Antimicrobial Effectiveness of Chlorhexidine Chewing Gums on Streptococcus Mutans Counts– An *in vivo* Microbiological Study

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*The **aim** of the present study was to evaluate the antimicrobial efficacy of Chlorhexidine chewing gums and to assess the effect of dosage and frequency of intake of Chlorhexidine gums on Streptococcus mutans(SM) count. Method: The **sample** consisted of 30 subjects, divided into two groups AI & AII. Each group consisted of 15 subjects. Group AI chewed 2 Chlorhexidine Chewing gum X Twice Daily for 20 minutes (Total = 4 gums Daily) & Group AII chewed 2 Chlorhexidine Chewing gum X Four times daily for 20 minutes (Total = 8 gums Daily) & saliva sample was collected & agar plates were inoculated for SM colony count. The study was carried for a week's time and salivary sample collected were Baseline, Day 1 morning and evening, Day 4 evening, Day7 morning and evening. **Results:** After the gum was chewed, it was observed that the colony count started to decrease when compared with baseline in both the groups. The fall in SM count was statistically highly significant with $p < 0.001$ in both the groups. When comparing between Group AI (Dosage 20mg daily) and Group AII (Dosage 40mg daily), the fall in SM count for both the groups was not statistically significant with p-value > 0.05. It was concluded that there was reduction in the level of Salivary SM, but was not statistically significant, by increasing the dosage and frequency of intake of Chlorhexidine containing gums. **Conclusions:** We recommend that dosage of Chlorhexidine containing chewing gums can be restricted to four gums instead of eight gums per day.*

Keywords: *Chlorhexidine, Chewing gums, Streptococcus mutans, children.*
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INTRODUCTION

Chewing gums is used by a large proportion of the population in many developed countries, and its use has increased in recent years. Human beings have been chewing gum – like substances since ancient time,[1] but the chewing of gum as we know it today has relatively short history. The ability to chew is fundamental for survival of man. The history of chewing gum can be traced back to ancient times. The pleasure of chewing is to clean the mouth or freshen the breath led to the habit of chewing a variety of gum – like substance Eg: Leaves, waxes, animal skin and artificially sweetened paraffin.

Tree resins, in particular, were chewed worldwide from ancient Egyptians to the Mayan Indians in Central America, who chewed a resin named Chicle from sapodilla tree. Greeks have chewed mastic gum (or mastiche), the resin obtained from the bark of the mastic tree.[2]

In 1896, a Dentist, Dr. William F. Semple from Ohio, first patented chewing gum. He considered that chewing gum was not only a tasty confectionary but also had potential as a dentifrice.[2] The first medicated chewing gum contained Aspirin (Acetylsalicyclic acid) and was commercially introduced in 1928 in US. World war II resulted in a shortage of natural gum bases thus synthetic gum bases were developed and they are still in use today. In 1991, Chewing gum was approved as a term for a Pharmaceutical dosage form by the commission of European communities.[2]

Chewing gum[3] is defined as a "Solid preparation with a base consisting of gum which should be chewed and not swallowed, providing a slow steady release of medicine contained.

Chewing gums has several properties that are potentially either beneficial or detrimental to the health of oral tissues.[4] Most chewing gum sold throughout the world is sweetened with sucrose and adds to the cariogenic 'Load' of dietary carbohydrate. On the other hand, Gum can act as a salivary stimulant and has been claimed to 'Cleanse' the mouth. Finally, chewing gum has been proposed as a vehicle for the delivery of therapeutic additives such as antibiotics, nicotine, phosphates and fluorides .

Chewing gum as a CHX Delivery System has the following advantages[5]: Ease of intake (without water, anytime,

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everywhere), less pronounced bitter taste, less impairment of taste sensitivity, better oral distribution, longer oral presence, less staining, less interference with surface active ingredients contained in toothpastes and can be used as an antiplaque and antibacterial agent.

Dental caries is a multifactorial disease with various etiological factors involved in dental caries. Pathogenic microorganism in mouth, fermentable carbohydrate that metabolize to organic acids and tooth surface that are susceptible to acid dissolution.[6] These etiological components (Host, Substrate and Flora) demonstrate the need for simultaneous presence before caries can occur.

Research has shown that chewing gums with CHX reduced the salivary levels of *SM*. As CHX chewing gums have already shown reduction in levels of *SM* in saliva[7] and plaque formation.[8,9,10,11] The knowledge of this confectionary item should be viewed against a background of existing published evidence on the dental effects of chewing gums. In the present age of cut throat competition, we see the market flooded with the formulations of drugs that differ only in their packaging or dosage forms.

