The effect of subgingival irrigation of *Gambir (Uncaria gambir* [Hunter] Roxb*)* catechin to the number of *Actinobacillus actinomycetemcomitans* in the gingival sulcus of periodontitis mice

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ABSTRACT

Periodontitis is one of periodontal disease that the main cause is bacteria, especially *Actinobacillus actinomycetemcomitans* (AA). One of the treatments on these conditions can be done with subgingival irrigation using *Gambir* catechin *(Uncaria gambir* [Hunter] Roxb*)*. Catechins are polyphenolic compounds and have antibacterial effects. Based on its advantages, cathecin effect may decrease the amount of AA in periodontitis and help the healing. The aim of this study was to know the effect of subgingival irrigation of the *Gambir* catechin to the amount of AA in periodontitis mice. Twelve mice, 3 months age, 200-250 gr in weight, with the anterior gingival sling with yarn was made for periodontitis condition. The mice was divided into four groups treatment: Treatment with aquadest, *Gambir* catechin I, *Gambir* catechin II, and chlorhexidin 0.2%. Subgingival irrigation performed after visible signs of periodontitis with 2 times daily for seven days. AA retrieval was done using paper points inserted in the gingival sulcus and grown in BHI agar with bacitrin 5 units/ml (modification). The number of AA was then counting with colony counters. Study result showed a significant difference ($p < 0.05$) between aquadest treatment group with *Gambir* catechin I, *Gambir* catechin II, and chlorhexidin 0.2%. But there was no significant difference between *Gambir* catechin I and II. The conclusion of this study indicated that subgingival irrigation of *Gambir* catechin I and II could decrease the amount of AA but there was no statistical difference between the two groups. This mean that *Gambir* catechin in solutions and effervescent didn't affect the activity of a substance in it. It was shown that catechins can be used as an alternative to the condition of periodontitis.

Key words: *Gambir* catechin, subgingiva irrigation, *Actinobacillus actinomycetemcomitans*

ABSTRAK

Periodontitis merupakan salah satu penyakit periodontal yang penyebab utamanya adalah bakteri, terutama Actinobacillus actinomycetemcomitans (AA). Salah satu perawatan pada kondisi ini dapat dilakukan dengan irigasi subgingiva menggunakan katekin Gambir (Uncaria gambir [Hunter] Roxb*). Katekin adalah senyawa polifenol dan memiliki efek antibakteri. Berdasarkan keuntungan, efek katekin dapat mengurangi jumlah AA pada periodontitis dan membantu penyembuhan. Tujuan penelitian ini adalah untuk mengetahui pengaruh irigasi subgingiva katekin Gambir terhadap jumlah AA pada jaringan*

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periodontal tikus yang mengalami periodontitis. Dua belas tikus, usia 3 bulan dengan 200-250 gr berat badan, diberi sling di anterior gingival dengan benang untuk membuat kondisi periodontitis. Tikus dibagi menjadi empat kelompok perawatan: Kelompok perawatan dengan aquades, katekin Gambir I, katekin Gambir II, dan chlorhexidin 0,2%. Irigasi subgingival dilakukan setelah terlihat tanda-tanda periodontitis, 2 kali sehari selama tujuh hari. Pengambilan AA dilakukan dengan menggunakan paper point *yang dimasukkan ke dalam sulkus gingiva dan ditanam di agar-agar BHI dengan bacitrin 5 unit/ml (modifikasi). Kemudian dilakukan penghitungan jumlah AA dengan* colony counter. *Hasil penelitian menunjukkan perbedaan yang signifikan (p <0,05) antara kelompok perawatan dengan aquades dengan katekin Gambir I, katekin Gambir II, dan chlorhexidin 0,2%. Tapi tidak ada perbedaan yang signifikan antara katekin Gambir I dan II. Simpulan dari penelitian ini menunjukkan bahwa irigasi subgingiva dari katekin Gambir I dan II dapat mengurangi jumlah AA tetapi tidak terlihat adanya perbedaan statistik antara kedua kelompok. Ini berarti bahwa katekin Gambir dalam bentuk larutan dan* effervescent *tidak mempengaruhi aktivitas zat di dalamnya. Hal ini menunjukkan bahwa katekin dapat digunakan sebagai alternatif untuk kondisi pasien periodontitis.*

Kata kunci: *katekin gambir, subgingiva irigasi, Actinobacillus actinomycetemcomitans*

INTRODUCTION

Periodontal disease is a pathological process in periodontal tissues. It mainly caused by bacterial infection, even though other factors may also influence periodontal tissues.[1] Predominant bacteria causing the damage in periodontal tissues, especially periodontitis, are *Porphyromonas gingivalis, Actinobacillus actinomycetemcomitans, Tannerella forsythia, Prevotela intermedia* and *Fusobacterium nucleatum.*[2]

