difference between green tea and honey in decreasing the quantity of *S. mutans*. Green tea polyphenol mainly consists of catechin and proanthocyanidins.[49-52] An *in vivo* study evaluated the protective properties of green tea against *S. mutans* count in plaque and saliva showed that there was a statistically significant difference of pre- and post-rinsing with 2% green tea for 5 min concerning *S. mutans* count among subjects.[3] Another *in vivo* study has reported that 60% of 12 to 18 years old patients using a green tea mouth rinse presented a significant less level of *S. mutans* compared with the control group using mouth rinse as placebo.[25] This is possibly because of the anti-bacterial properties of polyphenols linked to the inhibition of adherence of bacterial cells to the surfaces of the teeth.[30] The results from the present *in vivo* study on the action of green tea solution against *S. mutans* confirm the hypothesis that green tea act as anticaries agent with an antimicrobial action.[25,53] It was confirmed that anti-microbial properties of green tea is related to the epigallocathechingallate which reduce *S. mutans* and production of acid in dental plaque resulting in stopping lactate dehydrogenase-activity.[54,55] Currently evidence exists only for green tea as a functional food for health of oral cavity, partly due to its high content of catechins, especially epigallocatechingallate.[51,52,55] Tea polyphenols act as a source for slow- release of theaflavins and catechins, which prevent *S. mutans* growth and adherence to the surface of the tooth.[8,56]

Several methods of oral bacterial collection and sample type may be a problematical process when comparing results of different studies.[57,58] The techniques of collection and processing are aspects that may affect the difference and accuracy of the observed counts[59] and microbiologic quantification is the most accurate microbial test.[60] In the present study bacterial culture from saliva was used as microbiologic quantification and the method demonstrated the number of *S. mutans* within reasonable time.

In the present study all Saudi children enrolled (inclusion criteria) had dmft and DMFT equal or less than 4.2. This was based on the reported caries prevalence study in Saudi Arabia of 73.9% for 6-9 year olds with a mean dmft of 4.23 and a mean DMFT of 1.85.[61] Our results showed that the mean of dmfs and DMFS in the honey and green tea groups were 5.20 and 0.73 as well as 3.67 and 0.87 respectively. In considering the results of the present study, it should be mentioned that wide ranges of DMFS and dmfs were included in this study sample. In the present study, the mean of Di-S in the honey and green tea groups were 0.84 and 0.86 respectively and none of the children were plaque free. A survey of 5-12-year-old children in Riyadh, Saudi Arabia found plaque deposition in all children they examined.[62]

In the present study mouth rinsing was performed for 2 minutes only. Another study reported a tendency of decreasing the number of *S. mutans* in the green tea group which was directly proportionate to the exposure time to green tea.[25] However, the same study reported slight rise in the *lactobacilli* CFU density from saliva after repeated rinsing with the green tea, although not statistically significant and authors attributed this to possible institution of an internal mechanism of bacterial resistance.[25]

The findings of this study echoed the literature and indicated the association between decreasing of oral cariogenic bacteria and the use of specific foods.[63] Also the procedures of this experiment have proved its validity in assessing the aim of the study and was adequately sensitive in quantification of *S. mutans* as well as it is easy to perform. However, more *in vitro* and *in vivo* studies are needed to evaluate their mechanism of action against caries and identification of the anti-caries components of the prepared solutions. Also it is crucial to find out the distribution and nature of these compounds as well as the mechanism of absorption and metabolism.

There are some limitations in this study. One limitation is the age group which was limited to 7-10 years old. The other limitation was the convenient sample which included 15 children in each group. Future studies with more sample size and wider age group would strength the research. Also, the exact properties of honey and green tea that is responsible of reducing *S. mutans* were not investigated. In addition, wide ranges of DMFT and dmft as well as children with low DI-S score were included in this study sample. In the present study we did not use a rinse with water as control as previous study showed that using water rinsing did not affect test oral microorganisms.[64] Also, it would be interesting to expand this research to know the exact properties of honey and green tea solutions that is responsible of reducing *S. mutans* and possibly other cariogenic microflora. Furthermore, long term and repeated use rather than single use would be worth to assess.

CONCLUSIONS

Within the limitations of this population-based cross-sectional *in vivo* study, it was concluded that:

1. A single time mouth rinsing with honey and green tea solutions for two minutes effectively reduced the number of *S. mutans* in the saliva of 7-10 years old Saudi boys.
2. Mouth rinsing with honey and green tea solutions should be considered as a potential procedure in prevention of caries in children.

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