Effectiveness of Chitosan Tooth Paste from White Shrimp (Litopenaeusvannamei) to Reduce Number of Streptococcus Mutans in the Case of Early Childhood Caries

Harun Achmad[1], Yunita Feby Ramadhany[2]*

1. Department of Pedodontic Faculty of Dentistry Hasanuddin University, Makassar
2. Undergraduate Student of Dentistry Faculty of Hasanuddin University, Makassar

Abstract

Early childhood caries is one of the most common dental caries in children. One possible cause is the bacterium Streptococcus mutans. The most effective method for preventing the accumulation of plaque is brushing with toothpaste. Chitosan is a natural biopolymer as antibacterial and can be combined with toothpaste.

This study aims to determine the effectiveness of chitosan shell toothpaste white shrimp (Litopenaeusvannamei) in reducing Streptococcus mutans in cases of early childhood caries.

This research uses experimental field and laboratory research design pretest-posttest control group design. A sample of 30 children consisted of 3 groups: 10 children use toothpaste placebo, 10 children use toothpaste chitosan 2.5%, and 10 children use toothpaste chitosan 5%. Then bacteria sampling before and after brushing teeth. They are then taken to the laboratory for counting the number of colonies of Streptococcus mutans using colony counter with units of CFU. Processing and analysis of data using SPSS version 22.0 for Windows.

Paired t-test results showed a significant decrease in the number of colonies of Streptococcus mutans before and after brushing with toothpaste chitosan 5%. The number of bacterial colonies on is as much as 120.4 CFU pre and post as much as 11.4 CFU. The test results of data obtained p = 0.000 (p <0.05). This shows a significant reduction of the number of colonies of Streptococcus mutans in the case of early childhood caries.

Toothpaste chitosan 5% effective in reducing the number of bacterial colonies of Streptococcus mutans in the case of early childhood caries in children.

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Introduction

Oral health problems that are often found in children around the world, especially in developing countries including Indonesia are dental caries.[1]Caries is a chronic and regressive process that begins with the dissolution of the email by the formation of acid substrate and microbes, resulting in the destruction of the organic components of teeth.[2] Type of primary teeth caries are common, namely early childhood caries. Caries is often found in children aged under five years, with the highest prevalence in children aged three years.[3] The prevalence of early childhood caries according to the American Academy Of Pediatric Dentistry (AAPD), that children aged 12-36 months was 57.7%.[4] The prevalence of caries in Indonesia reached 90% of the population of children under five. According to research by the Health Research (RISKESDAS) in 2007, showed that dental caries has increased, especially in children ages toddler and pre-school children, ie from 24% to 28% whereas in children aged 2-5 years increased by 70% including early childhood caries.[5]

The process of caries is influenced by host factors (tooth surface), microorganisms (bacteria that cause caries), substrate (fermentable carbohydrates), and time. Caries can occur if all of these factors.[6] Dental plaque is a biofilm that forms naturally on the surface of the

Corresponding author:
Harun Achmad
Department of Pedodontic Faculty of Dentistry Hasanuddin University, Makassar
E-mail: harunachmader@gmail.com; febyr.yuferaa@yahoo.co.id

teeth and is supported by the ability of the host to resist the invasion bakteri.[7] If plaque builds up will appear grayed.[8] Some studies indicate that dental plaque play a role in oral disease and is associated with cariogenecity (ability substance that potentially form caries) of plaque bacteria, such as acid production, resulting from the production of polysaccharide intra and extra selular.[9]

Streptococcus mutans is a bacterium that is closely related acidogenicas a trigger for early childhood caries. Some types of carbohydrates, such as sucrose that can be fermented by Streptococcus mutans to form acids, which can lower the plaque pH and cause demineralization email.[10] caries prevention can be done by performing plaque control by means of mechanically removing plaque toothpaste to suppress the growth of *Streptococcus mutans*, antibacterial ingredient commonly used in toothpaste is phenol, hexetidine, fluorine and chlorhexidine.[11] That's why the alternative material of chitosan is an interesting material as antibacterial.[12]