The initial process of periodontitis is caused by colonization of pathogenic species in periodontal tissues. The entry of bacteria or its product into the tissues causes a destruction resulted from direct interaction with hospes cells[2]. Peridontitis is classified into several types. One of them is localisata aggressive periodontitis. In that condition, there is a strong link between certain anaerobic negative Gram bacteria with the occurrence of the disease. The other type is *A. actinomycetemcomitans* strain Y4. This microorganism produces a substance that plays an important role in the extensive tissues' damage in localized aggressive periodontitis. The substance is a leukotoxin which is, in nature, toxical to leukocyte, collagenase, endotoxin and fibroblast inhibiting factor. Besides the localized form, the overall form of the whole teeth is also found.[1]

Actinobacillus actinomycetemcomitans is an anaerobic facultative bacteria, negative Gram has the biggest role in the occurrence of periodontitis.[3] Level of virulence of *A. actinomycetemcomitans* is characterized by the modulation inflammation, tissues' damage induction and the inhibition of tissues' repair.[4] *Actinobacillus actinomycetemcomitans* produces the virulence factor by producing leukotoxin in the form of secreted lipoprotein, especially for killing PMN and macrophage by producing citolethal distending toxin *(CDT).*[5]

The treatment performed covering the effective plaque control, a thorough cleaning of root, and flap surgery to open up a wider field of view to the root surface and to eliminate granulation tissues. In addition, the use of antimicrobial chemical agent is also required in order to speed up the improvement the condition of periodontal tissues.[6]

Based on the research conducted by Putri[8], it was found that the formulation of *Gambir* catechin was harmless if swallowed. *Gambir* catechin has been traditionally used as a gargle rinse and has also been proven as an antibacterial. Therefore, Putri's *Gambir* catechin formulation was used as subgingival irrigation solution which was expected to reduce *A. actinomicetemcomitans* bacteria in periodontal disease.

This research aimed to determine the effect of subgingival irrigation of *Gambir* catechin preparation on the number of *A. actinomycetemcomitans* in periodontitis mice' gingival.

The benefit expected from this research was; subgingival irrigation with *Gambir* catechin preparation was expected to be able to inhibit *A.*

actinomycetemcomitans bacteria. *Gambir* catechin was expected to be an alternative of subgingival irrigation preparation derived from plants that have no side effects and can increase the added value of *Gambir* plants commodities.

METHODS

The subjects of the research were 16 male Wistar rats, aged 3 months and weighing between 200-250 grams. The main ingredients in this research were: Catechin (Andalas Farma Test Unit), citric acid, tartaric acid, Na benzoate, Mannitol, Polyvinyl pyrrolidone (PVP), Isopropyl alcohol, Tween solution 80, Menthol, and aquadest. The research preparation was making *Gambir* catechin preparation in the forms of effervescent (preparation I) and solution (preparation II).[8]

To created a periodontitis condition, all mice were injected with cetamine 60 mg/kg BW.[9] Next, binding using thread around the cervical of lower incisive teeth was performed for 7 days. To prevent thread loss, binding by sewing thread on the gingival was conducted. After 7 days, the thread was removed and evaluated to find out if there were any periodontitis signs, including change in color (dark red), teeth in the cervical region compared to normal (pink), enlargement or swelling of gingival and gingival sulcus got deeper. Bacteria identification was performed by conducting sample culture of pure *Actinobacillus actinomycetemcomitans*. The media used was modification of Brain Heart Infusion gel with bacitrin 5 unit/ml, and then biochemical test was performed for identifying *A. actinomycetemcomitans*. The biochemical tests performed were catalase, oxidase, phosphatase, gelatinase tests, H_2S production, indole production, D-mannose, sorbitol, sucrose, D-xylose, glucose fermentation, gas production, maltose and the growth in *Brain Heart Infusion gel* media with bacitrin 5 unit/ml (modification).[10]

White mice were in advance adapted to the laboratory environment for 1 week. They were given standard feeding and *ad libitum* for their drink. The mice were divided randomly into 4 big groups: Group I: the periodontitis mice were given aquadest therapy in gingival sulcus; Group II: the periodontitis mice were given preparation I of *Gambir* catechin in gingival sulcus; Group III: the periodontitis mice were given preparation II of *Gambir* catechin in gingival sulcus; Group IV: the periodontitis mice were given Chlorhexidine digluconate therapy in gingival sulcus.

The mice were treated at the 2nd week when they showed clinical periodontitis signs. Subgingival irrigation of *Gambir* catechin was performed by spraying it to the gingival sulcus of periodontitis mice with an injection syringe.

