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RESEARCH ARTICLE

A potential of *Jasminum sambac* (L.) *Aiton* leaf nano-extract as spray treatment of gingivitis-induced *Sprague Dawley* rats

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ABSTRACT

Gingivitis is the second most common disease that occurs in the oral cavity. The use of chlorhexidine as a gingivitis therapy agent has some side effects such as allergic reactions and bacterial resistance. *Jasminum Sambac* (L.) *Aiton* leaf nano-extract contains flavonoids, saponins, and tannins compounds reported to have an influence on wound healing process. The aim of this research was to determine the effect of observation time and concentrations of Jasminum sambac (L.) Aiton leaf nano-extract on the number of neutrophils, macrophages, and angiogenesis in the treatment of gingivitis-induced Sprague Dawley rats. Sixty male Sprague Dawley rats aged 2.5-3 months with body weight of 200-250 g were anesthetized with ketamine HCl (10 mg/kg BW) and xylazine (2 mg/kg BW) then induced using silk ligature 3.0 on the interdental of the mandibular incisor. The rats were divided into five groups and got daily spray using distilled water (negative control), nano-extract at a concentration of 40%, 45%, 50%, and 0.12% chlorhexidine (positive control). The rats were euthanized on the 1st, 3rd, 5th, and 7th days post-treatment. The tissues were processed histologically with HE staining. The number of neutrophils, macrophages, and angiogenesis were counted using a microscope (400x) and OptiLab Viewer® (13x) at five fields of view. The result of the Two Way ANOVA test showed that there were significant effect of concentrations and observation time, and interaction of both observation time and concentrations on the number of neutrophils, macrophages, and angiogenesis ($p < 0.05$). It can be concluded that observation time and concentrations of Jasminum sambac (L.) Aiton leaf nano-extract affected the number of neutrophils, macrophage, and angiogenesis in the treatment of gingivitis-induced Sprague Dawley rat ($p < 0.05$).

Keywords: angiogenesis; gingivitis; *Jasminum sambac (L.) Aiton*; macrophage; nano-extract; neutrophil

INTRODUCTION

The prevalence of periodontal diseases among Indonesian people is up to 90% in which gingivitis and periodontitis are included in periodontal diseases.[1] Gingivitis occurs in the oral cavity that is not properly cleaned which will induce accumulation of plaque.[2] Gingivitis is characterized by swollen, red gums, susceptible to bleeding when brushing.[3] A treatment for gingivitis is mechanical cleaning by scaling and root planning. However, topical application of antimicrobial substances to the gingivitis area can provide better treatment results. The antibacterial agent that is often used is chlorhexidine but this antimicrobial has several detrimental effects such as allergies and bacterial resistance.[4]

Jasmine leaves (*Jasminum sambac* (L.) *Aiton*) contain active substances such as flavonoids, saponins, and tannins which contain antiseptic, anti-inflammatory, and anti-bacterial agent. In addition, the use of nanoparticle technology has several advantages such as increasing the affinity of the system due to an increased contact area and penetrating the boundaries between cells. Previous research has proven that jasmine leaf nano-extract as a Piezoelectric scaler coolant agent is effective in increasing the number of blood vessels as a treatment agent of gingivitis.[5,6]

There are several stages of wound healing process as hemostasis, inflammation, migration, proliferation, and maturation. At the inflammatory stage, an increase in the number of inflammatory

cells such as neutrophils and macrophages can be found. Meanwhile, at the migration stage, angiogenesis, fibroblast proliferation, and re-epithelialization replace the process.[7]

Previous research has proven that jasmine leaf nano-extract as a Piezoelectric scaler coolant agent is effective in increasing the number of blood vessels as a gingivitis treatment substance. In this study, the effect of observation time and concentrations of *Jasminum sambac* (L.) *Aiton* nano-extract as a topical spray on the healing process of gingivitis-induced *Sprague Dawley* rats was tested. The test parameters in this study were the number of neutrophils, macrophages, and angiogenesis on the 1st, 3rd, 5th, and 7th days post treatment. The purpose of this study was to provide scientific data on the effect of observation time and concentrations of *Jasminum sambac* (L.) *Aiton* nano-extract used as a spray on the number of neutrophils, macrophages, and angiogenesis of gingivitis-induced *Sprague Dawley* rats.

