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Effectiveness of Acemannan Hydrogel Administration at Concentrations of 25%, 50%, 75% Against Decrease in the Number of Macrophages in Periodontitis Rats with Diabetes Mellitus

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Abstract: Acemannan activity as a natural polysaccharide from Aloevera has good biodegradability and biocompatibility and has an effect on cell tissue repair. The study's objective was to ascertain whether giving Hydrogel Acemannan at concentrations of 25%, 50%, and 75% would be beneficial in lowering the quantity of macrophages in diabetic periodontitis mice . 48 white Wistar rats were used as research samples, and they were split into four groups at two different observation intervals (days 3 and 7).One-way ANOVA and posthoc LSD statistical tests were used to assess the data. The number of macrophages in each group decreased on day 7 to 3.50 ± 0.354; 2.79±0.431; 1.54 ± 0.292; 0.54 ± 0.292. The results of the one-way ANOVAX test (p≤0.05) showed that the Hydrogel Acemannan concentrations of 25%, 50%, and 75% were effective in reducing the number of macrophages in periodontitis mice with diabetes mellitus. The results of the LSD posthoc test showed that Hydrogel Acemannan concentrations of 25%, 50%, and 75% were substantially more effective than the positive control in reducing the number of macrophages in periodontal mice with diabetes mellitus (p≤0.05).

Keywords: Diabetes mellitus; Hydrogel Acemannan; Macrophages; Periodontitis

Introduction

The multifaceted disease referred to as diabetes mellitus (IDMI) is characterized by elevated blood sugar levels, abnormalities in fat, protein, and carbohydrate metabolism, as well as inadequate or ineffective insulin (Faida et al., 2020; Rahmasari et al., 2019). The production of fat and glucose as energy sources is facilitated by the hormone insulin. As a result, insulin difficulties lead to the accumulation of glucose in the blood (hyperglycemia), which is then excreted by the body through urine (glycosuria) without any physiological benefit (Pertiwi et al., 2021).

The disease is ranked fourth in most developing countries as a cause of mortality and is one of the leading developing countries as a cause of death and is one of the most common non-communicable diseases worldwide diseases worldwide (Faida et al., 2020).

Currently there are 537 million DM patients globally, however, this number is expected to rise to 643 million by 2030 and 783 million by 2045 (Ng et al., 2022). Indonesia is ranked seventh out of ten countries with the highest number of people with of the ten countries with the highest number of people with diabetes, making it a type II diabetes alert status (Magdalena et al., 2021). According to the findings of Riskesdas (2018), type 2 diabetes is the most commonly diagnosed disease,

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which accounts for 90% of all cases of diabetes mellitus as a whole. The prevalence of diabetes mellitus in Indonesia is 8.5% (Husain et al., 2022). There are several factors that contribute to the year-on-year increase in Type II Diabetes Mellitus cases, including aging population, increasing obesity rates, and decreasing physical activity (Magdalena et al., 2021).

Of course, the likelihood of chronic complications will increase with the increase in the frequency of DM. Diabetes-related chronic hyperglycemia is associated with long-term damage, malfunction, or failure of several physiological organs, including the heart, blood vessels, kidneys, eyes, and nerves (Rahmasari et al., 2019). In addition, due to increased inflammatory response and impaired immunologic response, people with diabetes develop immunologic response, people with uncontrolled diabetes mellitus are more susceptible to periodontitis (Ng et al., 2022).

Periodontitis is characterized by the formation of periodontal pockets, the loss of loss of connective tissue attachment, alveolar bone resorption, and if left untreated, can result in tooth loss (Könönen et al., 2019). Several studies have revealed that the altered host immune response in patients with uncontrolled DM patients will result in excessive inflammation and increase the severity of periodontal tissue damage due to impaired macrophage cells, which are used by used by pathogenic bacteria that cause periodontitis as a defense (Kurniawan et al., 2018; Păunică et al., 2023).

Macrophages contribute to periodontitis to play diverse roles in the maintenance of tissue homeostasis through a cascade of signals that are induced by receptors (M. Li et al., 2023; Yin et al., 2022). One of the three types of of the three types of phagocytic cells in the immune system, macrophages are found in many tissues in the and perform debridement, microbicidal, and wound-healing functions through the release of cytokines, TGFs, and other inflammatory mediators such as IL-6 and TNF-α. (Indraswary et al., 2022; Soetjipto, 2018). With maximum numbers on day's two to three of the inflammatory phase, these cells are among the most dominating cells in that phase. A decrease in the number of macrophages on day 5 indicates that the inflammatory process has subsided significantly (Kurniawati, 2019).