According Kim[12] which states that the amino-polysaccharide chitosan is natural that has the molecular formula $C_8H_{12}NO_5$ with high levels of C, H, N, and O respectively 47%, 6%, 7% and 40%. The main source of chitosan obtained from the exoskeletons of marine invertebrate crustaceans (*crustacean*), such as shrimp, crab and lobster. The shrimp is a commodity fisheries sector, which has high economic value. One type of shrimp that is widely cultivated for export is white shrimp (*Litopenaeusvannamei*). There are currently a range of Indonesian shrimp waste reaches 298.642.25 tons every years.[13]

According to research conducted by Akbar[10], chitosan as an antibacterial to degradation in the cell walls of bacteria, then the resulting damage cytoplasmic membrane cytoplasm nucleus out of the bacterial cell wall. According Visveswaraiah[14] in his research proves that organic ion solubility chitosan inhibits hydroxapatite acid, which is highly reactive with cariogenic foods. According to the research results Rosdiani[15], proving that the anticariogenic properties of chitosan can act as a mechanical barrier to the enamel.

The basis of this study is the antibacterial properties of chitosan that will be combined with toothpaste that has the capability of remineralization, so the potential to be an agent of treatment in cases of early childhood caries. Results from this study is a toothpaste combined with chitosan leather waste white shrimp (*Litopenaeusvannamei*). However, until now there has been no research on the effects cariostatic of toothpaste combined with chitosan. Therefore, researchers interested in conducting a study on the effectiveness of a combination of toothpaste and white shrimp shell waste chitosan (*Litopenaeusvannamei*) in lowering the cariogenic bacteria *Streptococcus mutans* in cases of early childhood caries.

Materials and methods

This type of research is a quasi experimental. This study design using pre and post test with control group design. This research was conducted in kindergarten Cipta Mandiri Makassar for sampling the bacteria *Streptococcus mutans* on the teeth of children who experienced early childhood caries, Laboratory of Biochemistry, Faculty of Mathematics and Natural Sciences of Hasanuddin University to manufacture toothpaste chitosan from shrimp shell white (*Litopenaeusvannamei*) and in the Laboratory of Oral Biology, Faculty of Dentistry, University of Hasanuddin for counting the number of colonies of bacteria *Streptococcus mutans* in children who experienced early childhood caries. The study was conducted on 2 to 20 January 2017. The population was Cipta Mandiri Makassar kindergarten students who experienced early childhood caries aged 5 years. Samples numbered are 30 peoples. The sampling method used was simple random sampling for the research subjects homogeneous.

The criteria for inclusion in this study were children aged minimum 36 months and maximum 71 months, children with early childhood caries with caries at least 2 teeth, willing to be the subject of study by completing the informed consent, brushing teeth twice a day, did not have periodontal disease, and not taking drugs. Exclusion criteria in this study is in the process of sampling suddenly become the subject of child resists and does not adhere to the treatment process.

This study begins with the manufacture of chitosan from shrimp shell white (*Litopenaeusvannamei*). The first stage in the manufacture of chitosan is a sample preparation

process, ie white shrimp shell waste shrimp waste washed with water while cleaning. Furthermore, dried shrimp shells in the oven at a temperature of 80°C, and then pulverized using a dry blender. The second stage is the isolation of chitin from shrimp shells carried out in several stages. The first stage is demineralization, made by soaking the shrimp shells in a solution of hydrochloric acid 3%. The second stage is deproteinization, made by soaking the shells with a 4% solution of sodium hydroxide. The third stage is the formation and purification of chitosan. Then the process is carried out by soaking deacetylation shrimp shells in a solution of 60% sodium hydroxide. After that, chitosan which has been obtained is dried at a temperature of 65^{o}C. The results were Nihidrin, 0.1 g chitosan solution sprayed nihidrin then allowed to stand for 5 minutes. Chitosan has been cultivated in the laboratory of Biochemistry, Faculty of Mathematics and Natural Sciences University of Hasanuddin continued with toothpaste manufacturing chitosan 2.5% and 5%.

