

Role of Green Tea Catechins Toothpaste on Transforming Growth Factor- β 1 (TGF- β 1) and Bone Morphogenetic-2 (BMP-2) on Early Childhood Caries

by Fajriani _

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Role of Green Tea Catechins Toothpaste on Transforming Growth Factor- β 1 (TGF- β 1) and Bone Morphogenetic-2 (BMP-2) on Early Childhood Caries

Fajriani¹, Sartini², Sherly Horax¹, Adam Malik¹, Asmawati³, Atika Balqis¹, Dwi Putri Wulansari⁴

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¹Department of Pediatric Dentistry, Faculty of Dentistry, Hasanuddin University, Makassar, Indonesia

²Pharmaceutical Sciences, Faculty of Sciences, Hasanuddin University, Makassar, Indonesia

³Department of Oral Biology, Faculty of Dentistry, Hasanuddin University, Makassar, Indonesia

⁴Department of Oral and Maxillofacial Radiology, Faculty of Dentistry, Hasanuddin University, Makassar, Indonesia

Corresponding Author: fajriani1kg@gmail.com

ABSTRACT

Early Childhood caries (ECC) define as the most common dental health problem in children. ECC is severe tooth decay and develops quickly and causes long-standing dental problems among children. The objective of this study was to make toothpaste from green tea polyphenol for use among children with ECC. Next will be a beta growth factor transforming examination (TGF- β 1) to review tertiary dentin formation in children's teeth after using green tea catechin toothpaste so that it can be used as a topical treatment without any restoration. This study was an experimental study with a pre and post design, which was started by making green tea toothpaste catechins accompanied by an optimal inhibitory test for Streptococcus mutated bacteria. Further studies of patients with ECC were given Green Tea Polyphenol toothpaste and TGF- β 1 and BMP-2 measurements were taken before and after the experiment. The results showed an increase in TGF- β 1 and BMP-2 levels after using green tea catechin toothpaste in the first 5 minutes increased but decreased in 10 minutes. TGF- β 1 test results produced P < 0.05 (0.036) significant in the first 5 minutes after the use of green tea catechin toothpaste, BMP-2 P > 0.05 (0.705), 5 minutes or 10 minutes after the results of the first treatment test 2 TGF- β 1 produces P > 0.05 (0.086) and BMP-2 P > 0.05 (0.41). Based on the research, it was concluded that topical therapy was found to be more effective and safer natural ingredients to stop progressive caries and form a tertiary, especially among ECC's children.

Keywords: Early Childhood Caries, TGF- β 1, Polyphenol Green Tea Toothpaste

Correspondence:

Fajriani

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¹Department of Pediatric Dentistry, Faculty of Dentistry, Hasanuddin University, Makassar Indonesia

Corresponding Author: fajriani1kg@gmail.com

INTRODUCTION

Early Childhood caries is a disease that is most dental rampant common among children. American Dental Association or ADA described the ECC as the appearance of one or more decay (cavities lesion with or without cavity), loss of teeth due to caries or fillings, and deciduous tooth surface at preschool age between the ages of birth to 71 months. The prevalence of ECC achieves high levels in developing countries, and severity increases with age.¹ Household Health Survey (Survey) in 2004, the level of caries in Indonesia was 90.05% of the population of Indonesia. In 2011, the most significant incidence occurred in children aged 3-5 years 81.2%. To prevent the caries process from continuing, it takes a substance that can prevent demineralization. Some study approved that the development of caries process is determined by the balance between protective and pathological factors.²

The role of antibacterial activity of green tea catechins is very important in wound healing since the substance of phenols in green tea has the ability to denature bacterial proteins, forming a complex with the cell walls of bacteria and bacterial cell membrane damage. Death of bacteria results in reduced cell phagocytosis of bacteria by polymorphonuclear leukocytes. This resulted in the inflammatory phase of short duration so that the proliferative phase lasts too early. Similarly, the condition of the carious dentin, with the death of the bacteria will stimulate secretion dental papilla cell-matrix through which is played by TGF- β 1 and BMP-2.²

Transforming Growth Factor beta (TGF- β 1) and Bone Morphogenetic Beta-2 (BMP-2) is a protein that controls proliferation, cellular differentiation, and other functions