Tartar removal, root planing, and better oral hygiene as well as antibacterial mouthwashes (such as chlorhexidine gluconate) and, if required, systemic antibiotics constitute the first therapeutic phase of the treatment of periodontitis (Andriani et al., 2019; Deliana et al., 2023). However, long-term use of antibiotics can cause adverse effects such as allergies and local oral irritation (Andriani et al., 2019). Adverse effects such as allergies and local oral irritation, as well as impaired taste perception and resistance to mouthwash (Apriani et al., 2022; Lestari, 2024). Therefore, the use of herbal medicine is often an alternative option.

Acemannan is considered to be a natural polysaccharide that has good biodegradability and biocompatibility which is extracted from Aloevera as well as having effects on immunomodulatory, antiviral, antitumor, and tissue regeneration (Bai et al., 2023). Acemannan activity has an effect on cellssuch as lymphocytes, macrophages, and dendritic cells. Acemannan can also increase the response of lymphocytes to alo antigens by the mechanism of IL-1 from the cell nucleus under the protection from alo antigens (Susanto et al., 2022).

According to Bai et al. (2023), Acemannan is suitable in gel dosage form.Hydrogel is one of the gel biomaterials that has high hydrophilicity (S. Li et al., 2018; Y. Li et al., 2024; Susanto et al., 2022). Previous research results conducted by Susanto et al. (2022) have proven that Acemannan Hydrogel concentrations of 25%, 50% and 75% can increase collagen density as the concentration increases.

Research on the effectiveness of Acemannan Hydrogel administration on a decrease in the number of macrophages in periodontitis rats with diabetes mellitus has never been done before because it has never been done before. Mellitus has never been done before because it has never been done before. Carried out, the researcher is interested in conducting a study entitled Effectiveness of Acemannan Hydrogel Concentration 25%, 50%, 75% against Decrease in the Number of Macrophages in Periodontitis Rats with Diabetes Conditions Melitus.

Method

Research Design

This type of research includes laboratory experimental research with a post-test only control design.

Time and Place Research

This research was conducted at: (1) University of North Sumatra Herbarium Lab: a place to check Aloevera; (2) Plant Physiology and Tissue Culture Lab FMIPA University of North Sumatra: where Acemannan extracts and Acemannan hydrogels are made. North Sumatra University: where Acemannan extract and Acemannan hydrogel are made; (3) University of North Sumatra Animal Development Center Lab: where making wistar rats in diabetic and periodontitis conditions; (4) Applied Lab, Faculty of Medicine, University of North Sumatra: where the macrophage examination. The research was conducted from march to September 2023.

Research Sample

The sample in this study was a white Wistar rat. (Rattus norvegicus). The Federer formula is used to determine the size of the sample. The study involved four groups: the treatment group (25%, 50%, and 75% Hydrogel Acemannan) and the positive control group. (AllochlearTM). Because the observations were done on the third and fifth days, and the number of samples needed for this study was 48 samples.

Tools and Materials Used

The tools are gloves, mask, tissue, jar bottle, filter paper, funnel, plaster, bandage, 5 cc spuid, stirrer, vacuum, 15c blade, scalpel, nierbeken, microscope, object glass, spatula, filter, tweezers, glass tube, measuring cup, dropper pipette.

The materials using 48 Wistar rats, 96% ethanol, Aloe Vera Berbadensis Miller, CaCl, Aquades 2 liters, hypochlorite 65%, ketamine formalin, cotton roll, NHCl, high fat diet, alginate, colouring Hematoksilin Eosin.

Making Acemannan Hydrogel

First step is provision of the Aloe vera (L.) samples used came from the Berastagi area, Karo Regency, North Sumatra. Peel the skin from the Aloe Vera plant, then wash it with clean water and put the 65% Hypochlorite solution into the container containing the Aloe Vera. Drain the Aloe Vera then cut into cubes and blender to puree. Once smooth, mix the Aloe Vera and ethanol in a ratio of 1:4, stir until dissolved for 10 minutes at a temperature of 10°C. After that, put it in the refrigerator and let it sit for 10 hours so that it settles. The precipitate that has formed is separated from the solution using a sieve covered with filter paper. Then the filtered sediment is put into a vacuum dryer at a temperature of 50°C for 24 hours or a day.

