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RESEARCH ARTICLE

Biofilm Effectivity of Ethanol Extracts (Casuarina equisetifolia) Leaves of the Bacteria Enterococcus faecalis

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ABSTRACT

Background: *Enterococcus faecalis is one of the bacteria that can form biofilms that can cause persistent endodontic infections. Biofilm is the unity of microbial cell surface surrounded by a matrix of extracellular polymeric substances (EPS). The ethanol extracts of Casuarina equisetifolia leaves are known to have potentially bioactive compounds as antibacterials such as flavonoids, saponins, tannins, phenols, and alkaloids.* ***Objective:*** *To determine the power antibiofilm ethanol extracts of Casuarina equisetifolia leaves at multiple concentrations of the bacteria Enterococcus faecalis.* ***Methods:*** *This study is an experimental research design post-test only control group design. The sample divided into 5 groups consisted of positive control Enterococcus faecalis in media Trypticase Soy Broth (TSB)+DMSO 1%, and 4 treatment groups of ethanol extracts of Casuarina equisetifolia leaves a concentration of 0,5%, 1%, 1,5%, and 2%. Biofilm is made using Enterococcus faecalis ATCC 29212 bacteria were cultured on TSB media were incubated for 1x24 hours. 0,1 ml of the bacteria Enterococcus faecalis with a concentration of* 10^6 *put on a plate micro titter then done using crystal violet staining. Biofilm-checked by measuring optical density (OD) values using the ELISA reader. Data analysis used Kruskal-Wallis followed by Mann-Whitney test.* ***Results:*** *The ethanol extracts Casuarina equisetifolia leaves on the growth of Enterococcus faecalis bacteria indicate the presence of power antibiofilm. The results mean % mortality of bacteria ($p<0.05$) were significant in all groups that have the power antibiofilm effectiveness as against the growth of bacteria Enterococcus faecalis.* ***Conclusion:*** *The ethanol extracts Casuarina equisetifolia leave have antibiofilm power against bacterial growth Enterococcus faecalis.*

Keywords: *Enterococcus faecalis, Enterococcus faecalis antibiofilm, Casuarina equisetifolia leaves*

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INTRODUCTION

Dental caries is the process of crushing or softening enamel or dentin. The crushing process continues until the underlying tissue, and this is the beginning of the formation of holes in the teeth. Caries is a progressive, localized deterioration of the tooth structure, and most commonly causes pulp disease. It is currently generally accepted that for the development of caries, specific bacteria are required on the tooth surface.[1] Bacteria play a major trigger for inflammatory responses in the dental pulp.[2] Several studies have shown that obligate anaerobes are the predominant species in infected root canals. The failure of a root canal treatment can be caused by the presence of microorganisms left in the root canal. The bacterium that often causes infections resulting in root canal treatment failure is Enterococcus faecalis.[3]

Enterococcus faecalis is a gram-positive and fermentative non-spore-positive anaerobic facultative bacterium. The prevalence of infections caused by Enterococcus faecalis ranges from 24%-77%. This is due to various virulence resistance factors of Enterococcus faecalis, including its ability to compete with other microorganisms in their invasion of dentinal tubules and its ability to withstand low nutritional conditions.[1,4] Resistance of Enterococcus faecalis is due to its ability to form a biofilm layer that enables these bacteria to be 1000 times more resistant to phagocytosis, antibodies, and antimicrobials than organisms that do not produce biofilms.

A biofilm is a unit of the microbial cell surface that is covered by an extracellular polymeric substance matrix. A biofilm is a community of bacteria that is organized, communicating with each other, and attached to an inert or living surface.[5,6] The formation of a biofilm starts with several bacteria (planktonic cells) attached to a surface, then reproduces themselves to form a thin layer (monolayer) of the biofilm. Biofilms show very high resistance to antimicrobials, because of the adhesion of the EPS matrix and protection from barriers that inhibit the infiltration of the antimicrobial agent.

Root canal drugs are used to eliminate bacteria that cannot be eliminated by chemo-mechanical processes, such as the Enterococcus faecalis bacteria.[1] deficiency such as the Chlorophenol Kamfer Menthol (ChKM) class of drugs which are toxic, can cause irritation and soft tissue necrosis, pungent odor, and can cause allergic reactions.

Currently, many plants uses as an alternative are being developed, because root canal medicines have several shortcomings. One of the plants that can be used is Cemara Shrimp (Casuarina equisetifolia) which is a plant that is easy to grow and easy to find on the coast of Indonesia. Casuarina equisetifolia has no economic value and benefits, so far it is only used as an aesthetic plant, as a bonsai, and can be found along the coast and used as greening on critical land.[1]

Casuarina equisetifolia is a plant used in traditional medicine for the treatment of diarrhea, coughs, wounds, toothaches, and can be used as a lotion for swelling and diabetes.[7,8] Studies on the antibacterial activity of Casuarina equisetifolia conducted by Nehad [7], shows that Casuarina equisetifolia extract has antimicrobial effectiveness, at a concentration of 2.5% using ethanol and methanol extract can inhibit bacteria, a concentration of 10% shows the result in ethanol extract is 30.33 ± 0.33, and the result of methanol extract shows a result of 36.67 ± 0.33. The methanol extract of the leaves of Cemara Udang (Casuarina equisetifolia) has better results when compared to the ethanol extract, but it is widely reported that methanol has toxic properties.[9] So the researchers chose to use an ethanol solution to make the extract of the leaves of Cemara Udang (Casuarina equisetifolia).

Besides functioning as an antibacterial, Casuarina equisetifolia can be used as an antifungal, one of which is Candida albicans at a concentration of 10% which can inhibit Candida albicans. Research conducted by Nehad using leaf extract of Shrimp Cemara (Casuarina

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equisetifolia) on pathogenic microorganisms with concentrations of 2.5%, 5%, 7.5%, 10%, 15%, and 20% against Staphylococcus aureus bacteria.[7] At very low concentrations 2.5% can function as antibacterial, but there are no research data on the power of antibiofilm (Casuarina equisetifolia) against Enterococcus faecalis bacteria. This shows that the higher the extract concentration used, the higher the level of the extract's toxicity. [7]

Based on this background, this study determined the antibiofilm potency of the leaf extract of Cemara Udang (Casuarina equisetifolia) as an alternative drug for root canal sterilization to inhibit the growth of Enterococcus faecalis bacteria which is often found in failed root canal treatments.

MATERIALS AND METHODS

This study used experimental laboratory research. Research on Enterococcus faecalis bacteria was carried out in vitro using the microtiter plate assay method.

The sample used in this study was the bacterium Enterococcus faecalis ATCC 29212 obtained from the Surabaya Health Laboratory Center. Then, the Enterococcus faecalis stock was cultured on TSB media. Trypticase Soy Broth (TSB) media is used as a medium for biofilm formation by Enterococcus faecalis bacteria, because this medium is the most effective medium for biofilm formation by bacteria.

The number of samples used in this study was 45 samples, then divided into 5 groups, namely group 1 positive control using Enterococcus faecalis bacteria on Trypticase Soy Broth (TSB) + 1% DMSO media, group 2,3,4, and 5 treatment groups given an extract of Shrimp Cemara (Casuarina equisetifolia) leaves from Madura with a concentration of 0.5%, 1%, 1.5%, and 2%.

The method used to test biofilms is the microtiter plate biofilm assay method. The initial test suspension was made equal to the turbidity of 0.5 Mc Farland and diluted to reach a bacterial concentration of 106. The suspension of Enterococcus faecalis bacteria was cultured into the microplate and then incubated at 37°C for 6x24 hours. After 6 days, 0.1 ml of suspension from the ethanol extract of the leaves of Cemara Udang (Casuarina equisetifolia) was added at a concentration of 0.5%, 1%, 1.5%, and 2% into each microplate well. Then on the 7th day, the optical density value was measured using an ELISA reader.[10]

RESULTS

Data obtained from the measurement of optical density values, then calculated using the formula for % bacterial mortality:

% Bacterial mortality = $1-(\frac{OD7-KB-OD0}{PC7-PC0}) \times 100\%$

Explanation :

OD7: *Optical density (596nm) microtiter samples on 7th day of incubation*

OD0: Optical density (596nm) microtiter samples at 0 incubation day

KB: Optical density (596nm) control of materials on the 7th day of incubation

PC7: Optical density (596nm) positive control on 7th day of incubation

PC0: Optical density (596nm) positive control at 0 incubation days.

Tabel 1. Results% of mortality by the biofilm Enterococcus faecalis

Group	Mean ± Std. Deviation
K1	21,03±14,58
K2	94,38± 5,33
K3	92,94± 6,79
K4	73,83±11,55
K5	90,48± 8,75

Information: K1: K + (Enterococcus faecalis bacteria on TSB + DMSO 1% media), K2: (0.5% extract), K3: (1% extract), K4: (1.5% extract), K5: (Extract 2%) in%.

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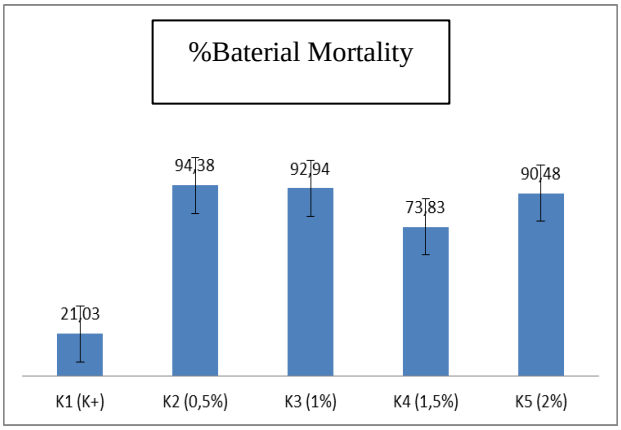
Tabel 2. Antibiofilm power of ethanol extract of shrimp pine leaves (Casuarina equisetifolia) graph of mean% mortality of Enterococcus faecalis bacteria. Information: K1 (Enterococcus faecalis bacteria on TSB + DMSO 1% media, K2: 0.5% extract, K3: 1% extract, K4: 1.5% extract, and K5: 2% extract.



Based on the table and graph of the average antibiotic power of Enterococcus faecalis, it shows that at a concentration of 0.5% it has the greatest effectiveness against Enterococcus faecalis biofilm compared to ethanol extract of shrimp pine leaves in various concentrations. This is evidenced by the results of% bacterial mortality, concentrations of 0.5%, 1%, 1.5%, and 2% that have antibiofilm power against Enterococcus faecalis bacteria. Positive control for DMSO 1% had the lowest percentage compared to the ethanol extract concentration of Cemara Udang leaves.

Tabel 3. Results of normality test with Shapiro-Wilk

Group	Mean ± Std. Deviation
K1	0,245
K2	0,007
K3	0,004
K4	0,208
K5	0,047

Based on the results of the normality test it can be seen that the antibiofilm power of the ethanol extract of the leaves of Cemara Udang (Casuarina equisetifolia) in the K1, K2, K3, K4, K5 groups have an abnormal distribution because it has a value (p <0.05). Then the non-parametric test was carried out, the hypothesis test used was the Kruskal-Wallis test, then the Mann-Whitney test was performed.

Tabel 4. Kruskal-Wallis Test Results

Group	Sig
K1	0,000

In Table 4, the significance value is obtained of 0.000 (p <0.05). This shows that there are differences between treatment groups.

Subsequently, a Post Hoc analysis was carried out to see a significant difference in the antibiofilm power of the ethanol extract of Cemara Shrimp (Casuarina equisetifolia) leaves against the growth of Enterococcus faecalis bacteria from each group. To perform Post Hoc analysis of the Kruskal-Wallis test is to use the Mann-Whitney test.

Tabel 5. Mann Whitney result

Kelompok	K2	K3	K4	K5
K1	.000*	.000*	.000*	.000*
K2		.340	.000*	.387
K3			.001*	.436*
K4				.003*

Remarks *: there is a significant difference.

The Mann-Whitney test results show that there is a difference between the K1 group as a positive control and the K2, K3, K4, and K5 groups as the treatment group. In the K2 group when compared with the K3 group, there was no significant difference. Likewise between groups K2 with K5, and K3 with K5 because they have a significant value> 0.05.

DISCUSSION

Biofilm is a unity of the microbial cell surface covered by a matrix of extracellular polymeric substances.[5]

The selection of Enterococcus faecalis ATCC 29212 is based on the research of Subbiya[8], where the Enterococcus faecalis ATCC 29212 bacteria have the same virulence

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as the Enterococcus faecalis bacteria isolated from root canals.

In this study, a concentration of 0.5% was used because based on the results of preliminary research, the concentration of 0.5% had the power of antibiofilm against the growth of the Enterococcus faecalis bacteria.

Enterococcus faecalis bacteria on TSB + DMSO 1% media was used as a positive control in this study because, DMSO 1% was used as a diluent for ethanol extract of leaves of Cemara Shrimp (Casuarina equisetifolia), the concentration of DMSO 1% did not have antibacterial properties that would affect the antibiofilm power of ethanol extract Leaf Cemara Shrimp (Casuarina equisetifolia) on the growth of Enterococcus faecalis.[11] This is proven based on the results of research that the ethanol extract of leaves of Cemara Shrimp (Casuarina equisetifolia) with a concentration of 0.5% has the greatest average value, namely 94.38% when compared to other concentrations. . DMSO 1% has a mean value of 21.03% which is the smallest mean value compared to other concentrations, so the addition of 1% DMSO as a diluent does not affect the effectiveness of the ethanol extract of Cemara Udang (Casuarina equisetifolia) leaves.

The ability of antibiofilm against the formation of Enterococcus faecalis bacteria is influenced by a concentration. The greater the concentration, the higher the inhibition of biofilm formation,[12] but in this study, it was shown that the higher the concentration of the leaf extract of Cemara Shrimp (Casuarina equisetifolia), the smaller the antibiofilm's power, on the contrary, the smaller the concentration, the greater the antibiofilm power produced. This research shows that 2% concentration has a mean value of 90.48%, a concentration of 1.5% has a mean value of 73.83%, a concentration of 1% has a mean value of 92.94%, and a concentration of 0.5% has a mean of 94.38%. . Concentration of 1.5% has a mean value of 73.83%, this shows a decrease in the power of antibiofilm when compared with concentrations of 0.5%, 1%, and 2%, this decrease is due to the concentration of 1.5% has a higher OD7 value. large when compared with a concentration of 2% so that the resulting mean value is small.

The ethanol extract of the leaves of Cemara Udang (Casuarina equisetifolia) contains flavonoids, saponins, tannins, phenols, and alkaloids.[7] Tannin compounds can inhibit the formation of biofilms by interfering with enzymes, inhibiting enzyme production, and reducing the concentration of calcium ions needed in the coagulation process of bacterial plasma. Bacterial plasma coagulation is needed by bacteria to form fibrin-rich biofilms so that biofilms do not form.[13]

Flavonoids are a phenol group and can kill bacteria by forming complex compounds with proteins through hydrogen bonds. At high levels of phenol, it causes protein coagulation and cell membranes to undergo lysis.[14] Saponins are characterized by their ability as a surfactant that can reduce surface tension to disrupt the integrity of bacterial cell membranes.[15] Alkaloids have alkaline groups that contain nitrogen. The presence of this base group when in contact with bacteria will react with amino acid compounds that make up the bacterial cell wall and also bacterial DNA. This reaction results in a change in the arrangement of the amino acid chains in DNA and will cause a change in the genetic balance of the DNA acid so that the bacterial DNA will be damaged. With the damage to the DNA, the bacterial cell nucleus will be damaged. This DNA damage in the bacterial cell nucleus will also encourage lysis of the bacterial cell nucleus. [16]

This research needs to be developed to find an effective concentration that can be applied as an alternative to root canal sterilization materials. In this study, the concentration of the extract used should not be more than 20% because the characteristic of the leaf extract of Cemara Udang (Casuarina equisetifolia) is sticky and dark brown, this can cause interference with the ELISA reader reading. The higher the concentration used can cause toxicity to the cells in the periapical area and around the non-vital teeth, causing further

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complications.[17] The next research can use another method to determine the antibiofilm power of the ethanol extract of Cemara Udang leaves (Casuarina equisetifolia) on the growth of Enterococcus faecalis bacteria. This research is preliminary, so it is necessary to develop research on the use of ethanol extract of Cemara Udang (Casuarina equisetifolia) leaves as an alternative material for root canal sterilization. It is feared that the color density of the extract can cause discoloration of the teeth so that further research is needed on the tooth discoloration test after applying the ethanol extract of the leaves of Cemara Udang (Casuarina equisetifolia).