Research in kindergarten Cipta Mandiri Makassar begins by asking the parents of children to fill out a questionnaire that asked about things pertaining to this study, to know the different types of variations that exist in every subject of study. Next, look for children who meet the inclusion criteria will be the subject of research. The next stage is to disseminate correct tooth brushing to kindergarten. It aims to unify the way of brushing teeth, so as not to be a moderator variable in the study. The study group was divided into 3 groups; there are 10 children brushing with toothpaste placebo, 10 children brushing with toothpaste chitosan 2.5%, and 10 children brushing with toothpaste chitosan 5%. First sample when the children are not brushing their teeth. Sampling of bacteria using a swab swab to smear technique. Then, the bacteria samples stored in physiological solution in NaCl0.9% is stored in a bottle vial of 60 bottles. After brushing teeth, children were instructed not perform activities of eating and drinking for 5 minutes. Then, the samples were taken after brushing and samples were kept in a NaCl 0.9% solution.

To calculate the number of colonies of bacteria *Streptococcus mutans* beginning with sterilization of equipment and materials used in oral biology laboratory. Then, the 10^{-3} dilution using NaCl 0.9% solution. Then, the manufacture of selective medium, namely TYSB20 (Tryptone Yeast Extract Sucrose Cysteine Bacitracin 20) which aims to select and identify the bacteria *Streptococcus mutans,* so that only this type of bacteria that can live as research variables. Then, do do pour plate method to isolate bacteria in a petri dish. 1 ml bacteria dilution has been carried put in a petri dish, then pour TYSB20 selective medium (Tryptone Yeast Extract Sucrose Cysteine Bacitracin 20) 10 mL sterile. Then the suspension was homogenized and put in an incubator at 37°C for 1x24 hours. After the bacteria were incubated, observations and calculation of the number of colonies to do with the method of colony forming units (CFU/ml) using colony counter. Analysis of the data in this study using the computer program SPSS 22.0 for windows. To test the significance difference in the number of colonies of bacteria Streptococcus mutans before and after each treatment group then tested the paired T-test.

Results

This research has been getting information escaped conduct with numbers: 8/H04.8.4.5.31/PP36-KOMETIK/2017 and registration number UH17010015 on January 6th, 2017. The results shown in the following table:

Gender	Pre	Post
	Mean ± SD	Mean ± SD
Male	120.8824 ± 6.07127	26.5882±14.30498
Female	121.8462 ± 6.14880	28.0000 ± 14.08309
Total	121.3000 ± 6.01808	27.2000 ± 13.98127

Table 1. Average Value Standard Deviation Number of Colonies of bacteria *Streptococcus mutans* by Sex

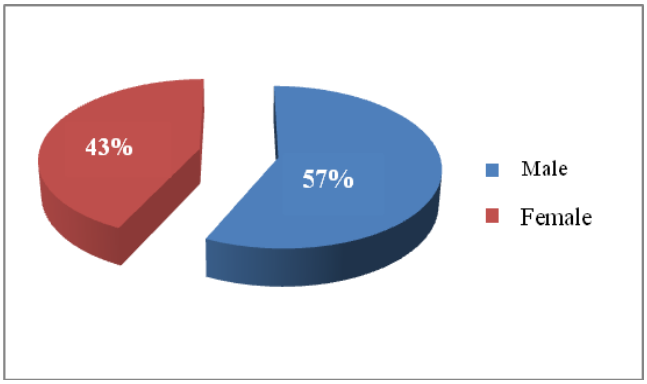

Table 2. Comparison Chart Period Subject Gender Male And Female.

On the characteristics of sex, reducing the number of colonies of bacteria *Streptococcus mutans* from pre to post in both sexes. *Streptococcus mutans* bacteria colony count obtained on the sex most female with pre total number of colonies CFU 121.84 and the post as much as 28.0 CFU.