Then, the Acemannan extract was weighed which was obtained at 25 grams, 50 grams and 75 grams. In a separate place, 25 grams of CaCl2 was dissolved in 500 ml of distilled water to become 25% CaCl2, the CaCl2 was stirred using a stirrer until homogeneous. Then, 15 grams of alginate was weighed for each treatment. To make 25% Acemannan Hydrogel, use 25 grams of Acemannan and 100 grams of distilled water, then stir using a stirrer until homogeneous, then take it using a spuid, then add it to CaCl2 and stir with a stirrer until it is homogeneous, then once finished, let it sit for 1 day, and so on to make Hydrogel Acemannan 50% uses 50 grams of Acemannan and to make Hydrogel Acemannan 75% uses 75 grams of Acemannan (Pereira et al., 2013).

Preparation of Test Animals

Mice were divided randomly into 4 groups, namely the treatment group (Hydrogel Acemannan 25%, 50%, 75%), and positive control (AloclairTM). Before being given treatment, all mice were adapted for a week. Alloxan induction was carried out on day 7 after adaptation of the experimental animals. The dose of alloxan for Wistar rats is 42 mg/grBW injected interperitoneally in the posterior 2/3 of the stomach and waited for 72 hours and given a high fat diet. Before measuring blood glucose levels, mice were fasted for 8-12 hours. If there is an increase in blood glucose levels exceeding 175 mg/dl, then the Wistar rat will have diabetes (Halim et al., 2022). Blood glucose levels were measured by disinfecting the tip of the Wistar rat's tail with alcohol, then wounding the tip of the tail, then touching the dripping blood to a glucometer test strip (Autocheck™) (Pertiwi et al., 2021). Blood glucose levels were measured 3 days after injection (Halim et al., 2021).

Then, subgingival binding with silk ligature is carried out on the lower incisors until periodontitis occurs, which is characterized by inflammation of the gingiva, and the bond is removed (Hariyani et al., 2021; Kurniawati, 2019; Prasetya et al., 2021). Hydrogel Acemannan 25%, 50% 75% was applied to the treatment group, and Aloclair™ gel was applied to the positive control group. All applications were carried out topically using a microbrush to the labial part of the mandibular incisors for 1 minute. This application is carried out twice a day, morning and evening with a time difference of 7 hours (Kartiningtyas et al., 2015).

On the third day, the mice were euthanized by neck dislocation, the area of the periodontal tissue on the mandibular incisors which had been tied with silk ligature using a scalpel was cut off from each mouse. The sections were placed in 10% buffered neutral formalin (BNF) fixation solution. Then dehydrate with graded alcohol (alcohol 70%, 80%, 90%, 95%), absolute alcohol (I and II), xylol (I and II), and paraffin (I and II) (Andayani et al., 2016; Lin et al., 2021). This process is carried out on each liquid for two hours. The next stage is clearing using xylol/benzol, each tissue is soaked for 1 ½ hours (Andayani et al., 2016).

Then the printing process (embedding), this process is carried out near a heat source with tools that have been warmed up first to prevent the paraffin from freezing before the process is complete. The substance used is histoplast paraffin which has a melting point of 56-57 ˚C. Tissue sample slices were soaked in liquid paraffin for 2 hours (Andayani et al., 2016). The mold is filled with liquid paraffin and then the tissue is placed in it with the help of tweezers. The semi-frozen paraffin blocks are labeled to facilitate tissue identification (Andayani et al., 2016). The next stage is cooling the paraffin block at a temperature of 4-5˚C. Once cool, the paraffin block is released from the mold and ready for the tissue slicing stage using a microtome (5µm). The resulting ribbon-shaped pieces are spread over a water bath with a

temperature of 46˚C. The tissue pieces were then attached to a glass object using albumin and placed in an incubator at 37˚C for 24 hours until the tissue adhered perfectly (Andayani et al., 2016).