CONCLUSION

Based on the results of this study, it is known that the ethanol extract of the leaves of Cemara Udang (Casuarina equisetifolia) has antibiofilm power against the growth of Enterococcus faecalis bacteria. The ethanol extract of Shrimp Cemara (Casuarina equisetifolia) leaves at concentrations of 0.5%, 1%, 1.5%, and 2% can inhibit the growth of the bacteria Enterococcus faecalis. The concentration of 0.5% is the most effective as an antibiofilm and inhibits the growth of the bacteria Enterococcus faecalis.

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RESEARCH ARTICLE

Choices and Reasons for RSGM Baiturrahmah Co-ass Student Batch 2018 in Continuing Specialization

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ABSTRACT

Background: *The dentistry profession continuously presents information aimed at improving its discipline consistently and measurably to improve the oral health of patients. Specializes in any field that is known to be associated with higher productivity. The same is true for the health sector in general and specifically for dentistry where general practice is being matched by the search for demand for specialists.* ***Purpose:*** *This study aims to look at students' choices and reasons for pursuing a dental specialist at RSGM Baiturrahmah.* ***Methods:*** *This is a descriptive study consisting of 71 college students at RSGM Baiturrahmah. Subjects and methods: consisting of students filling out a questionnaire containing 4 questions, the research time was in October 2020 using a questionnaire. Statistical Analysis: The data analysis in this study used the SPSS program with descriptive analysis.* ***Results:*** *showed that the specialist that students were interested in was periodontics as much as 22.22% and the reasons for students to continue to specialize at most were having skills/talents in specialists as much as 38.03%.*

Keywords: *Postgraduate training, Dental specialization, Intending dental residents.*

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INTRODUCTION

The dentistry profession continuously carries out self-assessments aimed at improving its discipline consistently and measurably to improve the oral health of patients, increasing efficiency in the health care system, and ultimately improving society as a whole.[1] Specialization in any field is known to be linked to productivity.[2] The same applies to the health sector in general and specifically for dentistry where general practice is being competed with by increasing demand for specialization. Dental specialization plays an important role in improving dental health. education and practice, stimulate research, and define quality expectations in various areas of expertise.[2]

Various specializations exist in the field of dentistry intending to improve oral health care and several studies have shown that there are a variety of factors that contribute to choosing a particular area of dental specialization.[2,5-7] These factors may be due to advice from friends or family, [8, 9] admiration for a particular mentor in the field or a genuine interest in the specialty.[8,9] Although, motivation varies by specialization, additional factors may include lifestyle choices, the possibility of personal practice, interest in a particular disease, space varied scope of practice, interest in research and teaching, financial benefits, perceived remuneration, how attractive this course is at undergraduate level or exposure level to specialization at the undergraduate level.[10] A study conducted in the same locality noted gender bias as a factor and the ability to be with families influencing the choice of specialization.[11]

Globally, research has been carried out at both the undergraduate and postgraduate levels. to assess reasons for preference in specific areas of dental specialization. [2.4-7,12,13] Increased socio-economic conditions and factors such as flexible working hours, financial benefits, good job opportunities abroad, self-employment, and having a family doctor teeth have been identified as significant predictors of career preference and specialization.[12,14,15] Besides, personal interest in specialization and the challenge of the complexity of cases or intellectual content were also among the predictors of specific choice.

In Nigeria, there has been concern about the skewed distribution of dental specialists and its potentially harmful impact on dentistry education.[4,5,13] Previous research has linked this imbalance to the degree of interest biased by dental graduates towards particular dental specialties, as they do not the availability of specialists in less favorable specialties.[4,10] There is, therefore, a need to understand the various factors influencing the choice of dental specialization among interested dentists to identify the reasons for the asymmetric distribution as well as explore ways of providing solutions for the distribution of specialists teeth that are angled on one side thereby increasing awareness of dental care. Based on the foregoing,

RESEARCH METHODOLOGY

This type of research is descriptive with a cross-sectional research design using a questionnaire. The research time was in October 2020. The sampling technique used was the total sampling technique, as many as 71 students agreed to participate in this research. The questionnaires were distributed to co-ass students of the 2018 class at the Baiturrahmah University Dental Hospital, the questionnaires that had been filled in by the subjects were collected, then data was grouped, data processed, and analyzed. The questionnaire consisted of student data, choice of specialization, and reasons for continuing to specialize, names, and other personal information were removed from the questionnaire to ensure confidentiality.

DATA ANALYSIS

Descriptive statistics were performed using SPSS version 24.0 on collected data.

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RESULTS

Based on the research that has been conducted, 71 respondents participated in the study with 6 men (8.45%) and 65 women (91.55%). Describing the specialization that respondents interested in, periodontics had the highest number (22.22%) and the least amount of interest was Prosthodontic respondents (1.39%). (Table 3)

The reason for selecting specialization by respondents, the reason has most often chosen to continue the specialization is "Having skills/talents in the specialization" (38.03%), followed by the intellectual content of specialization (14.08%), the existence of role models, and mentors in specialization (12, 68%), financial income (11.27), personal desires (9.86%), job opportunities (8.45%), opportunities for private practice (4.23%), flexible working hours (1.41%) and the remaining reasons were not chosen by the respondents. (Table 4)

Table 1. The gender of the student in choosing the reason for continuing to specialize at RSGM Baiturrahmah.

No.	Student gender	amount	Percentage (%)
1.	Man	6	8.45
2.	Women	65	91.55

Table 2. The most popular specialties at RSGM Baiturrahmah are based on Student' gender".

No.	**Specialties**	**Male (n%)**	**Female (n%)**	**Total (n%)**
1.	Oral and maxillofacial surgery	(12.5%)	(87.5%)	(100%)
2.	Oral pathology	(0.0%)	(100%)	(100%)
3.	Oral disease	(0.0%)	(100%)	(100%)
4.	Pediatric dentistry	(7.7%)	(92.3%)	(100%)
5.	Orthodontics	(9.1%)	(90.9%)	(100%)
6.	Community dentistry	(16.7%)	(83.3%)	(100%)
7.	Periodontics	(6.3%)	(93.8%)	(100%)
8.	Prosthodontics	(0.0%)	(100%)	(100%)
9.	Conservation dentistry	(14.3%)	(85.7%)	(100%)
10.	Family dentistry	(0.0%)	(100%)	(100%)
Total		**(8.5%)**	**(91.5%)**	**(100%)**

Table 3. A specialization favored by students in continuing their specialization at RSGM Baiturrahmah

No.	Specialties	amount	Percentage (%)
1.	Oral and maxillofacial surgery	8	11.11
2.	Oral pathology	2	2.78

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3.	Oral disease	4	6.94
4.	Pediatric dentistry	13	18.06
5.	Orthodontics	11	15.28
6.	Community dentistry	6	8.33
7.	Periodontics	16	22.22
8.	Prosthodontics	1	1.39
9.	Conservation dentistry	7	9.72
10.	Family dentistry	3	4.17

Table 4. The students' reasons for continuing to specialize at RSGM Baiturrahmah.

No.	Reasons for specialization	amount	Percentage (%)
1.	There are role models and mentors in specialties	9	12.68
2.	The intellectual content of specialties	10	14.08
3.	Private practice opportunities	3	4.23
4.	Have skills/talents in a specialization	27	38.03
5.	Financial income	8	11.27
6.	Flexible working hours	1	1.41
7.	Employment Opportunity	6	8.45
8.	Personal wishes	7	9.86

DISCUSSION

In this study, it was reported that the student data at RSGM Baiturrahmah were more than 65 people (91.55%) of the female sex, namely 6 people (8.45%). The specialization options that were of interest to men were oral and maxillofacial surgery by 1 person (12.5%), pediatric dentistry as much as 1 person (7.7%), Orthodontics by 1 person (9.1%), Community dentistry. as many as 1 people (16.7%), Periodontics as much as 1 person (6.3%), Conservation of dentistry as much as 1 person (14.3%), and specialties that were not selected were Oral Pathology, Oral Disease, Prosthodontics, Dentistry family (0. 0%). While the specialties that most women interested in were Periodontics as many as 15 people (93.8%), Pediatric dentistry as many as 12 people (92.3%), Orthodontics as many as 10 people (90, 9%), Oral and maxillofacial surgery as many as 7 people (87.5%), Conservation of dentistry as many as 6 people (85.7%), Community dentistry as many as 5 people (83.3%), Oral disease as many as 4 people (100%), family dentistry as many as 3 people (100%), oral pathology as many as 2 people (100%), and the least chosen is prosthodontics as many as 1 person (100%). A total of 6 men (8.5%) and women 65 people (91.5%) voted.

The specialization that most students interested in continuing their specialization in

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Periodontics, namely 16 people (22.22%), whereas, Pediatric Dentistry as many as 13 people (18.06%), Orthodontics as many as 11 people (15.28%), Oral Surgery and 8 people (11.11%), dental conservation as many as 7 people (9.72%), community dentistry as many as 6 people (8.33%), oral disease as many as 5 people (6.94%), Family teeth as many as 3 people (4.17%), oral pathology as many as 2 people (2.78%), and the least number is chosen by students in continuing specialization in prosthodontics as many as 1 person (1.39%).

The choice most favored by students in continuing their specialization at RSGM Baiturrahmah is Periodontics as many as 16 people (22.22%) and the least chosen by students, namely Prosthodontics as many as 1 people (1.39%) this may be because in the prosthodontics section there are many difficulties. and the length of completion, while in the periodontic section student work is easier and faster to finish.

In this study, it was reported that the reasons students continued to specialize were more, namely having skills/talents in the specialization of 27 people (38.03%), while the intellectual content of the specialization was 10 people (14.08%), the existence of role models and mentors in the specialization. 9 people (12.68%), financial income 8 people (11.27), personal desires 7 people (9.86%), job opportunities 6 people (8.45%), personal practice opportunities 3 people (4.23 %), Flexible working hours for 1 person (1.41%) and the remaining reasons are not chosen by students.

Students' reasons for continuing with more specializations are having skills/talents in specialization 27 people (38.03%), while what students do not choose (0.00%) is the variety of activities and procedures, undergraduate exposure to specialization, undergraduate readiness adequacy in specialization, complex and challenging procedures within a specialization, the possibility of passing examinations on time, availability of resident space, peer pressure, patient diversity, not much clinical work, lifestyle, less stressful work style, areas of special needs, research opportunities abundant, and family stress.

An assessment of these multiple factors may explain the underlying reasons behind the skewed distribution of dental specialists in a particular field of dentistry to curb trends and improve overall oral health care. The age and average age ranges observed in this study are similar to previous studies conducted in Nigeria, namely in 2018 students at the Baiturrahmah Dental Hospital, having skills/talents in specialization was reported as the most reason for the choice to continue specialization. This can be explained by the fact that people are intrinsically motivated towards their skills/talents compared to other external factors. This is also similar to previous research.

Family pressure or others has proven to have little or no influence on students in the reason for continuing to specialize, this further reinforces the fact that having the skills/talents possessed by the student in addition to other contributing factors are very important in influencing the choice of specialization for students who are interested in continuing specialization.

CONCLUSION

Based on the results of research on the reasons for students of class 2018 in continuing to specialize in questionnaire-based studies at RSGM Baiturrahmah, it was concluded that the most popular specialization was Periodontics, and having skills/talents in specialization was the most reason for the choice of continuing specialization from students of class 2018 at RSGM Baiturrahmah.

For dental clinic students, choosing a specialization at RSGM Baiturrahmah, so that more knowledge about the specialization will be selected so that the study is carried out easier and can be completed on time even faster.

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RESEARCH ARTICLE

Invitro Micro Encapsulation of Beta Tri Calcium Phosphate from *Anadara granosa* Shell Synthesis

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ABSTRACT

Background: *Calcium is a material that is mostly contained in the Anadara-granose shell. Beta-TCP can be obtained from the hydrothermal process from the Anadara-granose shell. Beta-TCP has a chemical composition that approximates the structure of bones and teeth.* ***Objective:****The microencapsulation technique aims to increase stability, reduce side effects and toxic effects of drugs, and prolong the release of ingredients. The encapsulation process is an attempt to inhibit the dissolution speed of Calcium to prevent tunnel defects.* ***Methods:*** *Anadara-granose shell powder was subjected to hydrothermal processing for 18 hours and sintering for 3 hours. The beta-TCP powder was dissolved with aquadest using a magnetic stirrer until it was homogeneous, Na-alginate was dissolved in aquadest until it was homogeneous with a magnetic stirrer then the two solutions were mixed and the $CaCl_2$ solution was dropped. The sample was divided into 3 groups; Pure Beta-TCP(K-); 7 hours stirring (P1); 8 hours stirring (P2). After completion of the stirrer, the samples were centrifuged at 2500 rpm for 6 minutes, then freeze-dried for 12 hours. The level test was carried out using complexometry comparing the pure Beta-TCP group with the Beta-TCP stirrer encapsulation process for 7 hours and 8 hours.* ***Results:*** *The data showed that the average calcium level in K(-) group with pure Beta-TCP was 8.63%, the P1 Beta-TCP group with 7 hours stirrer 2.86%, and the P2 Beta-TCP group with 8 hours stirrer 2.12%.* ***Conclusion:*** *In the Anadara-granosa shell nanoencapsulation process, the calcium level gradually decreased with the longer duration of stirring time.*

Keywords: *Encapsulated, Calcium Level, Natrium Alginate Polymer, Anadara granosa*

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INTRODUCTION

Indonesia has abundant land and sea natural resources. One of the natural resources that can be used as an alternative medicine/dental material is the shell of *Anadara granosa* (AG) which contains 98.68% Calcium Carbonate ($CaCO_3$). *Anadara granosa* (AG) shell can be synthesized by hydrothermal method for 18 hours at 200^{o}C and sintering for 3 hours at 900^{0}C to obtain hydroxyapatite (15%), Beta-tricalcium phosphate (B-TCP) (79%), and Ca $(OH)_2$ (6%).[1,2] Beta-Tricalcium Phosphate (β-TCP) (β-$Ca_3(PO_4)_2$) has a chemical composition that approximates the structure of bones and teeth.[3,4] β-TCP can be used as a potential bone substitute because it is biocompatible, bioresorbable, and has osteoconductivity.[4.5] TCP (Tricalcium Phosphate) is a material that has osteoconductive properties but has a faster resorption rate than hydroxyapatite.[6] TCP (Tricalcium Phospate) is also a porous bioceramic material that has non-reactivity and resorbability biological properties. Theoretically, the biocompatibility of TCP combined with calcium allows TCP to stimulate odontoblasts, thereby leading to the formation of dentin bridges. Because of it, Beta-TCP from the synthesis of *Anadara granosa* shell (AG) can be used as an alternative ingredient in dental care to maintain vitality and stimulate the pulp healing process.[2]

The material commonly used today is calcium hydroxide (Ca $(OH)_2$) which is the gold standard material. This material has a disadvantage in long-term use, its bond with dentin tends to be mild, crushed, and dissolved in the dentin fluid resulting in a tunnel defect in the formation of the dentin bridge which can facilitate the entry of bacteria, also because of the very high pH of 11-13 this can cause necrosis of the pulp tissue.[7]

One of the technological advancements that are currently being developed is the drug delivery system in the form of microparticles.[8,9] Microparticles can be defined as something small with a size of 1-1000 µm.[9,10] Microparticles formation aims to overcome the solubility of active substances that are difficult to dissolve, improve poor bioavailability, modify drug delivery systems, increase the stability of active substances and improve absorption. The advantage of microparticles is the ability to penetrate the spaces between cells that can be penetrated by colloidal particles.[12] Another advantage is the increased affinity of the system due to the increase in contact surface area by an equal amount.