MATERIALS AND METHODS

This was a quasi-experimental study that received ethical approval from the Health Research Ethics Commission of the Faculty of Medicine, Public Health, and Nursing, Universitas Gadjah Mada (Number: 00553KKEP/FKG-UGM/EC/2020). This research was carried out in November 2020-April 2021. *Jasminum sambac* (L.) *Aiton* leaves were collected from the jasmine plantation in Pemalang, Jawa Tengah. The samples were identified at the plant systematic laboratory, Faculty of Biology, Universitas Gadjah Mada (Number: 014612/S.Tb./VI/2019). The samples were extracted by the maceration method using 70% ethanol as the solvent. The extract was then made into nanoparticles using the ionic gelation method with chitosan and sodium tripolyphosphate (NaTPP) as an encapsulation. The nano-extracts were then made into spray preparations at concentrations of 40%, 45%, and 50% using distilled water as the solvent.

This research involved 60 gingivitis-induced *Sprague Dawley* rats as the subject. Treatment on the animal models was carried out at the Integrated Research and Experimental Laboratory, Universitas Gadjah Mada. Anaesthesia was done through intramuscular injection with a solution of Ketamine HCl 10 mg/kg body weight and Xylazine 2 mg/kg body weight. The male adult rats were induced by placement of silk ligature 3.0 in the interdental gingiva of the anterior mandibular teeth. This ligature promoted the accumulation of plaque as an aetiology of gingivitis. The rats were observed daily to check the ligature, gingival condition, and their nutrition until early clinical signs of gingivitis were found, namely inflammation and redness. The rats were divided into five groups: negative control, three treatment groups with different extract concentrations namely 40%, 45%, and 50%, and positive control group. They got daily gingival spray based on their groups. The subjects were decapitated on the 1st, 3rd, 5th, and 7th days after showing signs of early gingivitis and getting their first spray. Three rats were decapitated in every batch in each group.

The rats were euthanized by decapitation. The mandibular of the rats were stored in 10% buffered natural formalin (BNF) to prevent the samples from autolysis by enzymes and bacteria and to protect the cell structure. The samples were then soaked in formic acid solution before being processed histologically with Hematoxylin Eosin (HE) staining. Histological analysis was conducted on labial view of cemento-enamel junction of the gingiva of mandibular incisors, 5 fields from the cervical to the incisal region were selected to the gingival pocket at random as region of interest (ROIs).The number of neutrophils, macrophages, and angiogenesis were obtained by observing the specimens under a light microscope equipped with a digital camera (Carl Zeiss, Primo Star, Germany) at 400x magnification and Opti Lab Viewer (13x magnification). Histological evaluation was performed by three blinded investigators in triplicate. The agreement among the investigators was calculated with the Kappa test (K neutrophils = 0.67, K macrophages = 0.66, K angiogenesis = 0.64) indicating satisfactory intra-examiner and inter-

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examiner reliability. The total number of neutrophils, macrophages, and angiogenesis were obtained by calculating the mean across 5 ROIs from both incisors. The specimen was stained with HE, the neutrophils had a horseshoe-shaped nucleus and extensive pink cytoplasm. The macrophages were irregular in shape found near blood vessels, could carry out amoeboid movement towards the site of inflammation, and were phagocytic; angiogenesis was new blood vessels.