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in most cells. TGF- β 1 plays a role in the immune system, cancer, heart disease, diabetes, and syndrome Marfan. TGF- β 1 acts as a normal antiproliferative factor in epithelial cells and in the early stage of oncogenesis. In relation to dental health, TGF- β 1 is known as a growth factor that strictly regulates molecular and cellular events in the formation of tertiary dentin. Tertiary dentin is dentin formed in pathological processes which are classified into reactionary and reparative dentin.^{3,4} Some researchers have concluded that primary MMP-2 matrix metalloproteinases can be inhibited with green tea polyphenols and MMP-9 participates in dentin matrix degradation. MMP inhibition by inhibitors, particularly natural materials can provide the therapeutic potential of inhibiting the progressive carious dentin. Our previous studies have shown the effectiveness of the active substance catekin green tea can reduce MMP-8, and MMP-9, and TIMP-1 increases.⁵ Researchers propose to have toothpaste to make it convenient to use among children with ECC. Testing the Transforming Growth Factor beta (TGF- β 1) and Bone morphogenetic beta-2 (BMP-2) is important to test whether green tea toothpaste could be used as a topical treatment in the teeth of children with ECC.

MATERIALS AND METHODS

This study is a randomized experimental design with pre- and post-test control group design, as many as 30 patients with Early Childhood Caries disease are treated by administering a toothbrush with toothpaste green tea catechins. After the manufacture of toothpaste green tea catechins, we examine mutated Streptococcus bacteria and Lactobacillus, followed by measured growth factors

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TGF- β 1 dan BMP-2 in the saliva of patients ECC. Consecutive sampling is a selected sampling method, where all the subjects that come and meet the selection criteria for inclusion in the study until the required number of subjects are fulfilled.

Subjects were patients with Early Childhood Caries visiting Department of Pediatric Dentistry, Hasanuddin University Dental Hospital, Indonesia. With the inclusion criteria children aged 3-6 years in both men and women, people with ECC with a minimum of 4 carious dentin in the oral cavity, has not been done by the application of topical fluoride treatments to him and are willing to participate in research with parental consent and signed informed consent. Exclusion criteria were the study subjects suffering from systemic disease, taking antibiotics and cannot follow the procedure.

Governance Research

Phase 1: Preparation of green tea catechins toothpaste, minimal examination barrier against bacteria mutated *Streptococcus* and *Lactobacillus*, the examination of optimal barrier against bacteria mutated *Streptococcus* and *Lactobacillus*

Phase 2: Before making the subject of research, carry out a complete analysis of the subject and of forms regarding age, gender, health status and medications are used. Subjects who met the inclusion criteria will be a detailed explanation of the treatment to be received during the research process and sign an informed consent nature of this case is represented by a parent or guardian, dental status examination and clinical examination, saliva samples were taken before treatment, provision of toothpaste as a topical application procedure green tea catechins, taking saliva samples after treatment 5 minutes and 10 minutes after treatment to see the effect of interference toothpaste green tea catechins examined TGF- β 1 and BMP-2.

Saliva Sampling

Saliva sampling uses the Spitting method (standard method of Navazhesh, 1993). Before and during the collection of saliva, study subjects were not allowed to eat, drink and clean the mouth cavity, over a period of 90 minutes. During the process of collecting saliva subjects were not allowed to talk, move the tongue, or swallow movements. Subjects sit comfortably with the backrest upright, head bowed with his right hand holding the receiver tube of saliva. Saliva which is collected is whole saliva (whole saliva) with stimulation. Collecting saliva is done for 5 minutes, preceded by stimulation paraffin wax, then every 1-minute interval subjects were asked to remove the collected saliva in the mouth into the measuring tube through a glass funnel, saliva immediately deposited into a thermos of ice, then stored in a refrigerator at a temperature of -70°C for the measured levels of TGF- β 1 and BMP-2.

The level of TGF- β 1 (pg/ml) and BMP-2 measured in the laboratory of Hasanuddin University. Making green tea catechins toothpaste done at Biofarma Pharmaceutical Laboratories Hasanuddin University, and Inspection of mutated *Streptococcus* bacteria and *Lactobacillus* done at Microbiology Laboratory, Hasanuddin University. The data was analyzed by SPSS 15.0 for windows, performed multivariate analysis to see the effects of green tea catechins toothpaste against disease ECC. Ethical considerations remain to be done based on the guidance of the Council for International of Medical Sciences

(CIOMS) Geneva, 1991. It has gained ethical approval recommendation number: 01740 /H4.8.5.31/PP36-KOMETIK/2014.