Mechanical factors in the homogenization process can cause particle acceleration, vibration, emission pressure, and friction which are used to reduce the size of a particle and keep the particles small against the influence of particle growth and agglomeration.[12] Mechanical effects can be generated through rotation, magnetic stirrer vibrations, and the movement of aerated air bubbles (low energy technique), as well as from ultrasonic wave vibrations (utrasonicators / high energy techniques).[13] The small particle size is greatly influenced by the particle acceleration, vibration, emission pressure, and fast friction of the formulation technique used.[14] Researcher wanted to add to the magnetic stirrer process when carrying out the ionic glaciation process to see the particle size and calcium levels generated from the process and compare with the material without encapsulation

MATERIAL AND METHOD

The design of this study was the post-test-only control group design to determine the application of Beta - tricalcium phosphate (β-TCP) from *Anadara granosa* shell in the drug release process. The groups were divided into 3, which was the negative control group pure Beta - tricalcium phosphate (β-TCP) was K(-), the treatment group with 7 hours microencapsulated Beta - tricalcium phosphate (β-TCP) was P1 and 8 hours microencapsulated Beta - tricalcium phosphate (β-TCP) was P2.

Anadara granosa shells are obtained from Kenjeran coastal waters, Surabaya. The

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shells of *Anadara granosa* are boiled for 30 minutes. Then, brush the blood clam shells on the outside and inside using water and soap without bleach, then dried at room temperature. After that, it was crushed using a mortar and pestle and was sieved with 200 mesh to get smaller particles.

10 grams of *Anadara granosa* shell powder was added to 100 ml of aquadest to produce 1M and 6.9 grams of $NH_4H_2PO_4$ solution added with 100 ml of aquadest to produce 0.6M. Then the two were mixed with a magnetic stirrer for 30 minutes. Furthermore, the mixed solution was transferred to the reactor. The reactor was put in an electric oven to be heated to a temperature of 200 ° C for 18 hours. The result obtained was cooled at room temperature. Furthermore, the heating powder was washed with aquadest using a magnetic stirrer. Abstersion was carried out repeatedly until the reaction results were separated with distilled water, indicated by the pH to be 7. This action was done to remove acidic byproducts. Next abstersion was carried out with methanol to limit agglomeration of HA particles during drying. The samples were dried in an electric oven at 50 ° C for 3 hours and 4 hours. Sintering the sample at 900 ° C for 3 hours to remove impurities and increase the crystallinity of the sample to produce the active ingredient TCP.

β-TCP encapsulation process method

β-TCP was mixed with sodium alginate with a mass ratio for example β-TCP / sodium alginate which is 1/1. 2 gr, 2.5 g, and 3 g β-TCP powders were mixed with 2%, 2.5%, and 3% sodium alginate, and a solution was made with 100 ml aquadest for each sample. 16.65 g of $CaCl_2$ was dissolved with 100 ml of aquadest. Then the $CaCl_2$ solution was dropped into a solution of β-TCP and sodium alginate which was stirred for 7 hours for the P1 group and 8 hours for the P2 group. After that, centrifuged at 2500 rpm for 6 minutes and the sediment was taken. Then dried using the freeze-dry technique with 5% humidity. The freezing process was carried out by storing the sample in the freezer overnight to form ice crystals, then the drying process used a freeze dryer.

Particle size analysis

Measurement of particles or PSA beta-TCP which is encapsulated aims to determine the particle size. 1 gram of BTCP encapsulated powder was dissolved in 8 ml of distilled water and sonicated for 10 minutes to obtain a homogeneous sample. The sample is placed in a suitable single-use plastic cuvette. Samples were measured with a beta nanoparticle analyzer 5 times per group. The optimal attenuator gap width is 6 to 8. Overcast samples will appear 6 in the attenuator, which means that the sample needs to be dissolved. A sample that is too transparent will show 8, meaning that more sample material needs to be added.

Calcium Rate Analysis

Calcium rate analysis using the complexometric method. The sample was weighed 0.5 g. Then dissolved using distilled water using a measuring flask until the meniscus limit showed the number 100 ml. Furthermore, 2 ml of NaOH 8N was added. and the Calcon indicator. After that, the titration was carried out using a 0.1 N EDTA reagent until it changed color from orange to blue. The final result, record the volume of the EDTA titrant 0.1 N used.

RESULT

This study aimed to determine the differences in the content of calcium levels contained in β-TCP (β-Tri Calcium Phospate) which was carried out by the encapsulation process using sodium alginate polymer. This study used a calcium level test with the complexometric method. The data generated from this study were:

Table 4.1 The results of analysis of pure calcium β-TCP levels, β-TCP encapsulation using the complexometry method

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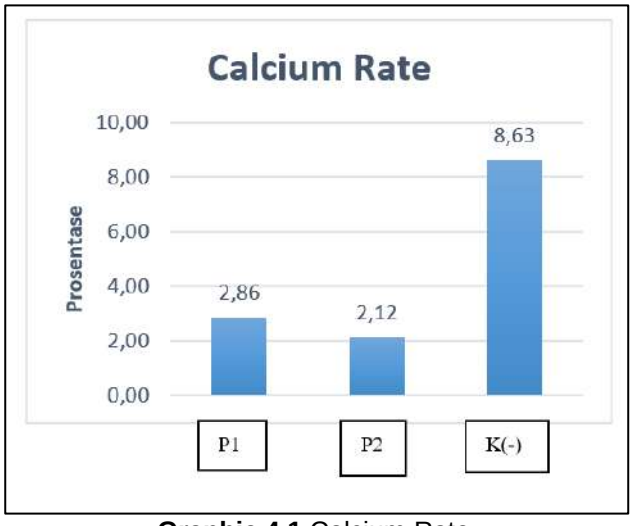
Sample	7 hours microencapsulated Beta Tricalcium phosphate	8hours microencapsulated Beta Tricalcium phosphate	Pure Beta Tricalcium phosphate
1	2,91	2,05	8,61
2	2,76	2,14	8,50
3	2,91	2,18	8,78

Table 4.2 The results of the average analysis of pure calcium β-TCP levels, β-TCP encapsulation using the Complexometric method

7 hours microencapsulated Beta Tricalcium phosphate	8 hours microencapsulated Beta Tricalcium phosphate	B-TCP 18 hours
2,86	2,12	8.63



Graphic 4.1 Calcium Rate

DISCUSSION

This study aimed to determine the differences in the content of calcium content of *Anadara granosa* shells which were carried out by the 18-hour hydrothermal process and 3 hours of sintering, which was then carried out by the β-TCP (β-Tri Calcium Phospate) encapsulation process with sodium alginate polymer for 7 hours and 8 hours, pure β-TCP (β-Tri Calcium Phospate).

In this study, the results of the calcium level test showed that the average calcium level in the 7-hour micro-encapsulation group β-TCP (β-Tri Calcium Phospate) was 2.86% greater than β-TCP (β-Tri Calcium Phospate) 8 hours by 2.12%. Meanwhile, in the pure β-TCP group the calcium content was 8.63%. Based on the results of this study, it was explained that microencapsulation was a way of using a relatively thin coating to protect the core material from being melt so that it was easy to handle and could protect the loss of core material and as a controlled release of drugs that entered the body so that the dissolution of the core material could be controlled .[9,10]

Microencapsulation is the process of using a relatively thin coating on small particles of solids, where the particle size ranges from 2-5000 μm. Microencapsulation or what is called a microcapsule is defined as a particle containing an active substance or core material surrounded by a coating or shell, with the presence of this polymer wall layer, the core substance will be protected from the influence of the outside environment. Microencapsulation techniques are commonly used to increase stability, reduce side effects and toxic effects of drugs, and prolong drug release.[15]

Microencapsulation consists of three main ingredients, that are core material, coating material, and solvent. The core material is the specific material to be coated, the core material should not dissolve or react with the coating material and solvent used. The coating material is a material that coats the core to cover unpleasant odors and tastes, protecting the environment, stability, and preventing evaporation. [15,16]

The coating material must be able to provide a cohesive thin layer with the core material, can mix chemically, does not react with the core (is inert), and has properties suitable for the coating.[16] The coating material or polymer used is alginate, which is a very

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good polymer in helping microencapsulation performance. Sodium alginate produces a more uniform gel structure which forms a stronger cross-linking structure and more trapped loading of the material. [15,17]

A simple way of making microparticles is the ionic gelation method which this method involves a cross-linking process between the polymer Na-alginate and $CaCl_2$ as a crosslinking ion. *Anadara* shells (AG) powder is micro-prepared and coating is carried out.[16] Ionic gelation method functions as a hardener and maintains the shape of the microparticles.[9,16] A widely used cross-linking agent is calcium chloride ($CaCl_2$), which this cross-linking agent can control the release of the active ingredient from the oral dosage form with alginate crosslinking.[9]

The nature of alginate gel formation with polyvalent ions such as calcium leads to the formation of cross-links. The addition of $CaCl_2$ to the alginate filtrate causes calcium alginate deposits in the form of white fibers and a large coarse size because the mixed Ca^+ ions will bind to the alginate and will form cross-links between molecules and then settle.[19] The formation of this cross-linked bond will strengthen the mechanical strength of the particles that are formed.[11]

Factors that can affect the properties of alginate solutions are temperature, concentration, and polymer size.[16] Alginate is a polymer that is often used with the ionic gelation method, because alginate produces a good shape, is biocompatible and the resulting matrix is non-toxic factors affecting stability. [20]

Aprilia's research stated that the beta-TCP particle size resulting from the synthesis of anadara granosa shell with sodium alginate polymer by stirring technique for 2 hours obtained a particle size that was closest to the ideal particle size determined for pulp capping material. Calcium levels test showed that the stirrer process for 1 hour had the highest calcium levels.[2] In another study, it was proven that the stirring technique using a magnetic stirrer could synthesize calcium alginate cross-links with various alginate concentrations.[20]

However in this study, the longer duration of microencapsulation process could reduce the calcium levels contained in the active ingredient, due to the influence of the encapsulating material or polymer that was given and also the effect of the crosslinker agent used. Polymers and crosslinkers would be cross-linked with the active ingredients, so that it could affect the amount of active ingredient.[10]

CONCLUSION

β-TCP (β-Tri Calcium Phospate) was produced from hydrothermal processes and sintering of Anadara granosa shells using the hydrothermal method for 18 hours and 3 hours of sintering. There was a difference in calcium levels which is pure β-TCP (β-Tri Calcium Phospate) material has more calcium levels than β-TCP (β-Tri Calcium Phospate) that encapsulated for 7 hours and 8 hours.

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CASE REPORT

Management of Herpangina

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ABSTRACT

Background: *Herpangina is an acute infectious disease with self-limiting properties. While herpangina may heal without any treatment, it may be followed by various complications such as meningitis, encephalitis, cardiomyopathy, or even death. In 2018, there was a 10.07% prevalence rate of viral infection in Banjarmasin city, South Kalimantan. One of which was herpangina.* ***Objective:*** *To elucidate the management of herpangina.* ***Case:*** *Young female patient, 11 years old, complained of multiple ulcerations on the posterior side of her mouth with excruciating pain when swallowing. Ulceration had appeared for three days which was initially commenced by fever and cough.* ***Case Management:*** *The patient was prescribed methisoprinol syrup 250 mg four times a day, ibuprofen syrup 250 mg four times a day, mouthwash containing aloe vera extract to be applied thrice daily, and explicit instruction for bed rest.* ***Conclusion:*** *Dental practitioners must differentiate herpangina from other differential diagnoses thus enabling the acquirement of final diagnosis through clinical examination. This will significantly assist dental practitioners to provide precise clinical therapy for the patient with herpangina.*

Keywords: *Herpangina, management, methisoprinol.*

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INTRODUCTION

Herpangina is an acute infectious disease with self-limiting properties. This disease is commonly occurred among children and tends to heal in five to seven days. While herpangina may heal without any therapy, this disease frequently induces various complications such as meningitis, encephalitis, cardiomyopathy, or even death. Asia is the region that is commonly affected by this condition. [1,2,3,4] Herpangina is caused by coxsackie A type 1-6, 8, 12, 20, and 22 and also Enterovirus (EV) 71.2,3 Of all viruses causing herpangina, EV 71 is the most frequent etiology of this disease with common complication such as neurological disease, cardiovascular collapse, and death.[2,4]

Enterovirus (EV) 39,8% and Coxsackievirus A genotypes 8 (CAV8) found 19,3% and from patients with these diseases in Thailand in 2012. In 2015, there was a large outbreak of herpangina in children caused by Enterovirus in Hangzhou, China. In 2018, there was a 10.07% prevalence rate of viral infection in Banjarmasin city, South Kalimantan. One of which was herpangina.[5,6,7]

EV 71 is categorized in the Picornaviridae family consisting of Ribo Nucleic Acid (RNA) genomes.[8] Therefore, any fecal-oral contact or respiratory secretion via direct person-to-person contact, droplets, and fomites may support the transmission of the disease. This transmission should also be supported by hygiene level, water quality, and crowding extension.[4]

Tropical and subtropical countries are frequently harmed by herpangina where the disease is transmitted in a year periodically. Including in Indonesia, this disease is more likely to be found in the rainy season. Clinical manifestations of herpangina are hardly distinguishable to other viral infections of the mouth, such as herpetic stomatitis and varicella-zoster.[9]

This resemblance will lead to misdiagnosis resulting in the inaccuracy of given therapy. This case report aims to clarify the case management of herpangina. Is required further discussion to differentiate herpangina from other differential diagnoses so that dental practitioners may prescribe accurate therapy.

CASE

A young female patient, eleven years old, complained about multiple ulcerations on the back of her mouth with excruciating pain when swallowing and resulted in difficulty eating. Based on history taking, the mother revealed that ulceration had occurred for three days. Before ulceration, the patient was experiencing a fever and cough. It was occurring for the first time. Due to unbearable painful sensations, the patient occasionally cried when dining with every food. Her mother had been administering Ibuprofen syrup 250 mg thrice daily for three days. Any improvement of the disease has yet been observed.

CASE MANAGEMENT

Extra-oral examination revealed that the right and left submandibular glands were palpable, soft, and painful. Intra-oral examination exposed the presence of ulceration, 2 to 3 mm in diameter, multiple, painful, around oropharynx with reddish macula surrounding the lesion. Based on clinical examination, the patient was diagnosed with herpangina. It was determined by assessing the fever and flu-like syndrome before ulceration, the location of ulcers which were merely found on palatum molle, and the absence of ulceration on other locations.

The patient was prescribed methisoprinol syrup 250 mg four times a day, ibuprofen syrup 250 mg four times a day, and aloe vera extract-containing mouthwash thrice a day. The patient was also instructed for bed rest and isolation to prevent the disease's transmission to others. High calorie and protein consumption were advised with lots of mineral water intake. The

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patient was requested to evaluate her condition for the following week.

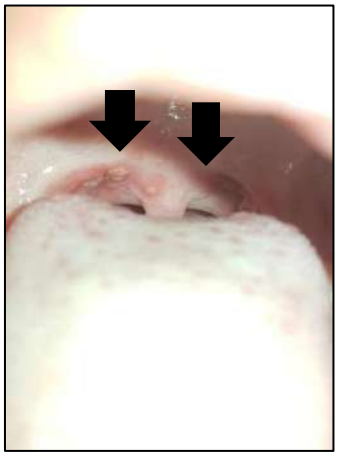

Figure 1. Ulcer, multiple, 2 to 3 mm, white in color, surrounded by reddish appearance, and painful

Second Visit (Day 8)

Based on anamnesis from her mother, the pain due to ulceration on the back of the patient's mouth had been subsided for the past three days. No fever was present and the patient was able to eat well. Extra-oral examination exhibited a normal submandibular gland. Intra-oral examination depicted no abnormal presentation. No lesion was present on the palatum molle surrounding the oropharynx which was normal in color and painless. The patient was healed and therapy was terminated.