RESULTS

From the particle size analyzer (PSA), the particle size of jasmine leaf nano-extract (*Jasminum sambac* (L.) *Aiton*) was 689.6 nm and the zeta potential was 54.8 mV. Based on the results of the test using PSA, the extract was classified as the nano-extract category with good stability.[8,9] The number of angiogenesis, macrophages, and neutrophils was counted on days 1, 3, 5, and 7 after treatment. Observations and calculations were carried out in five visual fields. The area of observation was from the junctional epithelium to the gingival pocket. The results of calculating the mean and standard deviation of angiogenesis, macrophages, and PMN neutrophils in each treatment group are presented in the following tables.

The normality and homogeneity tests were done. The results showed that the data were normally distributed ($p > 0.05$) and homogeneous ($p > 0.05$). After the data were shown to be normally distributed and homogeneous, the two-way ANOVA test was performed. The results of the two way ANOVA test showed that the number of angiogenesis was $p = 0.000$, the number of macrophages was $p = 0.024$, and the number of PMN neutrophils was $p = 0.002$. In addition, the results of the two-way ANOVA test showed $p < 0.05$, which means that there was an effect of observation time and concentrations of Jasminum sambac (L.) Aiton leaf nano-extract on the number of neutrophils, macrophages, and angiogenesis as a spray treatment for gingivitis-induced Sprague Dawley rats.