RESULTS

Research the role of toothpaste green tea catechins against transforming growth Factor -1 beta (TGF β -1) and bone morphogenetic beta 2 (BMP-2) in patients with early childhood caries (ECC) has been completed. The first step is to manufacture toothpaste green tea catechins and inhibition tests with toothpaste green tea catechins against mutated streptococci bacteria and bacterial *Lactobacillus*. The toothpaste process started with the green tea catechins with extraction, in this case we used Beard Head green tea. The extraction process is done with the help of services from the Royal Medica Pharma Lab Makassar Factory. Samples of 2.2 kg of powdered green tea and sieved with a sieve no. 60. The process of solvent extraction is done with water at a ratio of 1:10 (water 22 L). Extraction was carried out for 2 h at 50°C . Extracted results then evaporated with drier freezer for 24 hours at -60°C . The results obtained in the form of a fine powder of dry extract 178 g, acquired rendamen: $178\text{ g} / 2200\text{ g} \times 100\% = 8.1\%$.

Produce toothpaste green tea catechins product with a formula according to the test results and test the inhibition of toothpaste green tea catechins against the mutated *Streptococcus* bacteria and *Lactobacillus acidophilus*. Producing toothpaste green tea catechins is preceded by the manufacture catechin extract and test of minimal inhibitory concentration (MIC) and minimum kill concentration test (MKC) using liquid dilution method. They were compounding formula and manufacturing of green tea catechins toothpaste made in accordance with MIC test results. Toothpaste green tea catechins have measured levels of total flavonoids and total polyphenols, subsequent measurement of the inhibition test toothpaste green tea catechins done by using the good diffusion method (pitting) as the reservoir of the test sample to the mutated *Streptococcus* bacteria a *Lactobacillus acidophilus*. Hasil obtained toothpaste green tea catechins products with the content of flavonoid and polyphenol content 77% and 41%, whereas the diameter of the test results barriers toothpaste green tea catechins against the mutated *Streptococcus* bacteria 17.2 mm and 19.6 mm *Lactobacillus acidophilus* bacteria. These results show that green tea catechins produce this very effectively used daily as well as dental care and antibacterial cariogenic.

Further research conducted clinical trials on 30 samples of children aged six years sufferers childhood early caries, one drop out sample because it cannot continue the study to completion because of vomiting. Collecting saliva was performed before and after use of toothpaste green tea catechins, which is 5 and 10 minutes after a toothbrush with toothpaste green tea catechins, Examination of TGF β -1 and BMP-2 performed by ELISA. The results are shown in the tables and graphs below.

TGF- β 1 concentration in the saliva of children with ECC after using green tea catechins toothpaste was shown in Table 1. The average concentrations of TGF- β 1 current sample before treatment (290.53 ± 57.69), after treatment, mean average concentration of TGF- β 1 increase in the first 5 minutes (328.05 ± 82.28) and the second 5 or 10 minutes after the time of treatment is higher than the initial concentration but lower than the first 5 minutes (313.91 ± 73.96).

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The concentration of BMP-2 in the saliva of children with ECC after using green tea catechins toothpaste seen on Table 2 that average concentration of BMP-2 sample moments before treatment (439.26 ± 266.52) after treatment mean average concentration of BMP-2 increase in the first 5 minutes (447.01 ± 248.68) and at second 5 minutes or 10 minutes after the treatment is higher than the initial concentration but occurs a decrease than the first 5 minutes (429.42 ± 263.68).

Table 3 shows an increase in TGF β -1 levels after using green tea catechin toothpaste in the initial 5 minutes, then decreases in the 10 minutes thereafter. It is shown in Table 5 that the increase in TGF β -1 in the initial 5 minutes using the green catechin paste was significant ($p < 0.05$; = 0.036). Then the concentration decreased at 5 minutes after but was still higher than the initial concentration of $p > 0.05$ (0.086), and 10 minutes after the treatment $p > 0.05$ (0.430).

Table 4 shows an increase in BMP-2 levels after using green tea catechin toothpaste in the initial 5 minutes, then decreases in the next 10 minutes. Based on Table 5, there was a significant increase in BMP-2 in the initial 5 minutes using green tea catechin paste ($p > 0.05$; $p = 0.705$). Then at 5 minutes after, the concentration decreased but still higher than the initial concentration of $p > 0.05$ (0.600) and 10 minutes after the initial treatment $p > 0.05$ (0.247).

Table 5 shows the concentration difference of TGF- β 1 in the saliva of children with ECC based on the time use of toothpaste green tea catechins to describe the average of the statistical test results showed $p > 0.05$ (0.056).

Table 6 shows the concentration difference of TGF- β 1 in the saliva of children with ECC based on the time use of toothpaste green tea catechins to describe the average of the statistical test results showed $p > 0.05$ (0.255).

DISCUSSION

Research of the role of toothpaste green tea catechins against transforming growth factor-beta 1 (TGF β -1) and Bonemorphogenetic beta-2 (BMP-2) in patients with early childhood caries has been done. The study was conducted in children aged 6 years. During the sample collection process, there is one sample drop out due to the amount of saliva in the sample required is not enough for examination in 10 minutes or 5 minutes children can not continue collecting samples.