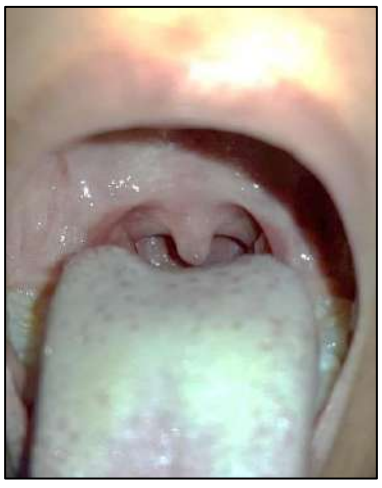

Figure 2. Palatum molle around oropharynx demonstrated no lesion, normal in color, and absence in pain.

DISCUSSION

This case presents a patient who is clinically diagnosed with herpangina. The diagnosis was established from particular clinical manifestations of herpangina such as multiple painful mouth ulcers which predominantly affect the posterior region of the oral cavity, including anterior pharyngeal fold, uvula, tonsils, and soft palate.[4,9]

Differential diagnoses of herpangina are herpetic stomatitis, hand foot and mouth disease (HFMD), and varicella-zoster.[9,10] Herpetic stomatitis is closely similar to herpangina ascribed its only occurrence in the oral cavity. Varicella-zoster and HFMD may easily be differentiated with herpangina through their clinical presentation on skin and ulcerations that occur on every surface of oral mucosal. In contrast, herpangina merely occurs in the posterior region of the oral cavity. Herpetic stomatitis that occurred in the oral cavity is a differential diagnosis which closely similar to herpangina. Both herpetic stomatitis and herpangina will be presented with high fever, prodromal symptoms, and flu-like syndrome before the emergence of ulceration in the oral cavity.[9,11] Clinical manifestations of both diseases include multiple yellowish ulceration with red surrounding and painful sensation.

A clear distinction between herpetic stomatitis and herpangina is presented in the type of virus causing the disease. Herpetic stomatitis is induced by herpes simplex virus type 1, while herpangina is caused by coxsackie A virus type 1-6, 8, 12, 20, and 22 and also Enterovirus (EV) 71. Clinically, herpangina only presents on the posterior of the oral cavity while herpetic stomatitis appears on all surface of the oral cavity and skin on the upper waist region.[4,9,10]

Enterovirus 71 (EV 71), the etiology of HFMD commonly found in the Asia Pacific region over the last decade, has been linked with neurological disease and mortality. Enteroviruses compose of small and non-enveloped RNA which are classified as

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members of the picornaviridae family. Categorized as an RNA virus as well, the Coxsackie virus also belongs to the picornaviridae family.[8]

To determine the final or differential diagnoses of herpangina, such as allergic stomatitis, the specific clinical manifestation may be exerted. It had any difficulty persisted in diagnosing clinically, so that should be obtained using additional testing such as isolation of virus specimens to be confirm by PCR examination, indirect immunofluorescence assay, and serological analysis.[1,9] Meanwhile, herpetic stomatitis may be diagnosed through additional examination in the form of viral culture isolation, direct immunofluorescence, or serology.[10]

In this case, methysoprinol syrup 250 mg was prescribed four times daily, along with ibuprofen syrup 250 mg four times daily and aloe vera extract-containing mouthwash to be applied three times a day for one week. Since herpangina is not induced by the herpes simplex virus, thus methisoprinol was prescribed instead of acyclovir. Methisoprinol possesses immunomodulatory and antiviral effects. The drug can enhance immune response, macrophage proliferation, and phagocytic activity against viruses. This will improve the immune system by repairing impaired mediated cell immune response into normal cells.[12] As the most common and the most frequent NSAID to be prescribed, Ibuprofen contains non-selective cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2) inhibitors which demonstrate weaker anti-inflammatory properties than other NSAID drug. It shows eminent analgetic and antipyretic activity due to cyclooxygenase inhibitory actions. Ibuprofen is also recommended to vanquish fever and malaise.[13,14,15]

The patient has also been prescribed an over-the-counter mouthwash containing aloe-vera extract that will accelerate the wound healing process and reduce pain sensation. The drug also holds antiviral, antibacterial, and antifungal activity with the capability of enhancing the re-epithelization process.[16,17] This prescription aims to prevent secondary infection, reduce painful sensation, and facilitate not only the masticatory but also the speech process. [18,19] It may be concluded that the knowledge of dental practitioners is essentially required to distinguish herpangina from other differential diagnoses so that final diagnosis may be acquired through clinical examination only. This is due to the many similarities of the lesions in herpangina with other infectious diseases of the oral mucosa.[20] This method will assist dental practitioners in managing herpangina patients effectively.

CONCLUSION

It may be concluded that is crucial for the dental practitioner to differentiate herpangina with another differential diagnosis thus enabling the acquirement of final diagnosis through clinical examination. This will significantly assist dental practitioners to provide precise clinical therapy for patients with herpangina.

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RESEARCH ARTICLE

The Effect of Addition of Nano hydroxyapatite Powder of Anadara granosa Shells on Surface Roughness of Heat-cured Acrylic Resin

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ABSTRACT

Background: *Heat-cured acrylic resin is one of the most widely used denture base materials. However, the residual monomer can reduce its mechanical properties, which affect physical properties such as surface roughness, resulting in high porosity—the results in the growth of fungal colonies. One way to improve these properties is to add a reinforcement material such as nano-hydroxyapatite (HAp) from the Anadara granosa shell. Nano-hydroxyapatite has a large surface area to act as a filler because of its strong bonding capacity and a smoother surface, and a higher density to improve mechanical properties.* ***Purpose:*** *This study aims to determine HAp powder's effect from the Anadara granosa shells on the surface roughness of heat-cured acrylic resin.* ***Methods:*** *This type of research used an experimental laboratory, research design posttest-only control group design. Twenty-four samples were used and divided into three groups: the acrylic resin group with 1%, 3% blood cockles shell HAp, and acrylic resin without HAp with simple random sampling.* ***Results:*** *The results showed that the lowest mean value of surface roughness was in a group with the addition of 3% HAp, at 1.696 ± 0.25058 µm. One-Way ANOVA test on roughness showed a significant difference between groups with p = 0.000 (p <0.05).* ***Conclusion:*** *This study concludes that there is an effect of HAp on the roughness of heat-cured acrylic resin.*

Keywords: *the Anadara granosa shell, heat-cured acrylic resin, nano-hydroxyapatite, surface roughness*

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INTRODUCTION

Heat-cured acrylic resin is one of the most widely used denture base materials. This type of resin has certain advantages such as lower toxicity, relatively prone to be processed and polished, more aesthetic values as well as affordable cost. However, the residual monomer can reduce mechanical properties which will affect physical properties such as surface roughness which results in higher porosity. This is due to higher growth on fungal colonies[1]. Surface roughness is one of the most significant physical properties of the denture base that needs further attention. Higher roughness on denture base will increase adhesions on bacterias, plaques and food leftovers. The tolerable surface roughness of the denture base shall be no more than 0.2 µm or less.[2]

Higher use of acrylic resin in dentistry has led to various measures in order to improve these properties, one of which is the addition of a reinforcing agent in the form of hydroxyapatite. Hydroxyapatite has osteoconductive properties due to approximately 65-70% of its components are bone-like components so that it shall not cause any allergic reactions. In addition, hydroxyapatite has also good biocompatibility and relatively stable thermodynamically.[3] Hydroxyapatite with calcium phosphate-based has its chemical compound $Ca_{10}(PO_4)_6(OH)_2$ (4). One of the most potential sources of hydroxyapatite is Anadara granosa shell/cockle. Cockle or Anadara granosa (A. granosa) is the most commonly consumed shellfish type by the public. The mineral compositions of anadara granosa shell are calcium carbonate and carbon, it content more than 98.7% of total mineral. The content of Mg^{2+}, Na^{+}, P^{3-}, K_{+}, Fe^{2+}, Cu^{+}, Ni^{2+}, it comprise approximately 1.3%[5]. Anadara granosa shell contains high calcium carbonate as well as with higher density on its particle size. Hydroxyapatite can be synthesized from Anadara granosa shell using low temperature hydrothermal method[6]. This material is widely used in Medicine and Dentistry sectors as a material to produce artificial bones and dentures due to it contains calcium phosphate with abundance of hydroxide.[4]

Hydroxyapatite can be made with certain forms, one of which is nanoparticle.[7] Nanoparticle is widely used as it has larger surface area so that it can be used as fillers.[8] The ratio between surface area and larger volume than similar particles on the nanometer scale makes its nano-particle become more reactive. Nano-hydroxyapatite has higher surface activity and a very fine structure similar to minerals found in hard tissues on the body and higher density which can improve its mechanical properties.[9,10]

Information on the addition of nano-hydroxyapatite on Anadara granosa shells to surface roughness of heat-cured acrylic resin is relatively small and has never been reported yet, nevertheless, certain researchers are becoming interested to carry out this study.

METHODS

This study is purely laboratory experimental with using post test only control group design. A total of 24 heat-cured acrylic resin samples were used as research samples and divided into 3 groups, namely acrylic resin group with no addition of nano hydroxyapatite, by the addition of 1% and 3% nano-hydroxyapatite on Anadara granosa shell. The study sample was conducted at the Basic Dentistry Laboratory, Department of Dentistry, University of Jenderal Soedirman. Surface roughness of heat-cured acrylic resin was tested at Material Laboratory, Mechanical Engineering Department, Vocational School of Gadjah Mada University.

Procedures on this research commences with manufacture of metal disc molds measuring 10 mm in diameter and 2 mm thick. The next stage is to manufacture nano-hydroxyapatite on Anadara granosa shells. The dried Anadara granosa shells were mashed

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using a ball mill and weighed for 15 g. and then calcination was carried out in a furnace at 1000^0 C for 3 hours. Calcined $Ca(OH)_2$ weighed to 18.03 g. $(NH_4)_2HPO_4$ was weighed to 16.63 g and then dissolved in 275 ml distilled water in a beaker, then to be homogenized in a magnetic stirrer with speed of 300 rpm for 150 minutes and to mix $Ca(OH)_2$ little by little. Formed precipitate is put into a petri dish and then dried in an oven for 12 hours with temperature of 120^0C. After drying it then mashed it using a mortar and to be weighed. After that, TEM test was carried out in order to confirm shape and size of Hap.[5]

Nano-hydroxyapatite was added according to the concentration determined in polymer, then to be mixed with heat-cured acrylic resin monomer. The polymer in group I (with no addition of nano HAp powder) was 5 g with 2.5 ml of monomer. Group II with 1% concentration with polymer for 4.95 g and HAp nano powder for 0.05 g. Group III with 3% concentration with polymer for 4.85 g and nano HAp powder for 0.15 g.

The acrylic resin mix was put into a mold that had been smeared with *could mold* seal. Polymerized acrylic resin was cured using water bath at 70^0C for 90 minutes and then continued to 100^0C for 30 minutes and allowed to stand with room temperature. Nonetheless, finishing is done with using a tungsten carbide bur with speed of 15,000 rpm for 60 seconds on each sample.[11]

Surface roughness testing on acrylic resin samples was conducted by using Surface Roughness Tester SE 1700® (Profilometer). The profilometer has a radius-shaped stylus tip with a radius of 2 µm and movement speed of 0.500 mm/s. This instrument works by means of stylus tip touching and moving horizontally on surface of acrylic resin sample and acrylic resin sample must be placed with stable position. Tests were also conducted on 3 sample groups on cylindrical-type sample with diameter of 10mm and thickness of 2mm.[12]

One of surface roughness parameters is to calculate mean roughness of quadratic (R_q). Calculation on roughness mean of quadratic (R_q) with equation as follows (13):

$$Rq = \sqrt{\frac{(Yi - Ym)^2}{N}}$$

where:

Yi = Reference profile distance to measures profile

Ym = Reference profile distance to average lines

N = Quantity of Y

Then, obtained data was tabulated and analyzed with using One-Way ANOVA test as well as advanced test of Post Hoc LSD.

RESULTS

The mean and standard deviation (SD) of surface roughness test results can be seen in Figure 1.

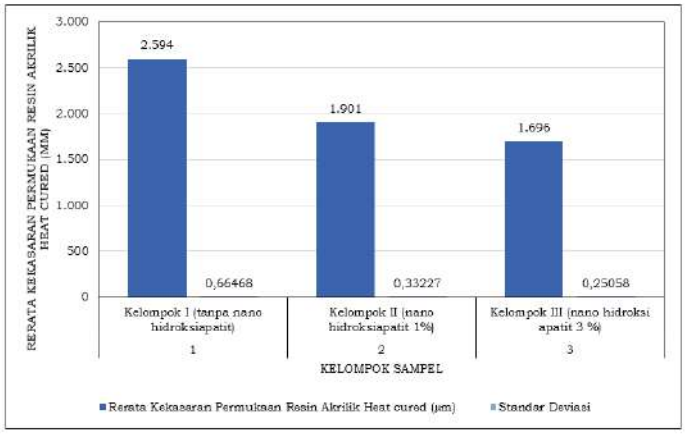

Figure 1. Mean and surface roughness on heat-cured acrylic resin.

Figure 1 shows that the highest surface roughness value is in group I with a mean of 2.594 ± 0.66468 µm and lowest surface roughness value is group III with a mean of 1.696 ± 0.25058 µm. Saphiro-Wilk test and Levene test showed that data were normally distributed and homogeneous ($p \geq 0.05$). Then, data on normally distributed and homogeneous were analyzed using One Way Anova test in order to determine is there any significant difference in surface roughness value between treatment group and control group. The results

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of One Way Anova test presented in Table 1 as follows.

Table 1. Result on *One-Way Anova* test on surface roughness value

Sample group	F	Sig
Group I (with no nano hydroxiapatite) Group II (1% of nano hydroxiapatite) Group III (3% of nano hydroxiapatite)	8.644	0.002*

Note :
*= there is a significant difference inter-groups (p<0,05)

Based on result in Table 1, p value is 0.002 (p <0.05), which indicates that there is a significant difference between surface roughness values on each sample group. Nevertheless, Post Hoc Least Significant Differences (LSD) test was carried out in order to identify groups with significant differences. The results of Post Hoc Least Significant Differences (LSD) tests are presented in Table 2 as follows.

Table 2. Result on *post hoc* LSD test for surface roughness

Group	Group I	Group II	Group III
Group I		0,006*	0,001*
Group II			0,376
Group III			

Table 2 shows that there is a significant difference in surface roughness of heat-cured acrylic resin (p≤0.01) in all treatment groups with control group. Meanwhile, there is no significant difference between groups II and III (p> 0.01).