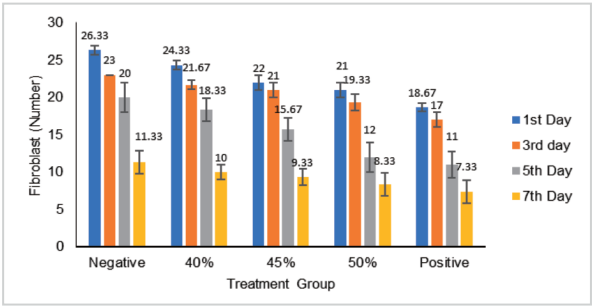


Figure 1. Diagram of mean and standard deviation of neutrophil

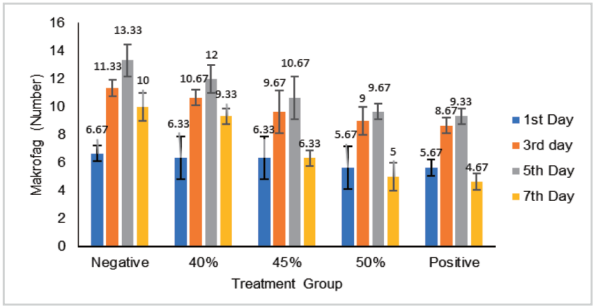


Figure 2. Diagram of mean and standard deviation of macrofag

