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Research Article

# EFFECT OF CHEWING MARSHMALLOW CONTAINING BETEL CHEW IN REDUCING *STREPTOCOCCUS MUTANS* AND PLAQUE INDEX ON CHILDREN

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**ABSTRACT**

**Objective:** Marshmallow is a soft candy loved by all levels of society, especially children. Sucrose contained in marshmallow increases the growth of *Streptococcus mutans* and plaque. Betel chew as Indonesian traditional plants add in marshmallow was believed to decrease *S. mutans* and plaque. The aim of this study was to evaluate the effect of chewing marshmallow containing betel chew in reducing *S. mutans* and plaque index in children.

**Methods:** This research was a quasi-experimental with pre-test and post-test control group design. It was conducted in the Laboratory of Agricultural Chemistry, Laboratory of Agricultural Technology Department, and Islamic School of Al-Amalul Khair. Thirty students were divided into two groups. Group A was chewing marshmallow without betel chew and Group B was chewing marshmallow containing betel chew.

**Results:** The results showed that chewing marshmallow without betel chew increased the growth of *S. mutans* and plaque index significantly. Chewing marshmallow without betel chew inhibited the growth of *S. mutans* and dental plaque formation.

**Conclusion:** Chewing marshmallow containing betel chew reduces *S. mutans* and plaque index on children.

**Keywords:** Betel chew, Marshmallow, *Streptococcus mutans*, Plaque.

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## INTRODUCTION

Marshmallow is a kind of soft candy with a texture-like soft foam, chewy in various forms of flavor, taste, and color. Marshmallow is a confectionery product that is loved by all levels of society, especially children because it tastes sweet, soft, and very colorful. This type has a relatively high water content (6–8%) and basic materials primarily sucrose and glucose syrup, whereas for the extra material that serves to form a chewy texture, usually used a mixture of fat, gelatin, and emulator materials [1].

Sucrose and glucose syrup in marshmallow are able to reduce the pH of saliva to below 5.5 drastically, lead demineralization, and cause cavity in the teeth [2]. Sucrose also has the ability to increase the growth of the number of acidogenic microorganisms that can form plaques such as *Streptococcus mutans*. *S. mutans* is one of the initial bacterial colonizers attaching to the pellicle. Pellicle provides receptors for specific surface adhesins of *Streptococci.* Using glucosyltransferase enzyme, the initial bacteria change the sucrose becomes glucans (dextran) and fructose becomes fructans (levan) that contribute to adherence of other microorganisms. The microorganism binds inorganic matrix and firmly attached to the surface of the tooth and causes dental plaque [3].

Dental plaque is a collection of microorganisms, albumin, glycoproteins, and mucins that cover the surface of the teeth. The microbial community in plaque increased the pathogenicity in the oral cavity [4]. It is associated with some dental diseases such as dental caries and periodontal diseases [5]. Simon-Soro and Mira stated that *S. mutans* colonized in dental plaque considered as the main causative agent of dental caries [6]. Another study reported that high *S. mutans* level in plaque was directly associated with increased severity of periodontal disease [7].

The control of dental plaque is needed to reduce the prevalence of dental and periodontal problems. The addition of Indonesian traditional plants such as betel chew in marshmallow was believed to decrease dental plaque. Betel chew consists of betel leaves (*Piper betle*), gambier (*Uncaria gambir*), and betel lime (calcium hydroxide). Those ingredients have antibacterial properties such as catechins, tannins, essential oils, and flavonoids. Mayasari *et al*. revealed that *P. betle* leaves in toothpaste are effective to inhibit the formation of dental plaque [8]. Dewi *et al.* reported that *U. gambir* reduced *S. mutans* colonies [9]. Baranwal *et al.* reviewed that calcium hydroxide had antibacterial properties and widely used in dentistry [10]. Verawati *et al.* revealed that concentration of betel chew inhibited the growth of *S. mutans* significantly [11].

The betel chew is able to be converted into a form of packaging favored by children such as marshmallow. The purpose of this study was to evaluate the effect of chewing marshmallow contained betel chew in reducing *S. mutans* and plaque index on children.