DISCUSSION

Objective of this study is to determine the effectiveness on adding nano-hydroxyapatite to Anadara granosa shells by reducing its surface roughness of heat-cured acrylic resin. In this study, nano-hydroxyapatite of Anadara granosa shells was used with concentration of 1%, 3% respectively and with no addition of nano-hydroxyapatite on Anadara granosa shells as a control group. Concentration selection is based on study of Kusumawardani et al.[3] by adding 2% of hydroxyapatite to acrylic resin can minimize residual monomers and increase its compressive strength, therefore, 1% and 3% concentrations were selected in this study.[3]

Results of this study indicate that lowest surface roughness value is in group with the addition of nano-hydroxyapatite for 3% and the highest is in group with no addition of nano hydroxyapatite. Significant differences were shown between groups with addition of 1% of nano-hydroxyapatite on Anadara granosa shells and 3% if nano-hydroxyapatite on anadara granosa shells and groups without addition of nano-hydroxyapatite on Anadara granosa shells. However, there was no significant difference between addition of 1% and 3% of nano-hydroxyapatite in Anadara granosa shells. Factors that can cause decrease of surface roughness include the presence of high interfacial bonding between nano-hydroxyapatite and polymer matrix, good dispersion and even distribution of nano-hydroxyapatite in polymer. Nanometer size and low concentration of nano-hydroxyapatite shall promote good dispersion in heat-cured acrylic resin.[14] In addition, nano-hydroxyapatite can play a role in binding heat-cured acrylic resin monomers.[15]

The capability of nano-hydroxyapatite on Anadara granosa shells can be made as a filler or nanofiller when manipulated polymer and monomer cause a decrease in surface roughness. Nano-hydroxyapatite on Anadara granosa shells has a large surface area so that its binding power is relatively solid. Based on TEM test conducted, it was found that particle size of nano-hydroxyapatite powder on Anadara granosa shells ranges from 20 to 200 nm. Nanometer particle size of nano-hydroxyapatite on anadara granosa shells will fill the porosity

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gap and increase inter-matrices binds. This conforms with the study of Chondro et al.[15] regarding addition of 2% and 5% concentration of hydroxyapatite powder which indicates that there is a decrease in porosity level of heat-cured acrylic resin.[15]

The addition of hydroxyapatite to heat-cured acrylic resin is able to minimize residual monomers as well as produce a homogeneous texture but without any chemical reactions (16). Nano-hydroxyapatite powder that fills porosity gaps on inter-matrices of heat-cured acrylic resin will bind mechanically. Reduced residual monomer in acrylic resin will affect physical properties such as decreased surface roughness.[17]

To mix with nano-hydroxyapatite during manipulation process into chain binds will not change its basic structure, however, it can react with polymethyl methacrylate. Nano-hydroxyapatite will fill gaps and bind to matrix walls in newly-formed gaps and fill gaps until it closed and form new surfaces (3). FTIR test result regarding the addition of hydroxyapatite to PMMA has no correlation chemically whatsoever. In PMMA spectrum, not only transformation on group C = C and C-C groups into polymers either before and after addition of hydroxyapatite. This means that addition of hydroxyapatite does not cause any chemical reactions, instead of a mechanical bond.[16]

On addition of nano-hydroxyapatite with a concentration of 3% resulted in number of hydroxyapatite nanoparticles becoming more and more evenly dispersed between chains of polymethyl methacrylate, causing some of microporosity to be closed and reducing surface roughness on acrylic resin.[13]

CONCLUSION

The addition of nano-hydroxyapatite on Anadara granosa shells can lower surface roughness of heat-cured acrylic resin, the best reduction was the addition of 3% nano-hydroxyapatite on Anadara granosa shells.

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RESEARCH ARTICLE

The Effect of Proanthocyanidin Cocoa Pod Rind Extract (Theobroma cacao L.) on MMP-8 Expession in Gingival Tissue of Periodontitis Rats Model

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ABSTRACT

Background: *Periodontitis is periodontal tissue inflammation due to Porphyromonas gingivalis which invades the gingival tissue due to the activity of virulence factors. This causes the host immune system changes and increases MMP-8 production in periodontal tissue so resulting excessive tissue destruction. Cocoa pod rind containing proanthocyanidin compound as anti-inflammatory drugs is given to reduce MMP-8 and cure periodontitis.* ***Objective:*** *To examine the effect of proanthocyanidin extract of cocoa pod rind (Theobroma cacao L.) on decreasing MMP-8 expression in gingival tissue of periodontitis rats model.* ***Materials and Methods:*** *The samples used were 16 male Wistar rats divided into 2 groups, placebo gel for the control group and proanthocyanidins gel for the treatment group.). Each group was induced by P.gingivalis bacteria every 3 days for 14 days and then smeared with placebo gel and proanthocyanidin gel for 7 and 14 days. Then all rats' gingival tissue were taken and made preparations to be observed under a microscope and analyzed using immunoratio software.* ***Result:*** *There were differences in MMP-8 expression based on observation time ($p<0.05$). MMP-8 expression in the 7^{th} day control group was lower than the 14^{th} day and MMP-8 expression in the 7^{th} day treatment group was higher than the 14^{th} day.* ***Conclusion:*** *Proanthocyanidin of cocoa pod rind extract (Theobroma cacao L.) can reduce MMP-8 expression in gingival tissue of periodontitis rats model.*

Keywords: *Periodontitis, MMP-8, Proanthocyanidin extract cocoa pod rind (Theobroma cacao L.)*

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INTRODUCTION

Periodontitis is an inflammation of the periodontal tissue affecting almost 75% of Indonesia's population of various age groups. Periodontitis caused by gram-negative bacteria is a continuation from gingivitis which will cause attachment loss and end in tooth loss[1].

Porphyromonas gingivalis is one of the proteolytic bacteria because the virulence factor of this bacterium contains protease enzymes, such as capsules, fimbriae, gingipains, and lipopolysaccharides. This protease enzyme is used by *P.gingivalis* bacteria to fight the host defense system and cause degradation of the structural proteins that make up the gingival and periodontal tissues, making it easier to invade deeper gingival tissue. Degradation of this protein will trigger damage and loss of attachment to the periodontal tissue, especially the gingiva[2].

The gingival tissue is composed of keratinized stratified squamous epithelium which functions to support the integrity of the gingival tissue. Gingival integrity is a form of the body's defense mechanism through physical defense in overcoming dangerous agents, including bacteria. The integrity of the gingival epithelium can be impaired or damaged by bacterial protease enzyme activity or the body's response activity in eliminating bacteria that cause periodontal disease, one of which is cytokine inflammation as a host response to *P.gingivalis* infection[2].

Colonization and infection of *P.gingivalis* will increase the production of proinflammatory cytokines, including matrix metalloproteinase-8 (MMP-8) which is a collagen-breaking enzyme in connective tissue and holds the greatest control of collagenase activity in the gingival tissue in the periodontitis[3]. MMP-8 as a biomarker of periodontal tissue inflammation is found in low levels because of the high levels of natural inhibitors of MMP-8 in healthy periodontal tissue and will increase if inflammation occurs[4].

In order to reduce the production of proinflammatory cytokines in periodontitis from excessive bacterial activity, the treatment that have an anti-inflammatory effect need to be made, one of the treatment comes from cocoa pod rind *(Theobroma cacao L.)* which is rich in polyphenol compounds and allegedly have these effects. The rind cocoa is very abundant in Jember as agricultural waste and has not been utilized properly. It contains water (85%), crude fiber (27%), and protein (8%). The polyphenol compounds including proanthocyanidin (58%), catechins (37%), and anthocyanins (4%)[5].

Proanthocyanidin is the most abundant polyphenol in the cocoa pod rind *(Theobroma cacao L.)* with utilization that is still not optimal. Previous studies have shown that 10% of proanthocyanidin in rind cocoa can reduce COX-2 levels in periodontitis, whereas COX-2 is related in the regulation of MMP-8 It is suspected that the decrease in COX-2 can reduce MMP-8 levels so that bacterial activity is also inhibited. Proanthocyanidin replaces an inhibitor of MMP-8 activity because of its ability to inhibit the secretion of MMP-8 by diverting the nuclear signal factor-kappaB (NF-κB)[6].

MATERIALS AND METHODS

This study is a type of experimental laboratory research with the post test only control group design. The code of ethics was obtained from the Health Research Ethics Commission (KEPK) at the Faculty of Dentistry, University of Jember with number 440 /UN25.8/ were observed on the 7th and 14th days. All rats were induced by *Porphyromonas gingivalis* (ATCC 33277) every 3 days for 14 days.

After that, the gingival margins of the mesial portion of the maxillary first molars of the right region were given placebo gel once a day for 7 and 14 days in the control group and were given a cocoa pod rind *(Theobroma cacao L.)* (100 mg/ml) extract once a day for 7 and 14 days

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in the treatment group. Then the gingival tissue was removed by cutting the maxillary region of the right region from the mesial portion of the first molar to distal the second or third molar after being anesthetized using ketamine anesthesia. Samples were put into formalin buffer for 24 hours so that the tissue were not damaged and then decalcified with 10% formic acid to make it soft. After that, the tissue samples were processing then coloring using IHC.

The research data were obtained from observations of histological preparations from each group. Analysis of MMP-8 expression in gingival tissue was done on the 7th and 14th days. Observations were carried out with a binocular microscope with the help of optilab with a magnification of 100X. Samples were observed for brownish color intensity arising from IHC staining and determined based on the percentage results that emerged from the Immunoratio software. The data obtained then analyzed using One-Way ANOVA test from SPSS.

Table 1. Calculation of MMP-8 expression in gingival tissue

Sample Groups	7th Day	14th Day	p
Control (K)	47.25±35.94	55.75±10.720	0.002
Treatment (P)	39.50±1.291	36.50±1.291	

KEPK / DL / 2019. The making of proantocyanidin cocoa pod rind extract took place in Phytochemical Laboratory of the Faculty of Pharmacy, Jember University, manufacture and rejuvenation of *P.gingivalis* bacterial suspension in the Laboratory of Microbiology, Faculty of Dentistry, University of Jember, maintenance and treatment of experimental animals in the Biomedical Laboratory of the Faculty of Dentistry, University of Jember and staining of Immunohistochemicals (IHC) in the Laboratory of Histology, Biomedics, Faculty of Dentistry, University of Jember.

The samples used were 16 male Wistar rats which were divided into two groups (control group and treatment group). Each group consisted of 8 rats which were divided into 2 sub-groups with each subgroup consisting of 4 male Wistar rats which The data shows the mean and standard deviation in each sample group.p is the result of the significance of the One- way ANOVA test.

The data obtained were tested for normality using Shapiro-Wilk and homogeneity tests using the Levene Test. After knowing the data are normally distributed and homogeneous, the data were analyzed using the One-way ANOVA test to find out whether or not there were significant differences regarding MMP-8 expressions in all groups. One- way ANOVA test results showed that there were differences in the mean expression of MMP-8 in the treatment and control groups (p <0.05) (Table 1).

Table 2. Summary of the Post Hoc Least Significant Difference (LSD) MMP-8 expression in each sample group

Groups	K7	K14	P7	P14
K7		0.058	0.080	0.021*
K14	0.058		0.002*	0.000*
P7	0.080	0.002*		0.473
P14	0.021*	0.000*	0.473	

Notes:
K7: 7th day control group K14: 14th day control group P7: 7th day treatment group P14: 14th day treatment group
(*): there are significant differences (p<0,05)

Furthermore, the data were tested to determine the average differences between the study groups using the Post Hoc Least Significant Difference (LSD) test. LSD test results showed that there were significant differences in MMP-8 expression between the 7th day control group and the 14th day treatment group and between the 14th day control group and the 14th day treatment group (p<0.05). While the expression of MMP-8 in the control group on the 7th day and the 14th day, the

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control group on the 7th day and the treatment group on the 7th day, the control group on the 14th day and the treatment group on the 7th day and the treatment groups on the 7th day and 14th day (P<0.05) (Tal

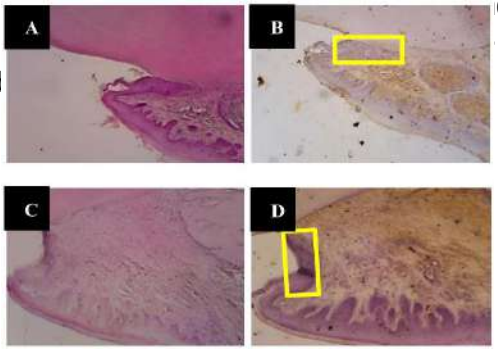

Figure 1. Histological pictures of rat gingival tissue of the 7th day control and treatment group (100X)
Notes:
Yellow squares indicate the epithelial tissue in which MMP-8 is expressed
A: Gingival tissue of the 7th day control group with HE staining (100X)
B: Gingival tissue of the 7th day control group with IHC staining (100X)
C: Gingival tissue of the 7th day treatment group with HE staining (100X)
D: Gingival tissue of the 7th day treatment group with IHC staining (100X)

Figure 1 are some histological pictures of periodontitis rat gingival tissue with HE and IHC staining of the 7th day control and treatment group. MMP-8 expressions were more visible on the 7th day control group (K) than the treatment group (P) because there were more cells expressing MMP-8 in the epithelial tissue in the yellow square from control group (K).

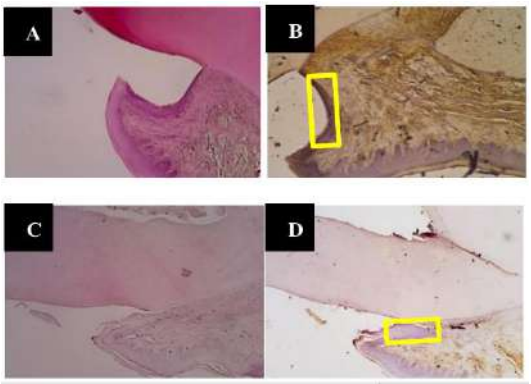

Figure 2. Histological pictures of rat gingival tissue of the 14th day control and treatment group (100X)
Notes:
Yellow squares indicate the epithelial tissue in which MMP-8 is expressed
A: Gingival tissue of the 14th day control group with HE staining (100X)
B: Gingival tissue of the 14th day control group with IHC staining (100X)
C: Gingival tissue of the 14th day treatment group with HE staining (100X)
D: Gingival tissue of the 14th day treatment group with IHC staining (100X)

Figure 2 are some histological pictures of periodontitis rat gingival tissue with HE and IHC staining of the 14th day control and treatment group. MMP-8 expressions were less visible on the 14th day treatment group (P) than the control group (K). This is indicated by the less number of cells expressing MMP-8 in the epithelial tissue in the yellow square from treatment group (P).

DISCUSSION

The results showed that there were differences in MMP-8 expression based on the time of observation in the control and treatment groups. MMP-8 expression on 7th day control group was lower than 14th day. This is probably caused by an inflammatory process in the gingival tissue due to the induction of *Porphyromonas gingivalis*. Inflammation due to *P.gingivalis* triggers collagen degradation involving the MMP-8 enzyme. MMP-8 activity will continue throughout the inflammatory process. The results of Ji (2015) showed that an increase in MMP-8 expression was in line with increased periodontal tissue damage in a rat model that had periodontitis for 14 days.

In the treatment group, MMP-8 expression on the 7th day was higher than the 14th day. It is possible that the administration of proanthocyanidin in cocoa od rind *(Theobroma cacao L.)* is able to inhibit the inflammatory process due to *P.gingivalis* induction through

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decreased MMP-8 activity. Inhibition of the inflammatory process will cause a decrease in the activity of enzymes involved in the inflammatory process, such as MMP-8 (Sorsa, 2016). The results of Lagha's research (2015) showed that the proanthocyanidin of cranberries was able to reduce MMP-8 expression so that there was no excessive tissue damage.

In addition, MMP-8 expression on the 7th day control group was higher than the 7th day treatment group, as well as on the 14th day. This is probably because the proanthocyanidin is able to inhibit the inflammatory process. Proanthocyanidin in grape seeds is known to have anti-inflammatory activity because it can inhibit the production of proinflammatory cytokines, one of which is MMP-8, so that there is no inflammatory process which can result in periodontal tissue damage[3,7,8].

Proanthocyanidin is the most polyphenol compound (58%) in the rind cocoa *(Theobroma cacao L.)* which is suspected to have anti-inflammatory activity. This is because proanthocyanidin is able to inhibit inflammation by inhibiting the activity of *P.gingivalis* virulence factors that invade the gingival tissue. This activity can reduce the production of proinflammatory cytokines, one of which is MMP-8. MMP-8 reduction will inhibit excessive tissue damage[3].