Next, the preparations were stained with Hematoxylin and Eosin (HE) staining. The preparations were deparaffinized by dipping gradually into xylol I and II solutions for 2 minutes each. Dipped in absolute alcohol for 2 minutes, 95%, 90% and 80% alcohol each for 1 minute. After that, the preparations were washed with running water for 1 minute. Hemaktocilin staining was carried out for 8 minutes and then washed in running water for 30

Data Collection and Analysis Methods

The data were analyzed using the SPSS Version 25 program, where in the initial stage the data was first tested for normality with Shapiro Wilk and homogeneity with the Levene Test. If the research data is normally distributed and homogeneous, then the statistical analysis used is Oneway ANOVA and post hoc LSD.

Result and Discussion

Average Number of Macrophages on Days 3 and 7

The complete results of research on the average number of macrophages on days 3 and 7 in periodontitis mice with diabetes mellitus can be seen in Table 1.

Table 1. Average Number of Macrophages on Days 3 and 7

Group	Day	
	The 3rd	7th
Acemannan Hydrogel25%	4.58 ± 0.408	3.50 ± 0.354
Acemannan Hydrogel50%	3.88 ± 0.345	2.79 ± 0.431
Acemannan Hydrogel75%	2.88 ± 0.345	1.54±0.292
Positive control	2.13 ± 0.379	0.54 ± 0.292

Based on the research results in Table 1, the mean and standard deviation of the number of macrophages on day 3 in periodontitis rats with diabetes mellitus treated with 25%, 50%, 75% Acemannan hydrogel and the positive control was 4.58 ± 0.408; 3.88±0.345; 2.88±0.345; 2.13 ± 0.379. The number of macrophages in each treatment group decreased on day 7 to 3.50 ± 0.354; 2.79±0.431; 1.54 ± 0.292; 0.54 ± 0.292. From the results of this study it can be stated that the minutes. For Eosin staining, the preparation is soaked in Eosin solution for 2-3 minutes then washed with running water for 30 seconds. The next process is to dip the preparations 10 times each into 95% alcohol and absolute alcohol (I and II). Then carried out for 1 minute and then in xylol II for 2 minutes (Andayani et al., 2016).

Closing the tissue is done by placing the object glass on tissue paper in a flat place. The object glass is dripped with an adhesive, namely Entellan. After that, the tissue was carefully covered with a cover glass and histological observations were carried out (Andayani et al., 2016). The same procedure was repeated on mice for examination on day 7. Analysis of counting the number of macrophages using a light microscope with 400x magnification with four fields of view (Wulandari et al., 2024). The macrophage cells observed were characterized as large cells with irregularly shaped cells, having round or kidney- shaped nuclei that were purplish in color and transparent pink cytoplasm (Prasetya et al., 2021). All data was collected, then statistical tests were carried out to produce research results and conclusions that greater the concentration of Acemannan hydrogel, the greater the decrease in the number of macrophages in periodontitis mice with diabetes mellitus.

Normality and Homogeneity Test Results

Before carrying out statistical tests, all data were first tested for Shapiro-Wilk normality and Levene Test homogeneity, the details of which can be seen in Table 2.

Table 2. Normality and Homogeneity Tests Result

Day	Group	Normality	Homogeneity
3	Acemannan Hydrogel25%	0.505	0.961
	Acemannan Hydrogel50%	0.191	
	Acemannan Hydrogel75%	0.191	
	Positive control	0.389	
7	Acemannan Hydrogel25%	0.960	0.888
	Acemannan Hydrogel50%	0.830	
	Acemannan Hydrogel75%	0.421	
	Positive control	0.421	

Description: *Significant (p ≤ 0.05)

Based on the research results in Table 2 above, it can be stated that the data is normally distributed and homogeneous so that the statistical tests used in this research are one-way ANOVA and post-hoc LSD.

Effectiveness of Giving Hydrogel Acemannan Concentrations of 25%, 50%, 75% on Reducing the Number of Macrophages in Periodontitis Rats with Diabetes Mellitus

The results of research on the effectiveness of administering Hydrogel Acemannan in concentrations of 25%, 50%, 75% in reducing the number of macrophages in periodontitis mice with diabetes mellitus can be seen in Table 3. Based on the results of the Oneway ANOVA statistical test in Table 3, it shows a value of p=0.000 (p ≤ 0.05) which means that there is a significant difference in the mean number of macrophages in periodontitis mice with diabetes mellitus conditions treated with 25%, 50% Acemannan hydrogel. , 75% and positive control on day 3 and day 7.