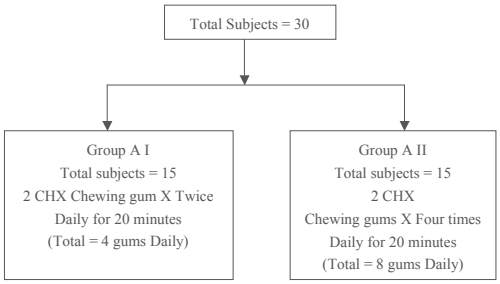
Therefore, keeping in mind these facts about CHX chewing gums, we undertook a study to evaluate the antimicrobial efficacy of CHX chewing gums and to assess the effect of dosage and frequency of intake of CHX gums on *SM* count.

MATERIALS AND METHOD

The study group in the present study consisted of 30 subjects in the age group of 6–12 years, irrespective of sex and socioeconomic status. Systemically healthy subjects with no history of antibiotic therapy within previous three months, no fixed or removable orthodontic appliance or removable prosthesis, no use of any regular or habitual use of CHX containing products, No history of oral prophylaxis done at least three months prior to the study were selected. The subjects volunteered to participate after verbal and written information. Ethical clearance and informed consent were taken.

After selection, Oral prophylaxis of all the subjects was done using Ultrasonic scaler. Then the subjects were instructed to abstain from any oral hygiene measures for next 24 hours.

Day 1 Morning: Baseline saliva sample was collected by spitting into a sterile collecting bottles for all the subjects. Subjects were then divided into two major groups: Group AI and Group AII



All the chewing gums were given before meals for both the groups. The study was conducted for a week's time. After collecting baseline samples, the subjects were given the respective CHX chewing gums (Fertin A/S, Denmark) as *per* the groups and were asked to chew as instructed under supervision and the saliva sample were again collected after 10 minutes. The subjects were then asked to start maintaining there oral hygiene as usual. The same procedure was repeated on Day 1 evening. All the 7 days, the same procedure was followed for all the groups under supervision and the sample collection was taken under aseptic conditions.

Days of sample collection: Day 1: Baseline, Morning and Evening; Day 4: Evening; Day 7: Morning and Evening. The sample were collected in sterile sample bottles and were carried in the ice box containing ice (used as transport media) to microbiology laboratory where the culture plates were inoculated for the Salivary SM counts.

Microbiological Procedure & Method of Inoculation: The Mutans Sanguis agar plates (HiMEDIA) were dried in the incubator for 20 minutes at a temperature of 37ºC in an aerobic chamber. Then, after drying of the plates, they were labeled and the salivary sample was inoculated on agar plates. The sample was taken in a loop of inoculating rod of diameter 1/1000 CFU/ml and was carried onto the agar plates and strains were made on the plate.

The plates were then kept in incubator for 48 hours at 37ºC in incubator for the growth of SM colonies. After 48 hours, the plates were removed from incubator for *SM* count and colony count was done manually using magnifying lens. Colonies of *SM* appeared rough, heaped, irregular resembling frosted glass - white, grey or yellow in color and 0.5 – 2mm in diameter.

RESULTS

The Data obtained was statistically analyzed by using Student's paired t-test and the following results were obtained. In both the Groups, a marked reduction in salivary *SM* count was observed on comparing the baseline with the 7th Day evening sample.

Microbiological results for both the groups are shown in Tables 1 to 4.

Graph 1 shows the mean values of Salivary SM (CFU/ml), at various intervals between the two CHX groups (Group-AI, Group-AII).

Table 1. Mean and Standard deviation values of salivary *Streptococcus Mutans* (CFU/ml), at various levels of Group A I & Group A II

Group	Level					
	Basal value	1st day Morning	1st day Evening	4th day Evening	7th day Morning	7th day Evening
Group-A I	152.33 ± 61.01	66.33 ± 48.39	26.33 ± 29.46	8.80 ± 12.09	5.80 ± 9.40	3.87 ± 6.63
Group-A II	143.33 ± 34.72	60.00 ± 34.45	13.33 ± 14.57	8.33 ± 13.86	6.00 ± 7.11	1.47 ± 2.85

Table 2. Statistical Comparison of Mean change and standard deviation (by Student's t-test, paired) of salivary *Streptococcus Mutans* (CFU/ml) observed from baseline value to various levels of Group A I

Group A I	Level				
	1st day Morning	1st day Evening	4th day Evening	7th day Morning	7th day Evening
Mean change ± Sd.	86.00 ± 58.31	126.00 ± 71.46	143.53 ± 65.16	146.53 ± 65.42	148.47 ± 65.13
P-value	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
Significance	HS	HS	HS	HS	HS