Porphyromonas gingivalis triggers inflammation through the activity of virulence factors that cause host immune deregulation. This process involves proinflammatory cytokines, one of which is MMP-8. MMP-8 is the result of activation of proMMP-8. *P.gingivalis* virulence factor causes MMP-8 production to increase, triggering degradation of type I and III collagen which is a key factor in excessive tissue damage in periodontitis[7].

This tissue damage process can be inhibited using anti-inflammatory drugs, such as non-steroidal anti-inflammatory drugs (NSAIDs). NSAIDs can prevent tissue damage by modulating the host immune response thereby inhibiting the inflammatory process. NSAIDs can inhibit proMMP-8 activation and reduce other proinflammatory cytokines, such as TNF-α and IL- 1β, by activating the NF-κB pathway. This pathway will inhibit MMP-8 production[9].
Proanthocyanidin as one of the herbal ingredients which is allegedly inhibit the inflammatory process through the NF-κB pathway. PAC resulted in inhibition of translocation of NF-kB as suggested by decreased protein expression of p65 in the nuclear fraction [10] Under normal circumstances, NF-κB binds to its inhibitor (IκB) as an inactive condition in the cytoplasm. NF-κB is then activated by several stimuli, such as TNF-α and IL-1β, resulting in an inflammatory process. When NF-κB is stimulated, IκB is phosphorylated by IκB Kinase (IKK) and is degraded. Degraded IκB will cause NF-κB to translocate from the cytoplasm to the nucleus to synthesize MMP-8 and cause excessive tissue damage[6].

Although in this study showed that the proanthocyanidin of cocoa pod rind extract is potent as an anti-inflammatory, but the results of this study still require further research, because the researchers only used a single dose and 2 periods of observation. Lack of observation time in the control group turn out insignificant results on 7th day and 14th day because the inflammatory process is still ongoing. The inflammatory process starts from injury to enter the proliferation phase on the 3rd day until the 21st day. In this phase, MMP-8 will be synthesized and will continue to increase until it reaches its peak on the 21st day then decreases until tissue recovery occurs. Therefore, administration of proanthocyanidin of cocoa pod rind extract *(Theobroma cacao L.)* can reduce MMP-8 expression during the proliferation phase so as to reduce excessive tissue damage[11].

CONCLUSION

Based on the research that has been done, it can be concluded that the proanthocyanidin extract of rind cocoa

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(Theobroma cacao L.) can give effect in the form of decreased MMP-8 expression in gingival tissue of periodontitis rats model.

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RESEARCH ARTICLE

The Effect of Purple Leaf Extract (Graptophyllum pictum L. Griff) to The Amount of Fibroblast in Gingiva Rat Wistar induced by Porphyromonas gingivalis

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ABSTRACT

Background: *The prevalence of periodontal disease ranks second in dental and oral diseases after caries. Periodontitis is inflammation with bacteria infecting the host and involving all parts of the periodontal tissue. If this condition is left untreated, it can lead to fibrosis and irreversible damage. Various types of periodontitis therapy have not been able to provide optimal results in healing periodontitis and that therapy can cause side effects. Because of this background, the researchers wanted to carry out research on alternative treatments for periodontitis with purple leaves as an anti-inflammatory with an indicator of an increase in the number of fibroblast cells in inflamed areas. The use of purple leaves has been used since ancient times for the treatment of wounds and inflammation.* ***Objective:*** *To determine the effect of purple leaf extract (EDU) on increasing the number of fibroblast cells in the gingiva of Wistar rats infected by Porphyromonas gingivalis (Pg).* ***Methods:*** *30 Wistar rats were divided into 5 groups, namely the normal group (KN), the control group Pg induced (K +) and the treatment group using EDU 2.5% (P1), EDU5% (P2), EDU10% (P3). All groups were induced by Pg except KN. EDU administration once a day for 7 days. On the 7th day the rats were decaputed and their gingivae were taken to make preparations and HE staining was carried out. Results readings in 3 different viewpoints were averaged and analyzed by one-way ANOVA.* ***Results:*** *The results showed that the K + group, 2.5% EDU, 5% EDU and 10% EDU groups increased the number of fibroblast significantly when compared to the Pg group ($p < 0.05$).* ***Conclusion:*** *EDU can increase the number of fibroblasts in Pg-induced mice*

Keywords: *Extract of Graptophyllum pictum L. Griff leaves, fibroblast, Porphyromonas gingivalis.*

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INTRODUCTION

Based on National Basic Health Research (RISKESDAS) in 2018, periodontal disease ranks second in dental and oral diseases suffered by many Indonesians with a prevalence of 74,1% [1]. Periodontitis is one of the periodontal diseases that can cause inflammation because bacteria infect the host and cause damage to all parts of the periodontal tissue. *Porphyromonas gingivalis* bacteria *(P. gingivalis)* is one of the microorganisms causing periodontitis.[2]

P. gingivalis bacteria invade the host and then damage the extracellular matrix, modulating both the inflammatory response and the immune response of the host.[3] At the time of inflammatory process occurs fibroblast cell production is increased compared to normal circumstances. Fibroblasts will respond to inflammation by proliferation of inflammatory areas. Inflammation that occurs for a long time and is not given therapy can cause irreversible damage. To prevent the severity of the disease can be done therapeutic treatment in patients with periodontal disease.[4] *P. gingivalis* bacteria invade the host and then damage the extracellular matrix, modulating both the inflammatory response and the immune response of the host.[3] At the time of inflammatory process occurs fibroblast cell production is increased compared to normal circumstances. Fibroblasts will respond to inflammation by proliferation of inflammatory areas. Inflammation that occurs for a long time and is not given therapy can cause irreversible damage. To prevent the severity of the disease can be done therapeutic treatment in patients with periodontal disease.[4]

One of the therapy periodontitis is the administration of medicines that are mouthwashes that are anti-inflammatory. Currently has been widely developed mouthwash with medicinal plant basic ingredients that are believed to have minimal efficacy and side effects. Mouthwash is a medicine that has a formula in the form of a solution and is generally diluted so that it can be used directly in the oral cavity.[5] Mouthwash containing benzydamine HCl and alcohol, can usually minimize inflammation, but the product has side effects such as numbness, burning sensation or stung in the oral cavity and vomiting nausea so that other alternatives are needed for the treatment of periodontitis that has lower side effects than the chemical mouthwash.[6]

One of the traditional medicinal plants that has been known to be beneficial for health is purple leaves *(Graptophyllum Pictum L. Griff*). Purple leaves are plants with purple leaves and are commonly found along the street or in people's yard because of their beauty. Purple leaf is one of 13 commodities developed by the Director General of Drug and Food Control (DITJEN POM) as the flagship medicinal plant.[7]

The active compounds contained in purple leaves *(Graptophyllum pictum L. Griff)* are flavonoid compounds, tannins, alkaloids, steroids, saponins, alcohol and calcium oxalate. The complex and diverse content causes purple leaves to have an anti-inflammatory effect. One of the content of purple leaves, namely flavonoids can inhibit the path of cyclooxygenase and lipooksigenase in inflammatory processes.[8] The inhibition is followed by the inhibition of inflammatory mediators so that the healing process of inflammatory tissue becomes faster which is characterized by a slowdown of inflammatory processes and acceleration towards the proliferation phase. Another content is alkaloids that work as anti-inflammatory by inhibiting the formation of prostaglandins [9]. The safety of purple leaves has been proven by some researchers and proven through toxicity tests conducted over 20 days that the administration of purple leaf extract is not toxic to the hematological profile of male white mice.[10]

Because of this background, researchers wanted to conduct research on alternative treatment of periodontitis with purple leaves that have lower side effects than chemical drugs. In addition, the use of purple leaves in the field of Dentistry is also still lacking so researchers want

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to prove whether purple leaf extract can work as an anti-inflammatory with indicators of increasing the number of fibroblast cells in the gingiva area that experience inflammation.

Some previous research that has been done kurniawati (2016), explained that purple leaf ethanol extract *(Graphtophyllum pictum L. Griff)* can affect the function of phagocyteocytes exposed Candida albicanss.[11] The study used several concentrations of purple leaf extract which is 2.5%, 5% and 10%. Researchers want to conduct research on whether purple leaves with such concentrations can work as an anti-inflammatory with indicators of increasing the number of fibroblast cells in areas that experience inflammation.

RESEARCH METHODS

This research is experimental laboratory type, with treatment in the form of induction porphyromonas gingivalis and administration of purple leaf extract (EDU). The research was conducted in November 2019 - January 2020. The research was conducted in several places, namely Medicinal Plant Education Tour (WETO) University of Jember to take samples of purple leaves, Center for Development Advanced Science and Technology (CDAST) University of Jember for the manufacture of purple leaf extract, Microbiology Laboratory Faculty of Dentistry University of Jember for the preparation of bacteria *P. gingivalis*, Biomedical Laboratory physiology section of the Faculty of Dentistry University of Jember for the treatment of mice wistar, Laboratory Histology Faculty of Dentistry University of Jember to examine the number of fibroblast cells. The purple leaf used is *Graptophyllum pictum L. Griff* which has been identified by the Indonesian Institute of Sciences (LIPI) through Identification Certificate No. 1110/IPH.06/HM/X/2019 dated October 22, 2019. There were 30 samples each divided into 5 groups namely: normal control group (KN), positive control group (K+) of *P. gingivalis*-induced group (Pg) and treatment group (P1, P2, P3) induced by Pg bacteria only and irrigation treatment group of purple leaf extract concentration of 2.5%, 5% and 10%. This research procedure has been approved by the Research Ethics Commission of the Faculty of Medicine, University of Jember with the number 633/UN25.8/KEPK/DL/2019.

The research procedure began with the manufacture of purple leaf extract with maceration technique using ethanol solvent 96% and diluted resulting in concentrations of 2.5%, 5% and 10%. Furthermore, the adaptation of male wistar mice for 7 days. After the mice were adapted, followed by the induction of pg bacteria for 2 weeks with the frequency of injection 1 week 3 times so as to create a condition of chronic periodontitis. Induction was performed using a tuberculine syringe with a needle size of 30G on the M1 teeth of the left lower jaw in the entire research group. After clinical symptoms of periodontitis were seen in rats, it was continued at the stage of treatment.

The stage of treatment in pgingivalis-induced groups only (K+) is the administration of aquades, in areas with chronic periodontitis. The treatment group was given EDU irrigation concentrations of 2.5%, 5% and 10% as much as 0.27ml. Furthermore, tissue preparation was carried out using ketamine in the entire treatment group until all the mice died which was characterized by a lack of reflexes in the eyes when given light and a heartbeat that was no longer beating. The next procedure is tissue cutting performed on the left regio of the lower jaw ranging from incisiv teeth 1 to molar 2 along with gingival tissue. Cutting on the network is done in the direction of buko – lingual. Furthermore, the stage of making histology preparations and then done painting HE and observation. Observation and calculation of the number of fibroblast cells is done after histological preparations are formed and carried out using a binocular microscope magnification of 400x. The number of fibroblasts was observed and recorded by calculating the fibroblasts found in three fields of view in each preparation and examined with three different people.

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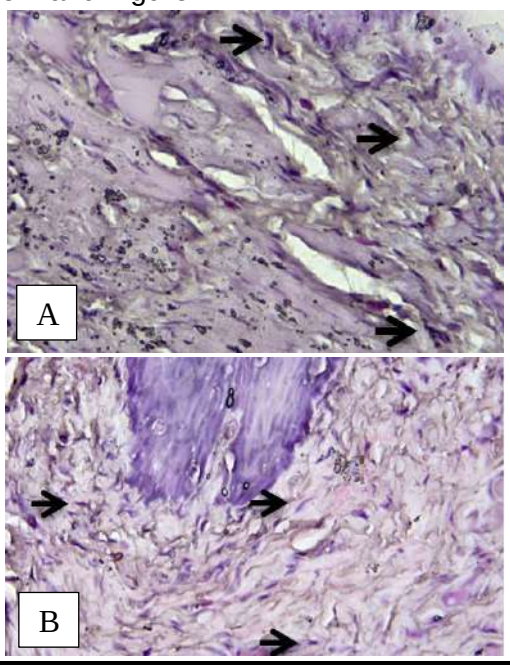
After obtaining the results of the research data, the data is then analyzed using SPSS 16 for windows software. Data is first conducted normality test using Saphiro Wilk test to determine whether the data is normally distributed or not. Furthermore, a variant homogeneity test was conducted to test the sample variation using Levene test. After obtaining normal and homogeneous distributed data, it is continued with one way ANOVA parametric test with a confidence level of 95% (α = 0.05). Furthermore, the LSD (Least Significant Difference) test was conducted to find out which group pairs had meaningful differences.

RESEARCH RESULTS

The observations showed semi-ovoid and ovoid fibroblast cells and dark-colored cell nuclei (Figure 1). The results of the calculation showed the number of fibroblast cells in the KP group of 10% namely fibroblast cells that were treated in the form of irrigation of purple leaf extract concentration of 10% had the highest number of cells compared to the control group and other treatment groups. While the normal group has the lowest number of fibroblast cells. The calculation results of each group can be seen in Figure 1. The average number of wistar rat fibroblast cells in each group is presented in Table 1 and Figure 2.



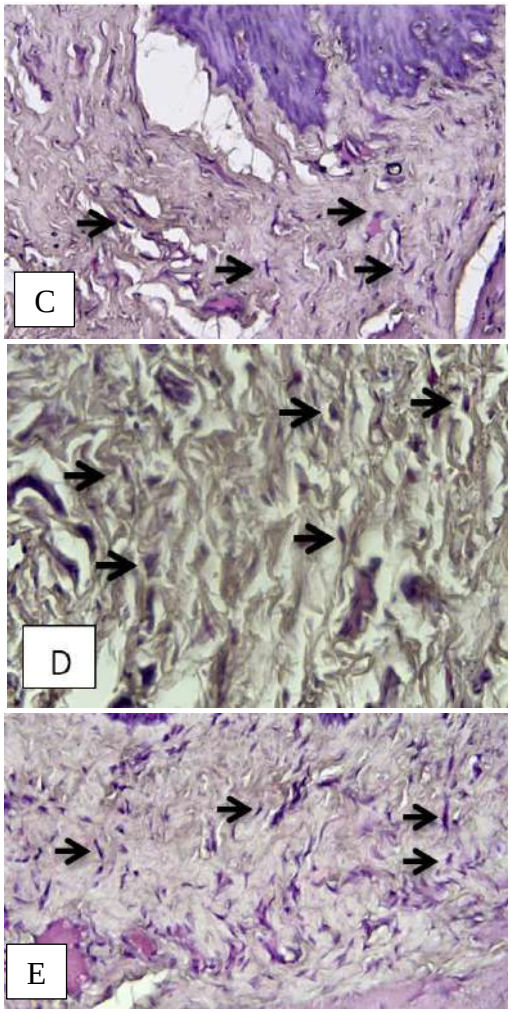


Figure 1. Overview of fibroblast cells in each research group

Description: A. Normal Group, B. Positive Control Group, C. Purple Leaf Extract Treatment Group 2.5%, D. Purple Leaf Extract Treatment Group 5% and E. Purple Leaf Extract Treatment Group 10%. The black arrow indicates fibroblast cells (400x magnification).