From the results of this study, it can be stated that there is effectiveness in administering Hydrogel Acemannan in concentrations of 25%, 50%, 75% in reducing the number of macrophages in periodontitis mice with diabetes mellitus ($p \leq 0.05$).

Table 3. Oneway *ANOVA* Results Test

Group	Day			
	3rd	p	7th	p
Acemannan Hydrogel25%	4.58 ± 0.408	0.000*	3.50 ± 0.354	0.000*
Acemannan Hydrogel50%	3.88 ± 0.345		2.79 ± 0.431	
Acemannan Hydrogel75%	2.88 ± 0.345		1.54±0.292	
Positive control	2.13± 0.379		0.54 ± 0.292	

Description: *Significant ($p \leq 0.05$)

Differences in the Effectiveness of Giving Hydrogel Acemannan Concentrations of 25%, 50%, 75% in Reducing the Number of Macrophages in Periodontitis Rats with Diabetes Mellitus Conditions between Two Different Groups

The results of research regarding the differences in the effectiveness of administering Hydrogel Acemannan concentrations of 25%, 50%, 75% in reducing the number of macrophages in periodontitis mice with diabetes mellitus between two different groups can be seen in Table 4.

Table 4. Posthoc LSD Test Results

Day	Group	25%	50%	75%	K is positive
3	25%	-	0.000*	0.000*	0.000*
	50%	0.003*	-	0.000*	0.000*
	75%	0.000*	0.000*	-	0.002*
	K is positive	0.000*	0.000*	0.002*	-
7	25%	-	0.002*	0.000*	0.000*
	50%	0.002*	-	0.000*	0.000*
	75%	0.000*	0.000*	-	0.000*
	K is positive	0.000*	0.000*	0.000*	-

Description: *Significant ($p \leq 0.05$)

Based on the results of the LSD posthoc test, it can be stated that there is a significant difference in effectiveness between administration of Hydrogel Acemannan concentrations of 25%, 50%, 75% and positive control in reducing the number of macrophages in periodontitis mice with diabetes mellitus ($p \leq 0.05$).

Discussion

Based on the research results, it showed that there was a difference in the number of macrophages between days 3 and 7 in the Acemannan hydrogel group with concentrations of 25%, 50% and 75%. From the results of this study, it can be seen that the greater the concentration of Acemannan hydrogel, the greater the decrease in the number of macrophages in periodontitis mice with diabetes mellitus. The 75% concentration is the best Acemannan Hydrogel concentration compared to other concentrations because it has the smallest number of macrophages, both on days 3 and 7.

Research by Carolia et al. (2016) also showed the same results as this study that the average number of macrophages in aloe vera extract at concentrations of 25%, 50%, 75% and 100% respectively in inflammation of the oral mucosa of male white Sprague Dawley rats was as high as 15.95; 14.88; 14.8, and 14.2. Research by Aldelina et al. (2013) found that there was a difference in the average number of macrophage cells in white rat periodontitis after application of young papaya leaf extract. This showed that there was a difference in the number of macrophages in each group of young papaya leaves with the extract concentration being the highest, namely 75% had the the fewest macrophages compared to other treatment groups.

Macrophages are cells that also appear at the beginning of inflammation, apart from PMN cells. Macrophages are capable of phagocytosing large quantities of bacteria, viruses, necrotic tissue, or other foreign molecules. In the chronic inflammatory phase, the formation of antigens is caused by persistent antigens from the continuous activation and accumulation of macrophages (Andayani et al, 2016). The decrease in the number of macrophages in the results of this study means that Acemannan hydrogel concentrations of 25%, 50% and 75% are effective in accelerating healing in periodontitis mice with diabetes mellitus.

This statement is supported by the results of the one- way ANOVA statistical test which can be stated that there is a significant difference in the mean number of macrophages in periodontitis mice with diabetes mellitus conditions treated with Acemannan hydrogel 25%, 50%, 75% and positive controls on day 3 and day 3. 7th. The results of this study are in line with research conducted by Carolia et al. (2016) which stated that there was a significant difference in the mean number of macrophages between groups (p= 0.014) with the most effective concentration of aloe vera extract as an anti-inflammatory in inflammation of the oral mucosa being 100%.