## METHODS

This research was a quasi-experimental with pre-test and post-test control group design. The study was conducted in the Laboratory of Agricultural Chemistry, Laboratory of Agricultural Technology Department, and Islamic School of Al-Amalul Khair. The protocol had been approved by Research Ethical Commission of Mohammad Hoesin General Hospital (RSMH), Palembang, and Medical Faculty of Universitas Sriwijaya with ethical certificate no. 325/kepkrsmhfkunsri/2018. Subjects were 30 students, taken with purposive sampling technic. The inclusion criteria were 12 years old students, DMFT score <5, and health physically and mentally. The exclusion criteria were using fixed appliance orthodontic, using dental prosthetic, and severe crowding and had one habitual chewing side. Subjects were divided into two groups. Group A was chewing marshmallow without betel chew and Group B was chewing marshmallow containing betel chew.

The betel chew consisted of betel leaf (*P. betle* L.), gambier (*U. gambir*), and calcium hydroxide, taken from Babat Toman, Sekayu, and Musi Banyuasin, South Sumatera Province. All components were identified and authenticated by Faculty of Agriculture, Universitas Sriwijaya, Indonesia.

**Procedure**

1. Parents or guardians were given the explanation of the research procedure to be carried out
2. Examination was performed to find out the subjects included in the inclusion and exclusion criteria
3. Examination of the oral cavity was done using sterile mouth mirror (Medesy, Italy) to evaluate the DMFT index
4. Examination of the bacterial colonies in saliva was assessed before and after chewing marshmallow
5. Selected subjects were explained about the procedure and asked to sign informed consents.

**Preparation of betel chew extract**

The betel chew was extracted using maceration methods. The betel chew ingredients were 2.5 g of gambier, 2 g of betel lime, and 8 g of betel leaves. All the ingredients were mixed, added with 40 mL of water. The blended materials were macerated for 24 h at a temperature of 37°C. The extract was vaporized to dryness using rotary evaporator (Rotavapor R300, Buchi, Germany). Ten mL betel chew extract was mixed with 90 mL of water.

**Preparation of marshmallow**

1. Thirty g of gelatin was soaked with 100 mL water for 10 min for Group A, while 30 g of gelatin and 5% betel chew were soaked with 100 mL water for Group B (Mixture 1).
2. Thirty mL of glucose syrup and 250 g of sugar were dissolved in 100 mL of water, heated at a temperature of 115°C for 7 min (Mixture 2).
3. Mixture 1 and 2 were mixed homogenously for 10 min and put into the mold then left for 4 h until it hardened.
4. Marshmallow was cut with the size of 2 cm×2 cm, covered with sweetener and maize flour.

**Examining saliva and dental plaque**

1. Subjects were instructed not to eat and drink an hour before assessment.
2. Subjects were instructed to sit in provided chairs, then spit their saliva as much as 2 mL into measuring saliva pots. The labeled saliva pots were put into box ice at temperature of 10°C (pre-test).
3. Subjects were instructed to open their mouth, calibrated examiners were retracted cheeks and tongues, then dried the teeth. The disclosing dye solution (GC Tri Plaque, ID Gel™, Jakarta, Indonesia) was applied on sterile cotton swab until the swab was fully saturated. The cotton swab was applied on the tooth surfaces gently. Excess solution was washed away by rinsing water once.
4. Dental plaque was examined using Turesky *et al*. Modified Quigley-Hein Plaque Index (TQHPI). Plaque was assessed on mesial, distal, and mid-surfaces of labial/buccal and palatal/lingual aspects of all teeth excluding third molars. Scoring was as follows:
   0 = no plaque; 1 = separate flecks of dental plaque at the cervical margin of the tooth; 2 = a thin continuous band of plaque (up to 1 mm) at the cervical margin of the tooth; 3 = a band of plaque wider than 1 mm but covering less than one-third of the crown of the tooth; 4 = plaque covering at least one-third but less than two-thirds of the crown of the tooth; and 5 = plaque covering two-thirds or more of the crown of the tooth (Fig. 1) [12,13] (pre-test).
5. After examining dental plaque, subjects were asked for rinsing off their mouth by a bottle of water (330 mL).
6. Subjects were divided into two groups. Group A was chewing marshmallow without containing betel chew and Group B was chewing marshmallow containing betel chew. Both groups were instructed to chew marshmallow using posterior teeth right and left alternately for 32 times.