Table 1. Average number of fibroblast cells

Research Group	Averange	Std.Deviation
KK(-)	128, 50	12, 069
KK (+)	145, 75	7, 890
KP 2,5%	152, 00	2, 380
KP 5 %	167, 25	8, 421
KP10%	208, 50	18, 120

Description :
Std.Deviation : Standart deviation
KK (-) : Normal Group
K (+) : Positive Control Group
KP 2,5% : Treatment Group 2.5%

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KP 5% : Treatment Group 5%
KP 10% : Treatment Group 10%

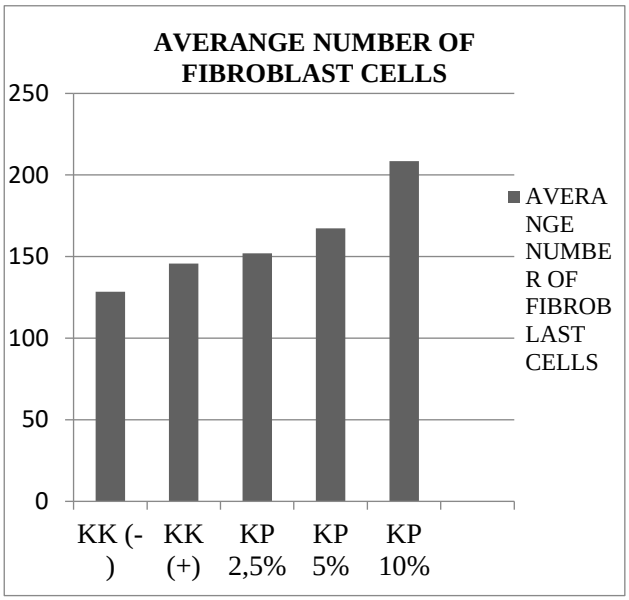

Figure 2. Diagram of the average number of fibroblast cells

Description :
KK (-) : Normal Group
K (+) : Positive Control Group
KP 2,5% : Treatment Group 2.5%
KP 5% : Treatment Group 5%
KP 10% : Treatment Group 10%

One Way ANOVA test results obtained a value smaller than 0.05 ($p<0.05$) which means there are significant differences in the entire research group. This suggests that there are significant differences in the number of fibroblast cells in the entire research group. The Least Significant Difference test showed that there were significant differences in the entire research group, except between the purple leaf treatment group concentration of 2.5% and the positive control group, the purple leaf treatment group concentration of 2.5% and the purple leaf treatment group concentration of 5%.

DISCUSSION

Purple leaves are chosen for their ability to affect the function of inflammatory cells. One of the anti-inflammatory effects is the inhibition of inflammatory processes and is expected to accelerate the proliferation phase characterized by an increase in the number of fibroblasts. Based on the results of the One Way ANOVA test, there were significant differences in the entire research group, with the average number of fibroblast cells in the treatment group 2.5%, 5% and 10% greater than the control group. The results showed that the administration of purple leaf extract can increase the number of fibroblast cells, which is suspected because there are various active compounds in purple leaves that are anti-inflammatory. This anti-inflammatory effect will speed up the healing process so that it can be interpreted that purple leaf extract has the potential to be used as an alternative treatment for periodontitis disease. This is in accordance with Rustini's research (2017) which states that purple leaves have an active compound that acts as an anti-inflammatory, namely flavonoid compounds, tannins, alkaloids, sitosterol, glycosides and saponins that are useful as anti-inflammatory.[12] In addition, Andiyani et al (2015) reported that open wounds on the skin of mice given purple leaf extract dried up faster than the comparison group. Clinically dry wounds indicate the healing process of wounds.[13] The process of healing wounds on the skin is not much different from that that occurs in the mucosa of the oral cavity.

The largest compound content in purple leaves is alkaloids as much as 65,480 mg / g and flavonoids as much as 54,043 mg / g. Alkaloids act as anti-inflammatories by suppressing the release of inflammatory mediators secreted by *P. gingivalis* bacteria through LPS. Other studies have stated that alkaloids act as anti-inflammatory by inhibiting the formation of prostaglandins, suppressing the release of histamine by mast cells, reducing the secretion of IL-1 by monocytes and PAF in platelets.[14] Barriers to the formation of prostaglandins will thus inhibit the work of arachidonic acid.[15] Arachidonic acid is the initial stage of inflammatory occurrence that will release into the tissues. Arachidonic acid will be metabolized through two pathways namely COX and LOX pathways that will produce inflammatory products, one of which is prostaglandins.[16]

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When prostaglandins are inhibited then fibroblasts that were originally low in proliferation during the inflammatory process, there will be an increase in the number. This is thought to occur because flavonoids inhibit prostaglandins which can cause macrophages to produce growth factors such as PDGF, FGF, TGF-β that will induce fibroblasts to proliferate on inflammatory areas. Next, fibroblasts will produce an extracellular matrix, primary collagen, and fibronectin that will close the wound.[13]

The results of the LSD test explained that the difference in the number of fibroblast cells in the normal group compared to the positive control group did not have a meaningful difference, which means that the two groups had the same effect on the healing process of inflammation. This is because the research was conducted for 7 days and the repair process conducted by fibroblast cells in the control group has not worked to the maximum so that the number is not much different from the normal group. It is shown from the results of table 1 the number of differences between fibroblast cell KN and KK (+) is not far linked, but the number of fibroblast cells in KK (+) is more than KN because the repair process performed by fibroblast cells will occur when the condition of inflammation.

P. gingivalis bacteria will secrete lipopolisakarida (LPS) and LPS will produce glycoprotein such as chemokin and interleukin which can cause inflammation [17]. When inflammation occurs, inflammatory cells such as macrophages will migrate to the inflammatory area to facilitate bacterialtosis and repair. This is in accordance with Rustiasari's statement (2017) which states that macrophages play a role in the process of repairing damaged tissues by secreting various pro-regeneration molecules, namely growth factors that can increase the proliferation of fibroblast cells [18]. So it can be said that even in the kk(+) group experienced inflammation and was not treated, fibroblast cells remained higher than normal due to inflammatory processes.

LSD test results showed that KP 2.5%, 5% and 10% showed meaningless differences. This means that the three EDU concentrations have the same effectiveness in increasing the number of fibroblast cells. It is thought that the content and composition of purple leaf extract such as flavonoids, alkaloids, tannins etc., in addition to having anti-inflammatory effect also has antibacterial effect. EDU is known to effectively inhibit the adhesion of *P. gingivalis* in neutrophils.[19] Indirect effects as an antibacterial against this *P. gingivalis*, causing the amount of *P. gingivalis* in gingival sulcus to decrease. A decrease in the number of bacteria will inhibit the occurrence of inflammatory processes that can lead to an increased number of fibroblasts.

The increase in the number of fibroblast cells in this study is thought to be due to the presence of flavonoid content in purple leaves. Purple leaves can increase immunity (immunomodulatory) and decrease existing inflammation. Increased immunity will stimulate the increase of fibroblast growth factor that plays a role in the proliferation of fibroblast cells, so the number of fibroblast cells will increase as well.[20]

KP 2.5% compared to KP 5% has no meaningful difference. In Table 1 can be seen from the average number of fibroblast cells between the two groups is not far apart so it can be said that purple leaf extract concentration of 2.5% and purple leaf extract concentration of 5% has wound healing and anti-inflammatory activities that do not differ significantly. This is because the difference between the two concentrations is not far away. This is reinforced by the research of Putri, et al (2017) reported that the difference in concentration is not too much causing results that do not different significantly to the number of fibroblast cells.[21] In addition, it can also be caused by a solution whose difference in concentration is not far apart means relatively diluted, so the molecules of chemical compounds are large and the penetration of extracts into the tissue becomes difficult. This can affect the reepitelization process and the number of fibroblasts in the wound healing process.

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KP 2.5% and KP 5% compared to KP 10% have a meaningful difference. It can be seen in Table 1 that the average number of fibroblast cells of the purple leaf extract group is 10% higher than the purple leaf extract group 2.5% and purple leaf extract 5%. This is thought to be because the concentration of purple leaf extract concentration of 10% contains the most active compounds compared to purple leaf extract concentrations of 2.5% and 5%. Increased active substances led to increased proliferation of fibroblast cells in the higher concentration treatment group.[22] Fibroblasts more actively synthesize extracellular matrix components in response to the presence of inflammation by proliferation and increasing the activity of fibrogenesis so that fibroblasts can be said to be the main agent in wound healing.[22] Based on this research, it can be said that along with the increase in concentration, the healing effect is further strengthened by the research of Atmajaya, et al (2019) which reports that the higher the concentration of cork fish extract, the higher the effect on wound healing and the number of fibroblast cells.[23]

EDU concentrations of 2.5%, 5% and 10% can increase the number of fibroblasts. The increase in fibroblast cells in this study cannot be said to be fibrosis although the number of fibroblast cells in purple leaf KP concentration is 10% much higher than the number of fibroblast cells under normal circumstances. This reason is because the proliferation phase will occur for 3-14 after inflammation occurs and the peak occurs on the 7th day. After the proliferation phase ends, it will be continued by the maturation phase. The maturation phase begins on the 21st day after inflammation occurs. In that phase there is a decrease in fibroblast cells and excessive collagen produced by fibroblast cells will be gradually degraded by the enzyme collagenase so that there will be a balance between the process of cell proliferation, collagen synthesis and collagen degradation and extracellular matrix.[24] It also caused a deficiency in this study because the research time is less long so that the healing process has not been seen until the number of fibroblast cells returns under normal conditions.

CONCLUSION

Based on the results of studies that have been done can be concluded that purple leaf extract can increase the amount of fibroblasts in wistar rat gingiva induced by *Porphyromonas gingivalis* bacteria.

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CASE REPORT

The Management of Transalevolar Surgery Teeth with Pulpal Polyps Condition

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ABSTRACT

Background: *The failure of intra-alveolar tooth extraction in cases of tooth extraction with complications is generally resolved by performing transalveolar tooth extraction. The open method of tooth extraction involves surgery by cutting the tooth or bone. Flap creation and partial removal of alveolar bone, refinement of alveolar bone, curettage, and suturing are the principles of this extraction method. The planning of the stages of the transalveolar extraction method must be made as carefully as possible to avoid complications of tooth extraction. Tooth extraction treatment in dental practice can be carried out due to various causes, including caries. Pulp polyps or also known as chronic hyperplastic pulpitis is another form of irreversible pulpitis in the chronically inflamed pulp so that the pulp tissue appears to the occlusal surface. Treatment in cases of pulp polyps varies widely depending on the remaining tooth crown wall. The usual treatments for polyps pulp patients are root canal treatment (RCT), pulpotomy to extraction. The transalveolar extraction method is performed by first taking a portion of the bone supporting the tooth.* ***Objective:*** *Knowing the treatment management of polyp pulp extraction with transalveolar surgery methods, indications and contraindications for transalveolar surgery and knowing the correct medical selection in cases of transalveolar surgical extraction of teeth with polyp pulp.* ***Discussion:*** *Chronic hyperplastic pulpitis or pulp polyp is a pathological condition that attacks vital pulp tissue so that it experiences a chronic inflammation as a defense response from the body to the pulp tissue against bacterial infection. The term use of the term chronic hyperplastic pulpitis occurs due to granulation of the pulp tissue covered with epithelial tissue due to chronic infection. The management of cases of pulp polyps for which restoration is no longer possible is to remove them. Extraction is the last step that can be done if restoration measures do not eliminate the source of infection. The transalveolar extraction procedure of the teeth located in the mandible is preceded by asepsis and anesthesia and removal of the polypoid tissue.* ***Conclusion:*** *In several cases of brittle teeth, extraction by intraalveolar method often failed so that it needs to be extracted by transalveolar method. Management of extraction with polyp pulp includes removal of the polypoid tissue first before extraction. The administration of medical therapy with the antibiotic amoxicillin 500 mg for 5 days, paracetamol for 5 days and dexamethasone for 3 days can control the patient's pain and help the healing process quickly.*

Keywords: *pulp polyps, transalveolar,pulpitis,teeth surgery*

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INTRODUCTION

Tooth extraction treatment in dental practice can be carried out due to various causes, including caries. Caries is a hard tissue disease of teeth that can be started from damage to the tooth surface tissue (pit, fissure and interproximal) which extends to the dentin and pulp. Infection of the tooth nerve tissue can cause pulpitis or inflammation of the pulp tissue. Pulp tissue infection can extend to the periapical area through the apical foramen and cause lesions in the periapical area. Untreated caries can result in loss of tooth structure such as crowns, leaving a residual root which is often referred to as the retained dental root. The dentical radicals are usually asymptomatic but in some cases can cause acute exacerbations due to secondary infection resulting in pain. Some of the periapical lesions that often occur include, periapical granuloma, radicular cysts and pulp polyps.[1] Pulp polyps or also known as chronic hyperplastic pulpitis is another form of irreversible pulpitis in chronically inflamed pulp so that the pulp tissue appears to the occlusal surface. Pulp polyps are granulation tissue consisting of many fibers and connective tissue. A person with pulp polyps usually presents with clinical signs such as spontaneous pain and persistent pain on thermal stimuli. This sensitive condition to thermal stimuli is due to the condition of the polyp pulp is still vital and there are many nerve fibers and blood vessels so that the pulp still responds well to the stimulus.2

Treatment in cases of pulp polyps varies widely depending on the remaining tooth crown wall. The usual treatments for root polyps pulp patients are canal treatment (RCT), pulpotomy to extraction. Various treatments can be performed in cases of pulp polyps, but they are carried out based on the operator / dentist's consideration in determining the prognosis of the case. RCT therapy can be performed in cases of pulp polyps either with periapical lesions or without periapical lesions. In addition, RCT therapy can be performed if the remaining wall is still strong to hold the restorative material. Pulpotomy treatment in cases of pulp polyps is usually performed on the teeth of children in cases where the seeds of the adult replacement teeth are still growing. Pulpotomy is the most commonly used pulp treatment technique for the treatment of extensive caries but without pathological conditions in the radicular area of primary teeth. Pulpotomy consists of removing the coronal pulp and repairing the radicular pulp with medicaments.[3] Extraction treatment is the final therapy that can be done if the tooth is untenable. Tooth extraction is one of the therapies in dentistry that helps to remove the source of infection. However, improper extraction management can lead to failure so that it can become a secondary infection, causing alveolar bone damage and psychological trauma. In some cases, especially the extraction by the intra alveolar method, it often fails so that it needs to be extracted by the transalveolar method. The transalveolar extraction method is performed by first taking a portion of the bone supporting the tooth. Apart from teeth with polypous pulp with brittle crown walls, this method is also used in cases such as: teeth that have hypersementosis or ankylosis, teeth that are germinated or brittle teeth that cannot be held with forceps or removed with a bein, especially the remains. roots associated with sinus maxillaris.[4]

Oral lesions can be a cause of systemic disease. However, the resulting infection can also be caused by local factors such as trauma. There are many traumas that can cause ulcers, namely mechanical trauma such as tooth malposition that causes traumatic ulcers, overhanging restoration, or biting of mucosal tissue due to sharp tooth fragments. Tooth extraction is one of the treatments in oral surgery in the field of dentistry which involves the extraction of teeth in the tooth socket in the alveolar bone. Tooth extraction should remove all parts of the tooth either the crown or the root of the tooth with minimal trauma and pain. All tooth extraction must be preceded by local anesthesia, so that it can make the patient feel comfortable during the extraction process. Tooth

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extraction is indicated as the preferred measure to prevent more widespread pathological conditions.5 Indications for tooth extraction include caries that leave roots, pulp necrosis that is impossible for root canal treatment, periodontal disease that causes loose teeth, extraction to support successful orthodontic treatment. , malposition of the teeth resulting in lession in table below :

Table 1. Difference between gingival polyp and pulp polyp.[12]

Gingival polyp	Pulpa polyp
The color same with the other tissue	The color is lighter than the surrounding tissue because it contains blood capillaries
Flat surface	Uneven surface
On vital teeth (proximal sides) or non vital teeth (on furcation).	On vital teeth
Derived from the gingiva and cavity of class 1 or 2 GV Black	Derived from the cavity pulp and Cavity Class 1 or GV Black