The first step is to manufacture toothpaste green tea catechins and inhibition test with toothpaste green tea catechins. Making the toothpaste process started with the green tea catechins, in this case we used Beard Head green tea sold in the market then extracted and minimal inhibitory concentration test against mutated streptococci bacteria and Lactobacillus we get is MKC 0.5%. We make toothpaste green tea catechins using a concentration of 2.5% extract of green tea catechins as an active ingredient. It was decided to take the concentration of 2.5% based on research by Wijaya, minimal inhibited power of green tea against mutated Streptococcus was 2.5%, as well as 1% concentration was capable of inhibiting the growth of mutated Streptococcus.

Toothpaste that has been created, then we do an inhibitory test to mutate Streptococcus bacteria and Lactobacillus, to see the effectiveness of green tea catechins have been made in the form of toothpaste against cariogenic bacteria. The purpose of this test is to look at the effectiveness of the active substance of green

tea catechins that have been made in the form of toothpaste against cariogenic bacteria. The average diameter barriers of green tea catechins toothpaste is effective and does not reduce the effectiveness of the active substances of green tea catechins, namely mutated Streptococcus bacteria 17.21 mm and the average diameter barrier by toothpaste green tea catechins against bacteria Lactobacillus acidophilus 19.57 mm.

Dental caries are the most widespread diseases in the world. It is a bacterial disease, oral microbes produce acids such as lactic, acetic and propionic acid, which dissolves inorganic mineral, hydroxyapatite crystals, which starts from the enamel, dentin next.^{4,6,7}

An acidic environment in the oral cavity is causing a demineralized dentin collagen matrix, which is evolved into a carious lesion. However, acid bacteria are not able to hydrolyze fibrous collagen, and there is also no evidence that the enzyme collagenase of bacteria plays a role in the occurrence of caries in degrading dentin organic matrix. Instead in vitro experiments indicated that the caries-causing bacteria could only lead to demineralization in dentin surface only, so that the organic degradation of dentin matrix, most likely caused by a proteolytic enzyme derived from the host's own example MMPs.^{7,8}

The next stage of clinical trials conducted on 30 children with ECC 6 years of age. It looks no significant obstacles when children toothbrush with toothpaste green tea catechins because the bitterness of catechins is not there anymore. Sampling saliva did before treatment with toothbrushing with green tea catechins toothpaste, 5 minutes after treatment, and 10 minutes after treatment. The timing is based on the properties of enzymes that will be investigated is TGF β -1 can already be detected with 18 minutes after treatment.⁸

ECC is the most common disease in children, during the period of deciduous dentition, children make the transition from breastfeeding/drink bottled milk to eat solid foods, starting from biscuits and cakes containing carbohydrates which are children's favorites. Foods with carbohydrate content accompanied with child independence in cleaning the teeth, plus the knowledge and awareness of parents who lack dental health of it, becomes important to many cases of ECC, and therefore required a material that is safe and effective for the treatment of this disease.⁹

TGF β -1 in the dentin can interact with a number of components that function as a modulator activity of the differentiation of odontoblasts in cytological and functional. Singly TGF β 1 stimulates the secretion of collagen type I and fibronectin without any cellular polarization. This observation is in line with previous studies with TGF β described as a regulator synthesis of extracellular matrix molecules such as collagen and fibronectin.¹⁰

Effect of TGF β 1 that combined with heparin (either alone had no effect) has a qualitative difference: polarized cells secrete large amounts of extracellular matrix containing collagen type I in peripheral explants. A number of reports have shown the possibility of a synergistic interaction between the extracellular matrix and growth factor (Schubert 1992). Heparin has been reported to increase the potential activity of TGF β .¹⁰

The results showed concentrations of TGF β -1 increased significantly in the first 5 minutes after treatment, whereas BMP-2 increase was not significant. At 10 minutes after treatment increased, but not significantly

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both at the TGF β -1 and BMP-2, this shows the green tea catechins bioactive substances play a role in the increase in enzyme TGF β -1 and BMP-2. Although not significant at the 10 minutes of this result is probably influenced by many factors, among others, some of the samples was found after sampling the first 5 minutes, quietly drinking water because thirsty so the possibility of residual water included in saliva making 10 after treatment affects the concentration of TGF β -1 and BMP-2 in the saliva of children. But does not affect the conclusions, because in the first 5 minutes results significantly increased in TGF β -1.^{4,10}