After the treatment, 4 mice from each group were taken. In the first place, the mice were anesthetized by cetamine, and then sterile paperpoint was inserted into the gingival sulcus of lower incisive teeth for 10 seconds. Next, the paperpoint was taken out and put into test tube containing 1 ml NaCl. The samples were suspended into a vortex for 10 seconds. Every 0.1 ml of the solution was diluted to 10^{-2}. One ml of dilution result was planted in *Brain Heart Infusion gel* media with bacitrin 5 unit/ml (modification). The media and bacteria were put into the anaerobic chamber and incubated in the air containing CO_2 5% and temperature 37°C for 2x24 hours. *A. actinomycetemcomitans* shaped were round, small, convex, white colored, and transparent sticks on Brain Heart Infusion *gel* media with bacitrin 5 unit/ml (modification).[11] Biochemical test on other bacteria similar to *A. actinomycetemcomitans* is conducted. Furthermore, calculation of the number of *A. actinomycetemcomitans* using colony counter is performed.

The result was statistically tested using the one way Anova test and proceed with the Least Significant Different ($LSD_{0.05}$).

RESULTS

The research result showed that all mice groups treated with periodontitis manipulation and were evaluated after 7 days indicated the signs of periodontitis, including the change of color which becomes redder on the gingival edge and papilla interdental, the enlargement and swelling of gingival with gingival recession and gingival sulcus gots deeper. The mean of the calculation result on the number of *A. actinomycetemcomitans* can be seen in Table 1. One way Anova test result can be seen in Table 2.

Table 1. The number of *A. actinomycetemcomitans* (CFU/ml) based on the treatment group after and before subgingival irrigation

Treatment Group	Mean of *A. actinomycetemcomitans*		Decreasing
	Before treatment	After treatment	
I	23.500 ± 4.796	21.000 ± 2.708	2.500 ± 4.123
II	25.500 ± 3.416	8.250 ± 3.304	14.750 ± 5.619
III	25.500 ± 1.915	11.250 ± 2.500	14.250 ± 3.594
IV	25.000 ± 2.582	7.250 ± 2.629	17.750 ± 3.594

Table 2. Summary of one way Anova test result, number decrease of *A. actinomycetemcomitans*

Sources of variation	Jk	Dk	MS	F	P
Inter group	542.188	3	180.729	9.714	0.002*
In the group	223.250	12	18.604		
Total	765.438	15			

Note: ss = sum square, df = degrees of freedom, MS = means square, p = probability, *= significant

Table 3. Summary of LSD result, number decrease of *A. actinomycetemcomitans*

Treatment group	I	II	III	IV
I		-12,250*	-11,750*	-15,250*
II			0,500	-3,000
III				-3,500
IV				

DISCUSSION

The research result showed that the average number decrease of *A. actinomycetemcomitans* in Group I was less than Group II, Group III, and Group IV. In Group II (*Gambir* catechin I) and Group III (*Gambir* catechin II) the difference of number decrease average of *A. actinomycetemcomitans* is not really different. This means the *Gambir* catechin contained in the preparation that has become solution and effervescent does not affect the activity of the substance in it.

Based on the average number decrease table of *A. actinomycetemcomitans*, it was seen that Group II and Group III got less decrease compared to Group IV; that was chlorhexidine preparation. The decrease occurred because Chlorhexidine was the preparation belongs to antiseptic group and possesses bactericide nature. Chlorhexidine is an antibacterial with broad spectrum and is very effective against positive Gram and negative Gram.[12,13]

The statistical test of number decrease of *A. actinomycetemcomitans* with *Gambir* catechin preparation does not show significant result. This happens because *A. actinomycetemcomitans* is anaerobic negative Gram that grows in subgingival area. Irrigation by spraying it on the subgingival area will easily dissolved due to the flow of gingival crevicular fluid and saliva. The use of antimicrobial agent in the form of solution was less effective because the antimicrobial agent has a difficulty in reaching subgingival area and the preparation will be immediately cleared by gingival crevicular fluid or the saliva from mucosal surface. Therefore, the decrease was not much, but it was estimated from how the *Gambir* catechin works in inhibiting bacteria by actively precipitating protein and damaging cell membranes as well as decreasing the surface voltage.[8,14] The previous research on the isolation and activity test of pure *Gambir* antibacterial against oral bacteria was conducted by Adra.[14] The result showed the inhibition on the growth of *Streptococcus mutans*, *Streptococcus sanguis*, *Staphylococcus saprophyticus*, and *Staphylococcus epidermis*. Other study on catechin as an antibacterial was conducted by Kozai et al. Liquid extract of *Gambir* is capable of inhibiting the growth of *Streptococcus mutans* in vitro. *Gambir* extract inhibits the synthesis of insoluble glucan (ISG) by *glucosyl transferase* (Gtase) up to 48.9% in 10 mg/ml concentration and up to 32.2% in 1.25 mg/ml concentration.[14]

The existence of the decreasing number of *A. actinomycetemcomitans* in periodontitis mice is also followed by the decrease of inflammation