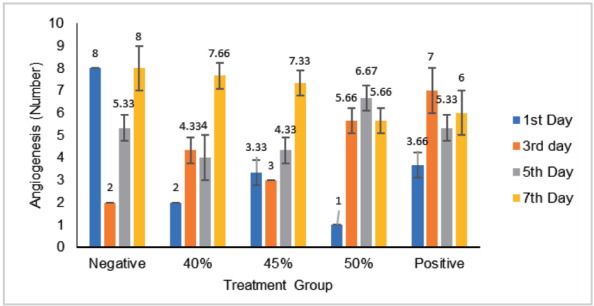


Figure 3. Diagram of mean and standard deviation of angiogenesis

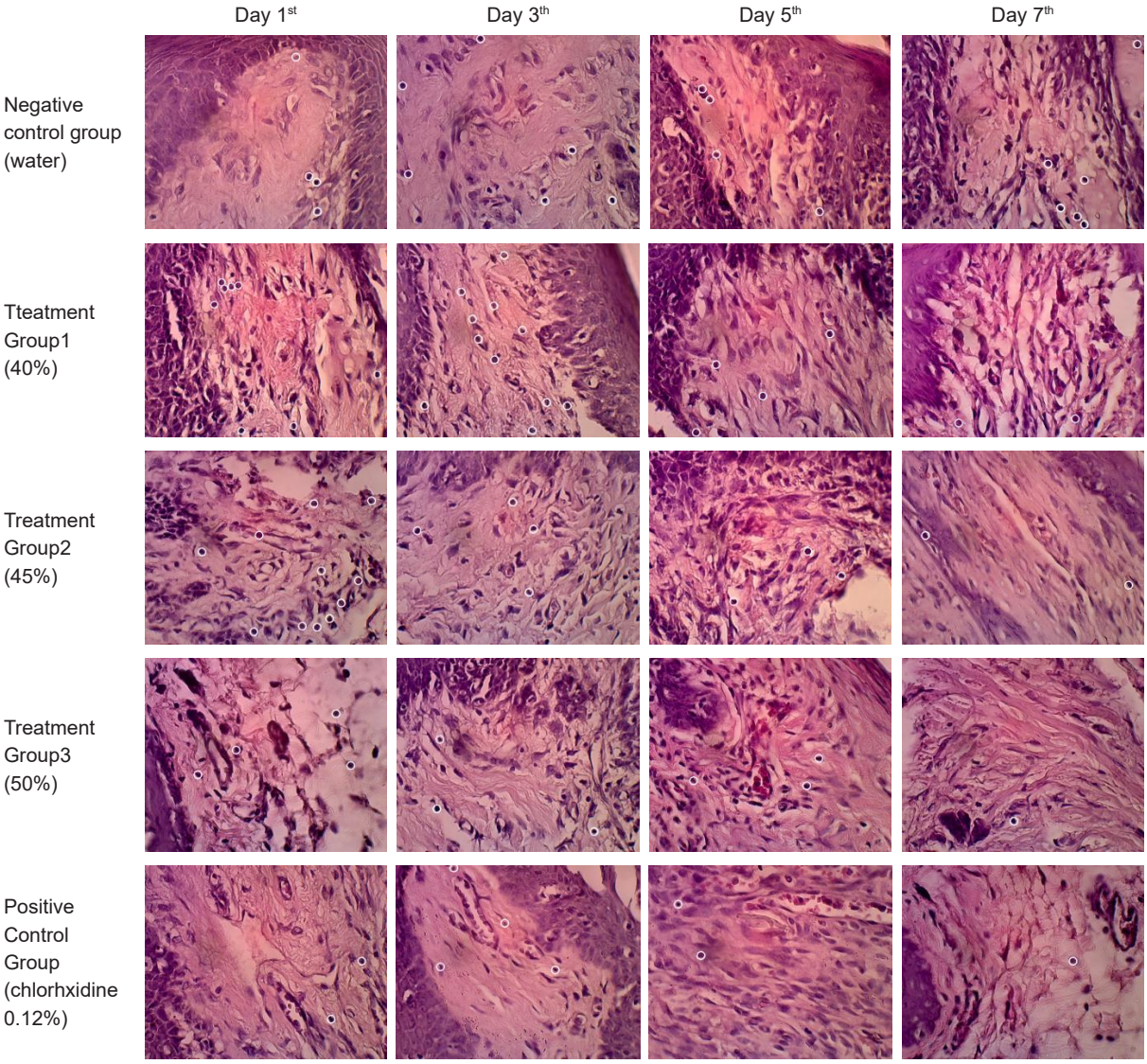
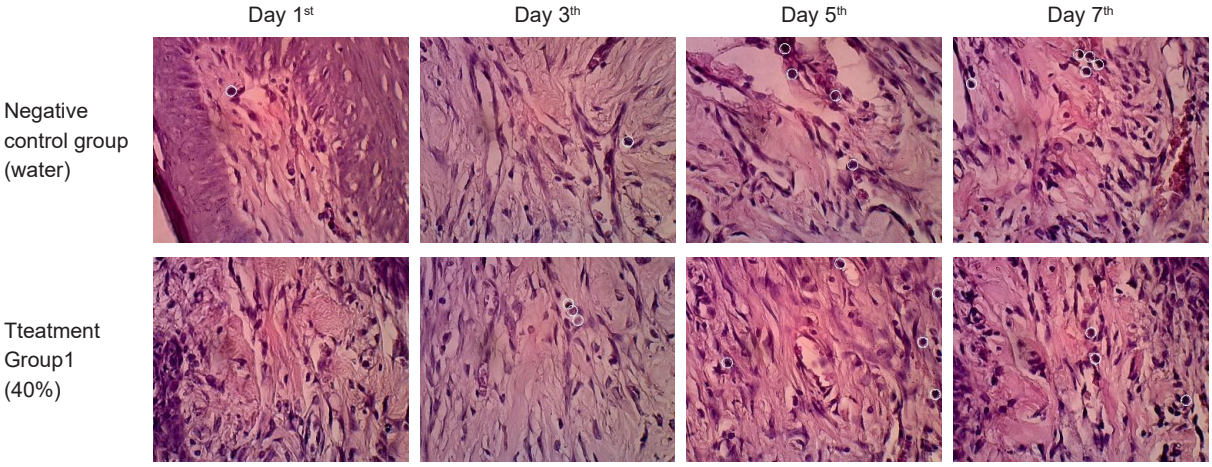