Transforming Growth Factor beta (TGF- β 1) and morphogenetic beta-2 (BMP-2) is a growth factor that strictly regulates a series of molecular and cellular events in the formation of tertiary dentin. Tertiary dentin is dentin formed in pathological processes which are classified into reactionary and reparative dentin. The role of TGF- β 1 and BMP-2 in the reactionary dentin formation is affecting the formation of extracellular matrix components including collagen in the dentin.^{11,12} Dissolution of dental tissues such as the carious lesions causes secretion of TGF- β 1 by odontoblasts which cells synthesize activities started in the dentin matrix. TGF- β 1 diffuses into the dentinal tubules towards the horn pulp region and serves to induce dentin matrix. TGF- β 1 also stimulates the formation of dentin matrix of other components that dentin sialoprotein (DSP), proteoglycan decorin and biglycan. TGF-1 was thought to stimulate the differentiation of odontoblasts in the dentin maturation process to increase the rate of mineral apposition. TGF- β 1 stimulates odontoblasts in vitro for deposition pre-dentin and stimulates differentiation of pulp cells. The formation of reparative dentin is much more complex than the reactionary dentin formation due to the involvement of progenitor cells that migrate and differentiate to form odontoblast-like cells that synthesize dentin repairing. TGF- β 1 together with Bone Morphogenetic Proteins (BMPs), Fibroblast Growth Factor (FGFs), and Insulin Growth Factor (IGF) form the TGF- β superfamily.¹¹⁻¹⁴

Conditions of the active substance in the form of green tea catechins toothpaste do not affect the effectiveness of these materials. Evident from mutated streptococcus bacteria test results and Lactobacillus edges in an effective barrier. Similarly, clinical trials were performed with enzyme tests TGF- β 1 dan BMP-2 in the saliva of children with ECC showed significant improvement results, green tea toothpaste effectively halt progressive caries and has the potential to improve and play a role in activating the formation of secondary dentin layer.^{15,16}

CONCLUSION

This study proves the role of green tea catechins to increased TGF- β 1 dan enzyme BMP-2 that was instrumental in the improvement of the human tooth dentin matrix.

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Table 1. ECC Child's Saliva Tgf β -1 Concentration After Toothbrushing with Green Tea Catechins Toothpaste

Time	n	Minimum	Maximum	Mean \pm SD
Early	29	227.4	499.9	290.53 \pm 57.69
5 minutes	29	204.1	526	328.05 \pm 82.28
10 minutes	29	168.3	512.2	313.91 \pm 73.96

Table 2. ECC Child's Saliva BMP-2 Concentration After Toothbrushing with Green Tea Catechins Toothpaste

Time	n	Minimum	Maximum	Mean \pm SD
Early	29	266.3	1439.2	439.26 \pm 266.52
5 minutes	29	270.5	1281.9	447.01 \pm 248.68
10 minutes	29	283.1	1545.5	429.42 \pm 263.68

Table 3. Saliva TGF- β 1 Concentration Different at ECC Child After Toothbrushing with Green Tea Catechins Toothpaste

		n	Mean \pm SD	p-value
TGF- β concentrations (pg/ml)	Early	29	290.53 \pm 57.69	0.036*
	5 minutes	29	328.05 \pm 82.28	
	Early	29	290.53 \pm 57.69	0.430
	10 minutes	29	313.91 \pm 73.96	
	5 minutes	29	328.05 \pm 82.28	
	10 minutes	29	313.91 \pm 73.96	0.086

Wilcoxon test

Table 4. Saliva BMP-2 Concentration Different at ECC Child After A Toothbrush with Green Tea Catechins Toothpaste

		n	Mean \pm SD	p-value
TGF- β concentrations (pg/ml)	Early	29	439.26 \pm 266.52	0.705
	5 minutes	29	447.01 \pm 248.68	
	Early	29	439.26 \pm 266.52	0.600
	10 minutes	29	429.42 \pm 263.68	
	5 minutes	29	447.01 \pm 248.68	
	10 minutes	29	429.42 \pm 263.68	0.247

Table 5. Saliva TGF- β 1 Concentration Different Based on Time At ECC Child After Toothbrushing with Green Tea Catechins Toothpaste

Time of Measure	Mean \pm SD	p-value
Early	290.53 \pm 57.69	0.056
5 minutes	328.05 \pm 82.28	
10 minutes	313.91 \pm 73.96	

Friedman test

Table 6. Saliva BMP-2 Concentration Different Based on Time At ECC Child After Toothbrushing with Green Tea Catechins Toothpaste

Time of Measure	Mean \pm SD	p-value
Early	439.26 \pm 266.52	0.056
5 minutes	477.01 \pm 248.68.28	
10 minutes	429.42 \pm 263.68	

Friedman test

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