Gender	Pre K	Post K	Pre X1	Post X1	Pre X2	Pre X2
	Mean± SD	Mean± SD	Mean± SD	Mean± SD	Mean± SD	Mean± SD
Male	1120.3333±6.91857	41.6667±6.15359	122.4000±6.69328	27.6000±5.85662	120.1667±5.56477	10.6667±3.38625
Female	22.5000±7.59386	44.7500±6.60177	122.2000±4.43847	27.0000±4.63681	120.7500±8.05709	12.5000±5.00000
Total	121.2000±6.86052	42.9000±6.17252	122.3000±5.35516	27.3000±4.98999	120.4000±6.2396	11.4000±3.94968

Table 3. Average Value Standard Deviation Number of Colonies of bacteria *Streptococcus mutans* by Sex in the Placebo group Toothpaste, Chitosan 2.5%Toothpaste, and Chitosan 5% Toothpaste.

On the characteristics of sex, reducing the number of colonies of bacteria Streptococcus mutans from pre to post in both sexes. Streptococcus mutans bacteria colony count most are found on the female gender by the number of colonies on the toothpaste placebo group pre many as 122.5 CFU and the CFU post as much as 44.75. In the group of toothpaste chitosan 2.5% pre and post as much as 122.2 27.0 CFU CFU. In the group of toothpaste chitosan 5% pre and post as much as 120.75 CFU 12.5 CFU.

Toothpaste Group	Pre	Post	t	p
	Mean ± SD	Mean ± SD		
Placebo Toothpaste	121.2000 ± 6.86052	42.9000 ± 6.17252	145.399	.000
Chitosan 2,5% Toothpaste	122.3000 ± 5.35516	27.3000 ± 4.98999	120.208	.000
Chitosan 5% Toothpaste	120.4000 ± 6.23966	11.4000 ± 3.94968	318.640	.000

Table 4. Difference Value Average Number of Colonies of bacteria Streptococcus mutans By Group Placebo Toothpaste, Chitosan 2.5%Toothpaste, and 5% Chitosan Toothpaste Before and After Brushing. *Paired sample t-test: p < 0,05; significants.*

The average value number of colonies of bacteria Streptococcus mutansin placebo toothpaste group as much as 121.20 CFU pre, post 42.9 CFU, and the difference in the average value of as much as 145.399 CFU. The average value of the bacterium Streptococcus mutans colonies in groups of chitosan 2.5% dentifrice as much as 122.30 CFU pre, post as much as 27.3 CFU, and the difference in the average value of as much as 120.208 CFU. The average value of bacterial colonies of Streptococcus mutans in the group of toothpaste chitosan 5% as much as 120.40 CFU pre, post as many as 11.4 CFU seen significant changes and differences in the average value of as much as 318.64 CFU.

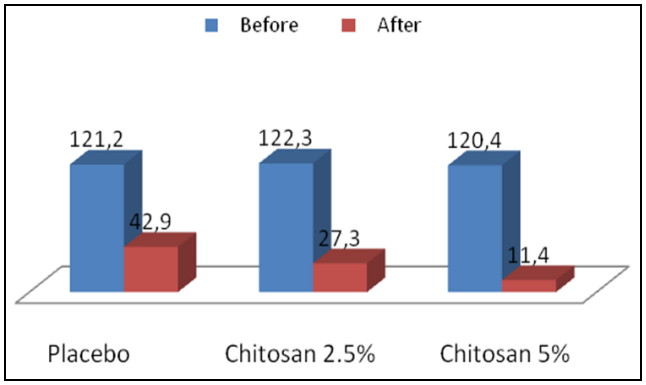

Table 5. Diagram Average Number of Colonies of bacteria *Streptococcus mutans* Before and After Brushing with Placebo Toothpaste, Chitosan 2.5%Toothpaste, and Chitosan 5% Toothpaste

All groups toothpaste has a significant change. The p-value is 0.000 and looks at p <0.005. However, the group toothpastes chitosan 5% had the largest difference between the groups and the placebo toothpaste toothpaste chitosan 2.5%.