Table 3. Statistical Comparison of Mean change and standard deviation (by Student's t-test, paired) of salivary *Streptococcus Mutans* (CFU/ml) observed from baseline value to various levels of Group A II

Group A I	Level				
	1st day Morning	1st day Evening	4th day Evening	7th day Morning	7th day Evening
Mean change ± Sd.	83.33 ± 50.45	130.00 ± 41.06	135.00 ± 39.46	137.33 ± 36.49	141.87 ± 36.07
P-value	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
Significance	HS	HS	HS	HS	HS

Table 4. Statistical Comparison (by unpaired t-test) of salivary *Streptococcus Mutans* (CFU/ml) of mean change at various levels between Group A I & Group A II

Group	Level				
	1st day Morning	1st day Evening	4th day Evening	7th day Morning	7th day Evening
Group-A I	86.00 ± 58.31	126.00 ± 71.46	143.53 ± 65.16	146.53 ± 65.42	148.47 ± 65.13
Group-A II	83.33 ± 50.45	130.00 ± 41.06	135.00 ± 39.46	137.33 ± 36.49	141.87 ± 36.07
P-value	>0.05	>0.05	>0.05	>0.05	>0.05
Significance	NS	NS	NS	NS	NS

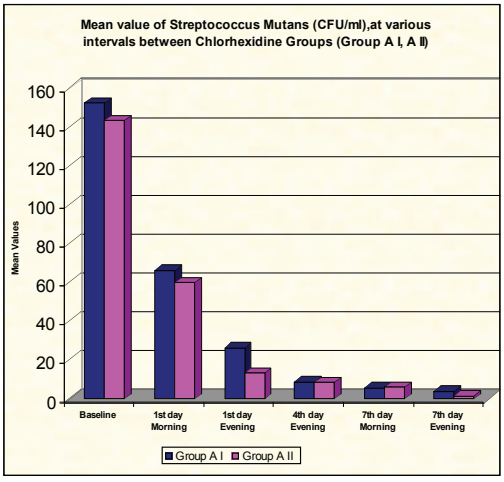


Figure 1. Mean values of Salivary Streptococcus Mutans (CFU/ml), at various intervals between 2 Chlorhexidine groups (Group-A1, Group-AII)

DISCUSSION

Dental caries is a significant public health problem for a large segment of society.[12]

SM is strongly associated with caries in humans and its level in the mouth can be a good indicator of caries-risk. However, caries is a multifactorial disease and the presence of high levels of *SM* at a particular site does not imply that such a site will inevitably develop a lesion.[13] In addition, *SM* is not found alone in association with caries.

For people with caries, the dental health care team needs to apply care strategies beyond restoration placement. Unless the underlying pathology is addressed, the excision and restoration of carious tooth structure alone will not prevent continued dental morbidity.[12]

A strategy is to suppress *SM* levels on dentition. It is a gram positive, facultative anaerobic bacteria commonly found in the human oral cavity.

SM represents the chief pathogen responsible for human coronal and root surface caries.[14,15] A means of predictability eradicating *SM* from the oral cavities of subjects harboring high levels of SM would represent a significant advance in the treatment of dental caries.

Today, it is generally accepted that chewing gums after meals and snacks may reduce plaque formation and gingival inflammation.[16] Chewing gums can also be a suitable delivery vehicle for various chemical compounds, including CHX acetate,[8] however the use of chewing gum is not widely accepted amongst all age groups.[9]

The antimicrobial agent, CHX, is well suited to the task of oral *SM* reduction. It exhibits significant substantivity (Retarded oral clearance), *SM* are more sensitive to it than are other types of oral flora, and it has a long history of safety with few side effects.[17,18] CHX shows different effects at different concentrations; at low concentration, the agent is bacteriostatic, whereas at high concentration the agent is rapidly bacteriocidal.[19] The actual level at which the bacteriostatic and bacteriocidal effects manifest themselves vary between bacterial species. The bacterial cell is characteristically negatively charged. The cationic CHX molecule is rapidly attracted to the negatively charged bacterial cell surface, with specific and strong adsorption of phosphate – containing compounds. This alters the integrity of the bacterial cell membrane and CHX is attracted towards the inner cell membrane. It binds to phospholipids in the inner membrane, leading to increased permeability of the inner membrane and leakage of low molecular weight components, such as potassium ions. At this bacteriostatic (sublethal) stage, the effects of CHX are reversible; removal of excess CHX by neutralizers allow the bacterial cell to recover.[20,21]