Acemannan is the main bioactive polysaccharide consisting of long chain polymers formed from glucose and mannose with good biodegradability and biocompatibility extracted from Aloevera and has excellent immunomodulatory, antiviral, antitumor and tissue regeneration effects. This polysaccharide has activity against macrophages and increases cell proliferation so it can help accelerate periodontitis (Bai et al., 2023; Liu et al., 2019; Primasari et al., 2023; Susanto et al., 2022). The results of this research show that the

greater the concentration of Acemannan Hydrogel used, the shorter the inflammatory process that occurs. This may be influenced by the higher concentration of Hydrogel Acemannan, so the anti-inflammatory properties will be higher.

According to Ruauw et al. (2019), topical application on the oral mucosa of Wistar rats was reported to accelerate the healing of incision wounds. However, topical preparations in the form of ointments are less effective for use in the oral cavity (Shah et al., 2020). Therefore, the Acemannan in this study is in the form of a hydrogel due to the nature of the hydrogel which is easy to use and clean, has intermolecular forces which can reduce molecular mobility, and produces good viscosity (Saputro et al., 2021).

Based on this research, it was found that the decrease in the number of macrophages in periodontitis mice with diabetes mellitus who were treated with Acemannan hydrogel at all concentrations showed a lower mean number of macrophages compared to the positive control group of mice (Aloclair) and according to the results of the LSD posthoc test, it was seen that there was a difference in effectiveness. There was a significant difference between administration of Hydrogel Acemannan at concentrations of 25%, 50%, 75% and positive control in reducing the number of macrophages in periodontitis mice with diabetes mellitus ($p \leq 0.05$). The positive control showed the greatest decrease in macrophage numbers on days 3 and 7.

This is different from the results of research conducted by Putri et al. (2023) which stated that Aloe vera extract could shorten the inflammatory process in wound healing which was characterized by a lower number of lymphocytes compared to the positive control group. This difference could be caused by the positive control used, the inflammatory cells observed, the concentration of Aloe vera, and the test material used was not the same as in this study, namely a combination of Aloe vera extract and chicken egg albumin with concentrations of 50% and 100% against lymphocyte cells.

Aloclair gel was used as a positive control in this study. It is an anti-inflammatory drug commonly used in the field of dentistry. Aloclair gel contains, among other things, polyvinylpyrrolidone (PVP) which has an antiseptic effect; sodium hyaluronate and Aloe vera in Aloclair support the natural healing process of damaged tissue by forming a layer of membrane as a mechanical protective barrier; Glycyrrhetinic acid has antiviral, antifungal, antiprotozoal and antibacterial effects, while Hydrogel Acemannan which comes from Aloe vera extract can only act as anti-inflammatory and tissue regeneration. Therefore, Aloclair has more benefits and advantages compared to Hydrogel Acemannan, so that the positive control ability is better than the treatment material in accelerating the decline of macrophages (et al., 2021). The same results can also be seen from the research results of Agusmawanti (2016) that the Aloclair group showed significant differences from the treatment group.

One of three types of phagocytic cells in the immune system and widely distributed in body tissues which have debridement functions, namely magrophages, microbicidal and wound healing by releasing various inflammatory mediators such as IL-6 and TNF-α, cytokines and Transforming Growth Factor (TGF) (Indraswary et al., 2022; Soetjipto, 2018). Magrophage cells are among the most dominant cells in the inflammatory phase with the highest numbers on days 2 to 3. On day 5, it showed that the inflammatory process had greatly reduced, decreasing the number of macrophages (Kurniawati, 2019).

Conclusion

Based on the researcch result, it can be concluded that Acemannan hydrogel concentrations of 25%, 50% and 75% can reduce macrophages in periodontitis mice with diabetes melitus. The higher the cooncentrations of Acemannan hydrogel, the greater the reduction in macrophages.

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Author Contributions

For Chandra Susanto conceptualized the idea of this research, Susanna Halim and Gusbakti Rusip designed methoodology, and Nada Aurelia analyzed the data, conducted the research and process, literature review. All authors read and approved the final version of manuscript.

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Conflicts of Interest

The authors declare no conflict of interest.

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