The tools used in transalveolar surgical extraction include mouth glasses, dental tweezers, sonde, cotton sticks, disposable injection syringes, gloves, masks, lower crown pliers, lower radix pliers, high speed, fissure burs, alveolar bur, bein and crayer. The materials used for transalveolar surgical extraction of teeth are anaesthetic agents, syringes, tampons, antiseptic solutions (10% povidone iodine solution) and alcohol. The management of cases of pulp polyps for which restoration is no longer possible is to remove them. Extraction is the last step that can be done if restoration measures do not eliminate the source of infection. The transalveolar extraction procedure of the teeth located in the mandible is preceded by asepsis and anesthesia and removal of the polypoid tissue. Asepsis is performed using new povidone iodine followed by anesthesia using Fisher's block technique because it is more effective and has a long duration so that the operator is more comfortable working. The work areas anesthetized in the use of Fisher's block technique are the mandibular half quadrant, buccal mucoperiosteum and mucous membranes at the injection site, the anterior two thirds of the tongue and floor of the mouth, lingual soft tissue and periosteum, mandibular body and the underside of the ramus and the skin over the zygoma, the posterior cheek and the temporal region. The initial phase in the treatment of transalveolar extraction with pulp polyps is to perform asepsis and anesthesia first on the work area using Fisher's block anesthesia technique and intrapulpal anesthesia technique. After the pulp does not respond to stimulation, removal of the polypoid tissue can begin. The removal of the polypoid tissue is intended so that the tooth extraction process is not hampered by the tissue that has pulp polyps. Tissue retrieval can be performed using surgical scissors, periodontal curette and excavator. Bleeding in the polyp can be controlled by compression using a tampon soaked in epinephrine. Epinephrine has a vasoconstrictor effect so that it can cause narrowing of peripheral blood vessels and control the rate of seepage in the blood. Making an incision in the maxilla is easier to apply because there is less risk of damage this is because in the maxilla there are no large blood vessels or nerves passing through one tooth. The standard incision procedure should be modified according to cases where fistulas, incision wounds, damage to mucous membranes, scar tissue and incisions for unoperated teeth should be considered.[12]

The removal of the polyp pulp should be thorough in one visit. Management of teeth with root residual conditions must pay attention to the possibility of periapical abnormalities that occur in the teeth. The buried root will be easier to extract by reducing the bone so that it can minimize the occurrence of trauma. The transalveolar technique extraction of the buried roots minimizes the occurrence of fracture of the teeth, more severe damage to the alveolar bone

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and gingiva.[13] Multiple transalveolar surgery requires the flap method. The flap is divided into 2 according to its thickness, namely the partial thickness flap and full thickness methods. The full thickness flap consists of the gingival, mucosa, submucosa, and periosteum. This flap is made by separating the soft tissue from the bone with a blunt cut. The flap opening technique can be performed by making an internal bevel incision, from near the edge of the gingiva to the top of the alveolar bone, keeping the gingiva keratinized as much as possible. No.11, 12b, 15 or 15c blades are commonly used to make this initial incision. No.11 or 15c blades with modified shafts can be well used to make incisions in the lingual or palatal area. This initial incision should be extended around the neck of the tooth and the interproximal area to maintain the height of the interdental papilla tissue for suturing, after which the tissue is separated from the bone by a periosteal elevator (rasparatorium) or chisel (blunt dissection), so that the flap can be opened and easy to move, and provides adequate access to the underlying structures, such as the crest of the bone, bone defect area, necrotic cementum.[14]

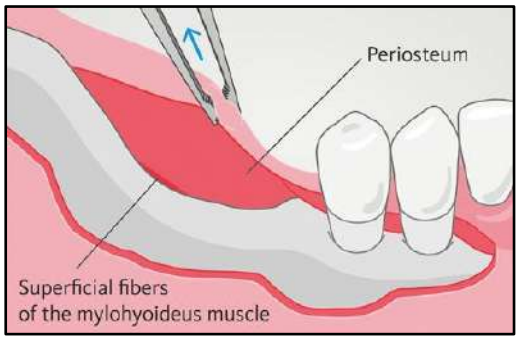

Figure 1. Mukoperiosteal.[14]

Bone reduction was performed using a tapered bur using water. Water can help cool the affected bone. In addition, bone removal is not done with a high speed bur because it can cause excessive heat, which can lead to hard tissue necrosis. The next step is to remove the bone that covers the dental crown and the distal part, then the bone in the buccal part of the tooth crown is also removed to the cervical part, remove the bone that covers the tooth crown and the distal part, then the bone in the buccal part of the tooth crown is also removed to the cervical part. The tooth was cut vertically using a tapered bur, separating the crown to the bifurcation. After the tooth has been separated into mesial and distal sections, make a bur notch in the cervical region of the distal tooth, then using a cryer to lift the distal tooth out of the socket.[13] The next step is to debridement the work area with physiological solutions and and povidone iodine. suturing the buccal flap area. The technique is performed by suturing using simple interrupted methods. After the procedure, the patient was given analgesic medication in the form of paracetamol 500 mg for 3 days and amoxicillin 500 mg for 5 days and dexamethasone 0.5 mg 3 days 2 times a day and paracetamol 500 mg for 5 days 3 times a day. Further control patients 1 week later if needed.[14]

CASE REPORT

A 31-year-old female patient came to want to remove the left lower back tooth with a large cavity and visible tissue that protruded above it, and did not feel pain. Clinically, it was seen that tooth 37 was left apical 1/3 crown and there was a polyp pulp in the occlusal part. Percussion (-), palpation (-), vitality (+), mobility degree 1. Blood pressure 120/80 mmHg, respiration 20x / minute, pulse 60x / minute and temperature 36 degrees Celsius. The results of periapical radiographs appear radiolucent in the crown area, it appears that the bicurcation has not been separated so that the assessment in this case is chronic hyperplastic necrosis (K04.0) with a planned extraction treatment with transalveolar surgery.

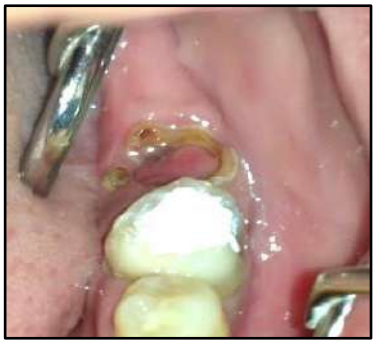

Figure 2. Clinical Case

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The case in this report was diagnosed as chronic hyperplastic pulpitis (K04.0). The differential diagnosis in this case is the gingival polyp that can be pushed aside because it refers to the difference between the polyp and gingival polyp in Table 1. A treatment plan is needed to determine what stages must be done for hard tissue surgery. Preoperative examination of the patient's medical condition and social history will help ensure that there are no contraindications for extraction. Intra oral radiographs with periapical techniques can be performed to see the condition of the roots and the direction of the roots. Patients need to sign informed consent or action consent to protect patients from arbitrary actions and protect doctors from post-treatment demands. Medical therapy ibuprofen 400 mg and 1000 mg paracetamol given to patients before the procedure. If there were signs of infection prior to the extraction procedure, 500mg of amoxicillin was also used before starting the extraction procedure.

Asepsis action on the work area is absolutely necessary before administering local anesthesia. Administration of anesthetic agents to the buccal and lingual aspects of the teeth using Fisher's block anesthesia technique by anesthetizing the mandibular buccal and lingual areas, if necessary intra-pulpal anesthesia for removal of the polyp tissue using tissue scissors. Control bleeding by using cotton swabs soaked in adrenaline solution. Making a flap to get a good field of view. In this case, the full thickness method was used with a triangular flap model so that the bone was seen properly and could control the bleeding maximally.

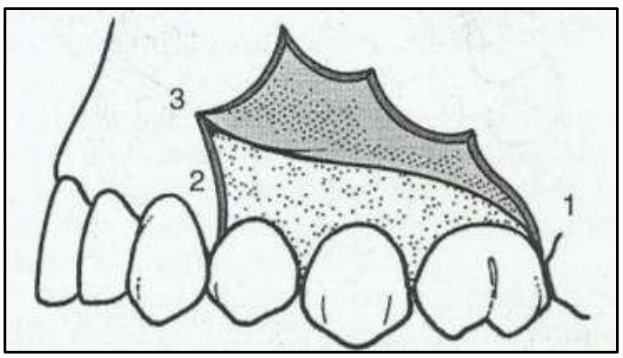

Figure 3. Triangular *flap*[5]

Drilling the bones using a bone bur until the root of the tooth is visible. Cutting teeth using a large bur or elevator to cut the tooth into 2 parts in the bifurcation area. Separation of the periodontal ligament using a periotome device that surrounds the root of the tooth, if there is no periotome, you can use an excavator to separate the periodontal ligament. Remove each separate root with a luxator. Root pliers may be used to remove each root from the socket. Debridement of the work area with physiological solutions and povidone iodine then suturing the buccal flap area. The suturing technique uses simple interrupted methods. Prescribing analgesics and antibiotics amoxicillin 500 mg for 5 days and dexamethasone 0.5 mg 3 days 2 times a day and paracetamol 500 mg for 5 days 3 times a day. Further control patients 1 week later if needed.

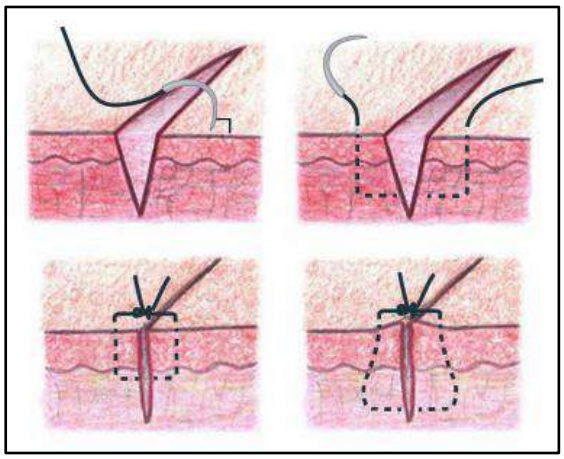

Figure 5. Simple interrupted suture.[16]

The treatment for this case is surgery with a transalveolar technique with removal of the polypoid tissue in the pulp polyp lesion. Polypoid tissue removal was initiated using sterile tissue scissors under local and intra pulpal anesthesia. Local anesthesia uses Fisher's block technique to anesthetize the inferior alveolaris, N. Buccalis longus and N. Lingualis, while the intra pulpal technique is used to anesthetize the pulp tissue so that the patient is more comfortable in the procedure. The removal of polypoid tissue is prone to bleeding because the polyp tissue lesion is rich in blood vessels, so that the action of stopping bleeding in the

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wound by pressing the open part with gauze soaked with epinephrine to control the bleeding is absolutely necessary. The transalveolar technique with the separation method was chosen with consideration of the hard tissue that was already brittle and prone to fractures. To avoid this, an open flap was performed using the triangular flap technique to open bone tissue to visually widen the work area. Medical administration after surgery using antibiotics, analgesics and corticosteroids for swelling control.[13] Chronic hyperplastic pulpitis or pulp polyps is a pathological condition that attacks vital pulp tissue so that it experiences a chronic inflammation as a defense response from the body to the pulp tissue against bacterial infection. The infected pulp forms granulation tissue where this tissue is only in young infected pulp with large cavities. The condition of good pulp vascularity makes the granulation process more developed than pulps with poor vascularity. The term use of the term chronic hyperplastic pulpitis occurs due to granulation of the pulp tissue covered with epithelial tissue due to chronic infection. Pulp polyps usually occur in patients who are still adolescents, this is because the pulp in adolescent patients has good blood vessels. Perforation in the roof of the pulp causes the opening of the pulp tissue and the entry of bacteria which can cause chronic inflammation. [2]

The pathophysiology of the pulp polyp is starting from the opening of the pulp chamber due to chronic and progressive caries which causes the tooth to become non-vital. The open pulp chamber results in tissue proliferation resulting in pulp cell activation which is protected by epithelial cells that grow to cover the surface and form inflamed lobes. Pulp polyps often occur in large open cavities, young, resistant pulp and chronic low-level stimuli, so mechanical irritation caused by chewing and bacterial infection are often the main causes of pulp polyps. Pulp polyps can be caused by mechanical irritation and invasion of bacteria into the pulp chamber, chronic caries, trauma to the tooth resulting in tooth fracture and secondary caries at the edge of the restorative material. which is rich in blood vessels. This granulating blood vessel will be covered with epithelium. The distinctive red color of the polyp pulp is due to the proliferation of blood vessels. This causes the polyp to bleed easily. Polyps are generally asymptomatic but pain can arise due to the mastication process, heat and electrical stimulation.[1] Clinically, the pulp polyp is seen as a mass in the tooth cavity resembling pink to reddish gingiva, in the form of lobes, single and multiple lesions and protruding from the pulp chamber, including open cavities in posterior teeth that have long experienced deep and chronic caries.[11]

DISCUSSION

The pulp polyps in this case report were caused by a chronic carious condition. The chronic condition makes the pulp tissue create a defense that is to form polypoid tissue. The choice of treatment for this patient includes removal of the polypoid tissue with tissue scissors. Control bleeding during the extraction process and so that the blood in the polyp is not too much and the patient is comfortable in the process of taking the polyp from. Pulp polyps bleed very easily and feel pain when exposed, so removal of polyp tissue before tooth extraction is highly recommended.[11] The condition of the teeth cannot be treated with root canals or direct or indirect restorations, so tooth extraction must be carried out. The absence of support from the hard tissue of the tooth which allows for retention of the restorative material is the main cause of extraction in patients with pulp polyps. Often the absence of a supporting wall on the hard tooth tissue becomes a problem in the extraction process. The process of extraction of posterior teeth without surgery relies on bifurcated tissue to be the support when the extraction process is carried out, but in the case of polyps, usually the hard tissue of the teeth is already brittle so that it is prone to fracture.[17] Surgical extraction of teeth and making flaps has a function to broaden the operator's perspective in the operation process. [17] Flap reflexology and

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bone reduction can result in trauma to both soft and hard tissues leading to postoperative complications such as pain, swelling and trismus. Several clinical studies on treatments that can reduce complications after dental surgery have been conducted. Among them, Dexamethasone and Methylprednisolone are examples of corticosteroids that are often used in dentoalveolar surgery because they have a pure glucocoticoid effect (they do not have a mineralocorticoid effect). The ability of glucocorticoids to suppress the inflammatory response is well known.[18]

Glucocorticoids can increase the release of multinucleated leukocytes from the bone marrow, thereby increasing the number of leukocytes in the bloodstream. Glucocorticoids can also inhibit the accumulation of leukocytes at the site of inflammation causing substances involved in the inflammatory response such as prostaglandins to be released from leukocytes. Glucocorticoids can inhibit fibroblast proliferation, such as the production of collagen and fibronectin. This combination is responsible for the healing of difficult wounds, increased susceptibility to infection and the response to typical inflammation is reduced by excess.[9] Use of oral antibiotics is indispensable to control post-extraction infection by surgery. The antibiotics most often used in dentistry are the penicillin class. Penicillin is still the gold standard in treating dental infections. Among the penicillin group, penicillin V, amoxicillin, and amoxicillin and clavulanic acid have been recommended to treat odontogenic infections and studies have shown that there are no differences in the clinical results of using the three types of antibiotics. the use of antibiotics should be stopped when the immune system has been able to control the infection.[18] The use of antibiotics for 2-3 days has been recommended. Studies have shown that the patient's condition improves after 2-3 days of antibiotic use. The Center for Diseases Control and Prevention (CDC) recommends the shortest possible use of antibiotics, which is 1-3 days after clinical signs and symptoms disappear. Therefore, in general, antibiotic doses are given in dentistry for a duration of five days. Prolonged use of antibiotics can cause damage to the body's normal flora. The use of antibiotics for more than 21 days is also suspected to cause antibiotic resistance.[19]

CONCLUSION

Tooth extraction with polypoid tissue can use transalveolar techniques to avoid complications of extraction. Extraction by the intraalveolar method often fails so that it needs to be extracted by the transalveolar method. Management of extraction with polyp pulp includes removal of the polypoid tissue first before extraction. So that patients are more comfortable in the process of removing hard tissue. Making a full thickness flap with a triangular model is absolutely necessary before the teeth are separated. The flap functions so that the bones are well visible and can control bleeding optimally.

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