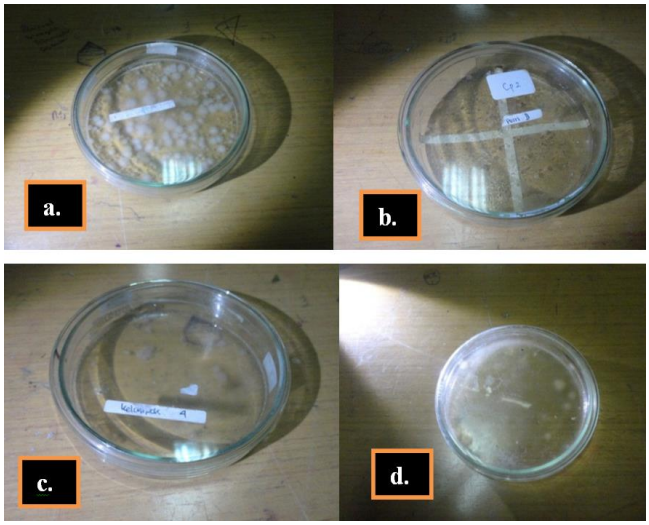

Figure a. The number of bacteria colonies of *Streptococcus mutans* Before Brushing; **b.** Toothpaste Placebo group; **c.** Toothpaste group Chitosan 2.5%; **d.** Toothpaste group Chitosan 5%.

Discussion

Antibacterial effect seen by the ability of toothpaste to kill bacteria colonies plaque measured by calculating the decrease in the number of bacterial colonies plaque. Samples of bacteria deposited into physiological solution of NaCl 0.9%, followed by a 10^{-3} dilution method because it is more effective in reducing the number of bacterial colonies. Previous research breed *Streprococcus mutans* in the blood so that, while in this study *Streptococcus mutans* cultured on selective media, namely TYSB20 (Tryptone Yeast Extract Sucrose cysteine Bacitracin 20).[16]

This study uses a chitosan concentration of 2.5% and 5% based on the research that has been done by Visveswaraiah[14] (2016) says that chitosan is effective and safe for the body with a concentration of 2.5% and 5%. That statement is the same to Soeroso (2016).[17] Table 4 shows that the average value of colonies of bacteria Streptococcus mutans in the group of toothpaste chitosan 5% as much as 120.40 CFU pre, post as many as 11.4 CFU seen significant changes and differences in the average value of as much as 318.64 CFU. Toothpaste chitosan works as an antibacterial agent by combining the capabilities of toothpaste in inhibiting bacterial cell division and chitosan disrupt bacterial metabolism through inhibition of glycolysis bacteria. According to research conducted by Indrani[18] (2016), chitosan as an antibacterial to degradation in the cell walls of bacteria, then the resulting damage cytoplasmic membrane cytoplasm nucleus out of the bacterial cell wall.

Based on research Yuliati[19] (2017) proved that chitosan has antibacterial properties because it inhibits bacteria to produce enzymes glucosyltransferase (GTF). GTF enzyme serves to convert sucrose into two types of glucans, namely glucan soluble and water insoluble glucan water. Water-soluble glucans also called mutant (α-1,3glucan), while water insoluble glucan called dextran (α-1,6 glucan). The glucan role in initiating the attachment of bacteria in dental plaque. Chitosan can also inhibit the expression of m-RNA that induces the formation of the enzyme COX-2 (Prostaglandin-endoperoksida synthase 2) that play a role in the mechanism of inflammation and pain.[15]Accordingto Olga[20] (2017) say that which proves that the antibacterial effect of chitosan derived from amine groups groups free amine (NH_2) chitosan protonated become cationic amine group ($-NH_3$) and can interact with the surface of the negatively charged bacterial cell. Therefore, chitosan has antibacterial toothpaste excellent in reducing the number of colonies of bacteria *Streptococcus mutans* in cases of early childhood caries.

Conclusions

Chitosan 5% toothpaste more effective than chitosan 2.5% toothpaste and placebo toothpaste. So in this study, chitosan 5% toothpaste have a more significant influence on the growth of *Streptococcus mutans.*

Declaration of Interest

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