Figure 4. The histology specimen of neutrophils



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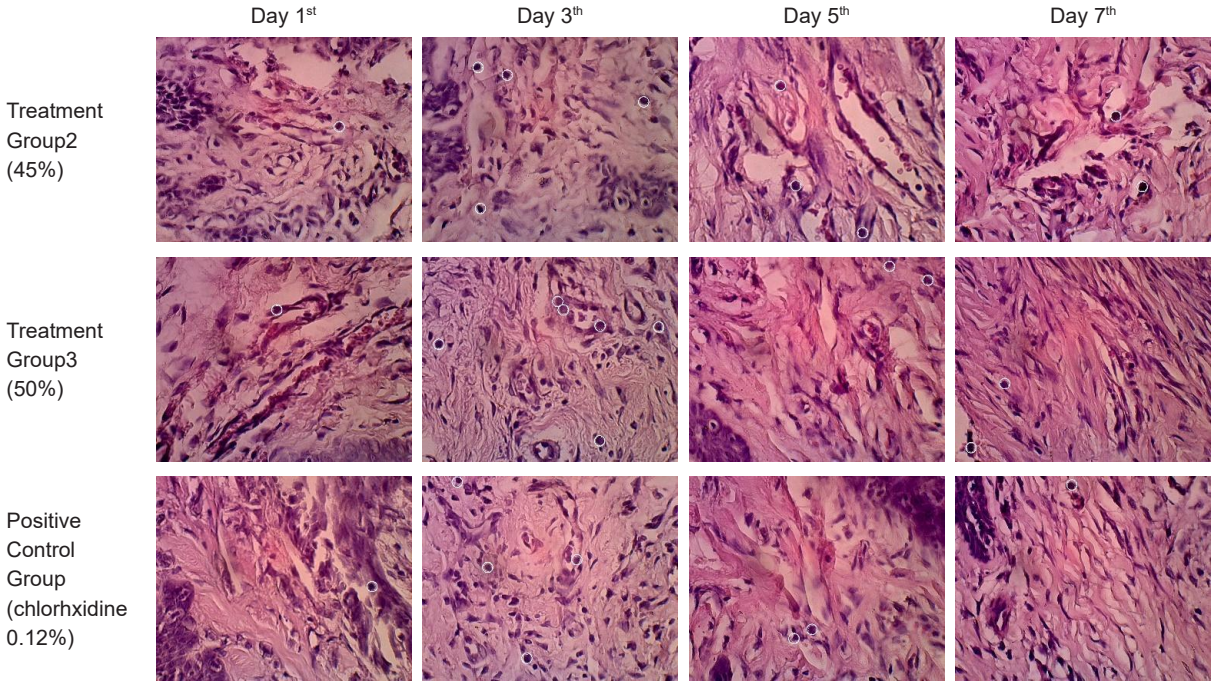
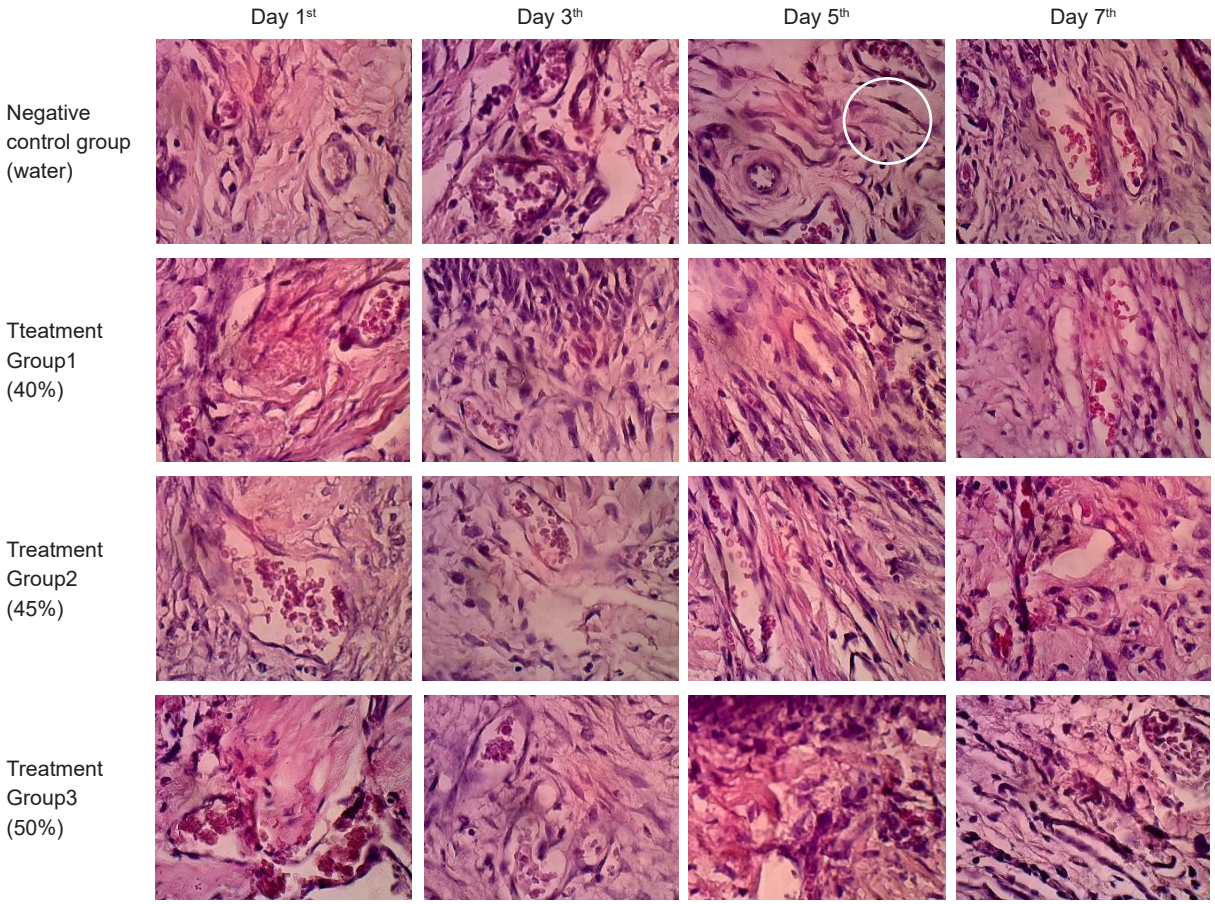