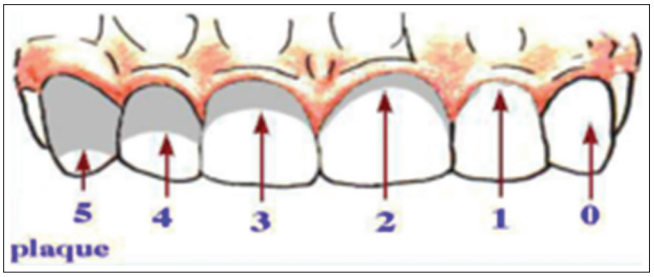


**Fig. 1: Turesky *et al*. Modified Quigley-Hein Plaque Index scoring [13]**

7. After 6 h, subjects were instructed to sit in provided chairs, then spit their saliva as much as 2 mL into measuring saliva pots. The labeled saliva pots were put into box ice at a temperature of 10°C (post-test).
8. Post-test dental plaque score was assessed by TQHPI. The clinical parameter was examined by the same independent calibrated examiners.

**Examining *S. mutans***

The samples of saliva were homogenated, diluted, and thinned for twice. Ten µL was taken using micropipette and inoculated in selective media of Tryptone Yeast Extract Cystine w/Sucrose and w/o Bacitracin Agar Base, then spread using loop inoculation. Subsequently, the media were inoculated for 24 h at room temperature of 37°C. The colonies were counted using Colony Counter (SC6 Plus, Stuart®, London, UK).

**Statistical analysis**

Data were recorded in the form, processed, and analyzed. Data were tested with Levene's test to know the homogeneity of samples. If $p>0.05$, it meant that data were homogenous. Kolmogorov–Smirnov test was used to know the normality of samples. If p=0.0.05, it meant that data were normal. Paired t-test was used to evaluate the effect between "before and after" chewing marshmallow. To test for differences in plaque scores between chewing marshmallow contained betel chew and no betel chew, the independent test was used. If data were not homogenous and normal, so Wilcoxon and Mann–Whitney U-test were used. *$p<0.05$ was considered statistically significant. All statistical analyses were performed using the SPSS (ver. 22, IBM Inc. USA).

**RESULTS**

The evaluation of the homogeneity test and normality test for the number of *S. mutans* showed $p<0.05$. Hence, it meant that the data were not homogenous and normal. Wilcoxon test showed that there was a significant effect between before and after chewing marshmallow without betel chew ($p<0.05$). Chewing marshmallow containing betel chew did not increase *S. mutans* while chewing marshmallow without betel chew increased *S. mutans* (Table 1). Chewing marshmallow enhanced the number of *S. mutans* in saliva, whereas there was no significant effect between before and after chewing marshmallow containing betel chew. It meant that chewing marshmallow containing betel chew inhibited the growth of *S. mutans*.

Mann–Whitney U-test showed that there was a significant effect of chewing marshmallow containing betel chew in inhibiting the growth of *S. mutans* in children (Table 2).

Plaque index was observed and evaluated in both groups. The homogeneity and normality test showed that $p>0.05$. It meant that data were homogenous and normal. From Table 3, it could be seen that chewing marshmallow without betel chew led dental plaque formation while chewing marshmallow containing betel chew did not form dental plaque in children's teeth. Paired t-test showed that plaque index increased significantly when chewing marshmallow without betel chew.

Independent t-test exhibited that there was a significant effect of chewing marshmallow containing betel chew in reducing plaque index

($p<0.05$). These findings showed that chewing marshmallow inhibited dental plaque formation (Table 4).

## DISCUSSION

Marshmallow with betel chew was able to reduce *S. mutans* and inhibit plaque index. The decrease was due to their active compounds contained in betel chew such as alkaloids, flavonoids catechins, tannins, and other bioactive molecules. Shah *et al.* reviewed that *P. betle* leaves contained variety of active compounds, especially essential oil and tannin that were effective against *E. coli*, *S. mutans, S. aureus, Pseudomonas aeruginosa*, and *Staphylococcus cerevisiae* [14]. Nalina and Rahim exhibited that *P. betle* extract inhibited the growth, adhering ability, glucosyltransferase activity, and cell surface hydrophobicity of *S. mutans* [15]. The loss of cell surface hydrophobicity caused the loss of adhesion ability to the tooth surfaces. It is because the presence of active molecules contained in *P. betle* alternates the cell surface protein of *S. mutans* [16]. Glucosyltransferase facilitates the formation of insoluble glucan, thereby providing binding sites for *S. mutans* and other oral pathogens. The reduction of glucosyltransferase activity may contribute to the reduction of water-insoluble glucan [17]. The capability of *P. betle* in reducing all virulence factors of *S. mutans* in forming biofilm leads the inhibition of dental plaque formation. The previous study reported that *P. betle* L. leaves extracted contained in mouthwash and toothpaste decreased plaque score [18].