CHX mouth rinses have the potential to suppress SM to very low or undetectable levels. Not all people harboring high *mutans* levels, however, respond optimally to Chlorhexidine treatment.[17] Also, once CHX treatment ceases, how quickly people return to pre-treatment *SM* levels varies considerably from subject to subject[22] and appears to be primarily related to incomplete eradication of, rather than re-inoculation with, the pathogen.[23]

Reduction of oral *SM* by topical CHX, has been shown to significantly reduce caries activity.[24] To maintain *SM* suppression for several months or years requires either repeated CHX treatment or some other form of intervention.[25] Repeated use of CHX at tight intervals for an indefinite period is not a viable therapeutic option, as staining of teeth and restorations and taste alteration can be expected to interfere with long-term compliance.[24]

Ainamo and Etemadzadeh (1987)[26] concluded that two pieces of CHX containing gum, each containing 5mg of CHXacetate, chewed five times daily were found to inhibit plaque growth on the teeth completely and the *in vivo* release of CHXacetate from chewing gum was 40% after 5 minutes and approximately 65% after 15 minutes of chewing. Therefore, there was a longer oral presence of CHX with chewing gums compared with CHX mouthwash.

In the present study, salivary SM levels in both the groups AI and AII has shown drastic reduction when compared with baseline. Fall in salivary *SM* level in group AI was observed after chewing of CHX containing chewing gums and the possible reason attributed could be that the cationic CHX molecule binds to anionic compounds such as free sulfates, carboxyl and phosphate group of the pellicle and salivary glycoprotein and will reduce the adsorption of protein to the tooth surface needed for the formation of dental pellicle. Coating salivary bacteria with CHX molecule also alter the mechanism of adsorption of bacteria to the tooth. CHX molecules bound to salivary proteins will be released in 8–12 hours in active form. Low concentration of CHX can still be recovered after 24 hrs. This prolonged bacteriostatic effect of CHX is an important complement to its high initial bacteriocidal activity. It is active against gram positive and gram negative microorganism and against yeast cells. Because of its cationic nature, it has a great affinity for the cell wall of microorganism and change the surface structure. Osmotic equilibrium is lost, as a consequence, cytoplasmic membrane is extruded, vesicles are formed and the cytoplasm precipitates. These precipitations inhibit the repair of the cell wall and the bacteria are no longer able to recover.[27]

We observed that the use of CHX chewing gum twice daily (Total number of gums = 4 per day, Total Dosage of Chlorhexidine acetate = 20mg per day) for a week, reduces the salivary SM count, highly significantly when comparing the baseline values with other samples. It goes in accordance with the study of Simons D, Kidd EAM, Beighton D, Jones B (1997),[28] who also concluded the same that the CHX gum significantly reduced the salivary levels of SM ($p< 0.0001$). Georg Tellefsen *et al* (1996)[10] suggested that regular use of CHX containing gums appear useful to control dental plaque formation.

The possible reason attributed for the reduction in *SM* count goes in accordance to explanation reported by Niklaus PL and Michel CB (1986).[27]

In Group AI and AII, a Total Dosage of 20 mg and 40 mg of CHXacetate was given daily respectively. We observed that there was reduction in the level of Salivary *SM*, but was not statistically significant, by increasing the dosage and frequency of intake of CHX containing gums. The possible reason attributed could be the mechanism of CHXacetate which, when released from chewing gums, has an antibacterial effect and antiplaque effect.[26] But the retention time of CHX in the mouth is known to be about 12 hours that is the CHX molecules bound to the salivary proteins and will be released in 8–12 hours in active form. This prolonged bacteriostatic effect of CHX attributes to its initial bacteriocidal activity.[29]

In our study, no direct relationship was observed between dosage and reduction of SM counts. Therefore, we recommend that Dosage of CHX containing chewing gums can be restricted to four gums instead of eight gums *per* day.

CONCLUSION

According to the study:

1. In antibacterial efficacy, CHX containing chewing gums has shown reduction in salivary *SM* count.
2. When comparing between intake of CHX chewing gums twice a day and four times a day, equal reduction in *SM* count was observed.
3. Finally, based on our study, CHX containing chewing gums has shown significant reduction in salivary *SM* count and therefore, we recommend that two CHX chewing gums twice daily (Total dosage = 20 mg CHXacetate per day) is sufficient enough to reduce the colonization of SM in oral cavity.
4. Further studies, can be conducted with a larger sample size and also to see its effects on any other microorganisms directly or indirectly associated with dental diseases.

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