*U. gambir* contained in marshmallow has potent antibacterial Gram-positive activity, due to catechins and tannins [9]. Catechins and tannins caused membrane damage by phenolic interaction to bacterial membrane, leakage of intracellular constituent, inhibition of enzyme activities, and nucleic acid formation [19,20]. Dewi *et al.,* 2018, reported that gambier extract had antiseptic effect in mucosal wound [21]. This antibacterial effect will lead to reducing the formation of dental plaque.

Calcium hydroxide has a wide range of antibacterial activities against oral pathogens [22]. The antimicrobial activities of calcium hydroxide are damaging to the bacterial membrane, protein denaturation, and damaging to the DNA [23]. Javidi *et al.* reported that calcium hydroxide was able to reduce intraluminal and intratubular pathogens significantly in treated tooth [24].

All active molecules contained in betel chew synergistically reduced *S. mutans*. These bacteria play an important role in dental plaque formation. *S. mutans* adhere to the pellicle with specific cell-to-surface interactions. The growth of *S. mutans* leads to the formation of dental plaque. They produce acidic environment and create tooth cavities. The toxins induce some periodontal diseases such as gingivitis and periodontitis. By inhibiting the number of *S. mutans,* it causes the reduction of dental plaque formation [25].

Marshmallow is a popular confectionery product, a special kind of soufflé [26]. Marshmallow is consumed by many people from children, teenagers, and adults. Its consistency provides a comfortable and pleasant feeling to the consumers. However, consuming marshmallow without adequate cleaning of oral cavity will cause some dental and periodontal diseases. The role of sugar as one of the marshmallow compositions is overwhelming as a risk factor of dental caries. Gupta *et al.* reported that there was a significant effect between daily sugar intake and dental caries in 12-year-old students [27]. Skafida and Chambers found that there was a positive correlation between sugar consumption in soft drinks, sweets, chocolates, snacks, and tooth decay in pre-schoolchildren [28]. The addition of betel chew extract in marshmallow is capable to reduce the number of *S. mutans* and dental plaque, so oral health problems will be decreased.

**Table 1: Mean of *Streptococcus mutans* before and after chewing marshmallow**

| Groups | Mean±SD ($\times10^3$) | | p value |
|---|---|---|---|
| | Before | After | |
| Marshmallow without betel chew | 7.00±0.99 | 9.11±0.55 | 0.00* |
| Marshmallow containing betel chew | 7.06±0.68 | 7.01±0.89 | 0.41 |

Wilcoxon test, p=0.05* significant

**Table 2: Differences of *S. mutans* between chewing marshmallow without betel chew and containing betel chew**

| Groups | Mean of *S. mutans*±SD ($\times10^3$) | p value |
|---|---|---|
| Marshmallow without betel chew | 9.11±0.55 | 0.00* |
| Marshmallow containing betel chew | 7.01±0.89 | |

Mann–Whitney U-test, p=0.05* significant. *S. mutans: Streptococcus mutans*

**Table 3: Mean of plaque index before and after chewing marshmallow**

| Groups | Mean±SD ($\times10^3$) | | p value |
|---|---|---|---|
| | Before | After | |
| Marshmallow without betel chew | 2.43±0.19 | 2.94±0.17 | 0.00* |
| Marshmallow containing betel chew | 2.49±0.15 | 2.47±0.14 | 0.09 |

Wilcoxon test, p=0.05* significant

**Table 4: Differences of plaque index between chewing marshmallow without betel chew and containing betel chew**

| Groups | Mean of *Streptococcus mutans*±SD ($\times10^3$) | p value |
|---|---|---|
| Marshmallow without betel chew | 2.94±0.17 | 0.00* |
| Marshmallow containing betel chew | 2.47±0.14 | |

Mann–Whitney U-test, p=0.05* significant

## CONCLUSION

This study has shown that chewing marshmallow contained betel chew reduces *S. mutans* and plaque formation in children.

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## AUTHORS' CONTRIBUTIONS

Prof. Dr. Rindit Pambayun conceived the presented idea, developed the theory, investigated, and supervised the findings; Ms. Ade Putri and Mr. Meidi Tri Yudha carried out the experiments and administered ethical clearance and informed consents; Dr. Siti Rusdiana Puspa Dewi conceived the idea, carried out the experiments, and wrote the manuscript; Mrs. Tri Wardani Widowati and Mr. Budi Santoso contributed in making, performed, and analyzed the quality of marshmallow. All authors had contribution to the final manuscript.

## CONFLICTS OF INTEREST

There is no conflict of interest among all authors regarding the publication of this manuscript. All authors had contributed substantially to the manuscript.

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