Figure 5. The histology specimen of macrophages



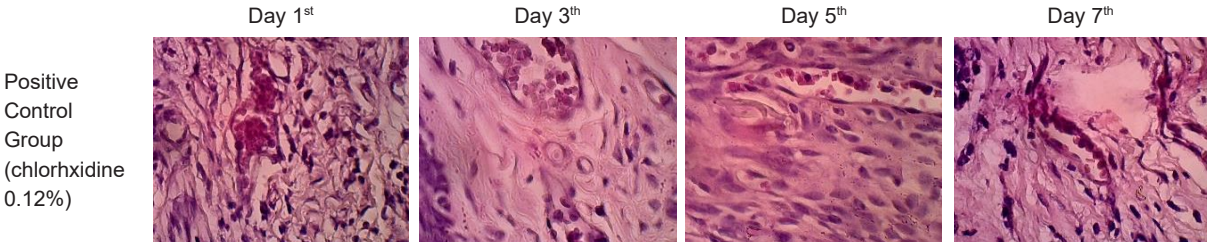


Figure 6. The histology specimen of angionesis

Table 1. Two way ANOVA test results on the number of neutrophils

Source	df	Mean square	F	Sig.
day	3	518.772	338.330	0.000*
treatment	4	84.233	54.935	0.000*
treatment*day	12	3.633	2.370	0.020*

(*) : sig. (p<0.05)
df : degrees of freedom
Sig. : significance (probability)

Table 2. Two way ANOVA test results on the number of macrophages

Source	df	Mean square	F	Sig.
day	3	78.861	77.568	0.000*
treatment	4	23.892	23.500	0.000*
treatment*day	12	2.347	2.309	0.024*

(*) : sig. (p<0.05)
df : degrees of freedom
Sig. : significance (probability)

Table 3. Two way ANOVA test results on the number of angiogenesis

Source	df	Mean square	F	Sig.
Treatment	4	4.517	10.423	0.000*
Day	3	30.372	70.090	0.000*
Treatment*Day	12	11.872	27.397	0.000*

(*) : sig. (p<0.05)
df : degrees of freedom
Sig. : significance (probability)

Then the post hoc LSD (Least Significant Difference) test was done. The results of which show a significant effect if the significance is less than 0.05 ($p < 0.05$). The following is a list of groups whose mean differences were not significantly different.

DISCUSSION

Based on the results of the two-way ANOVA test, there was a significant effect ($p < 0.05$) of observation time and concentrations, and a significant interaction between observation time and concentrations of Jasminum sambac

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Table 4. Summary of LSD test on the number of neutrophils (p > 0.05/ not significantly different)

A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T
D1C-	D1T1	D1T2	D1T3	D1C+	D3C-	D3T1	D3T2	D3T3	D3C+	D5C-	D5T1	D5T2	D5T2	D5T3	D5C+	D7C-	D7T1	D7T2	D7C+
	A																		
					B														
			C		C	C	C			C									
					D	D	D	D		D									
								E	E	E	E								
						F	F												
							G			G									
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																	N	N	N
															O	O	O		
																P	P		
																	Q	Q	
																		R	R
																			S

D1/D3/D5/D7 : day1st/day3th/day5th/day7th
C- : Negative control group
C+ : Positive control group
T11/T2/T3 : Treatment (40%/45%/50%) group

(L.) Aiton leaf nano-extract on the number of neutrophils, macrophages and angiogenesis as a spray treatment for gingivitis-induced Sprague Dawley rat. On day 1, the highest number of PMN neutrophils appeared in each concentration group. This is because on day 1, PMN neutrophils migrate into the tissues as the first defense mechanism when inflammation occurs. Neutrophils have intracellular defense (phagocytosis) and extracellular defense (degranulation and neutrophil extracellular traps).[10] PMN neutrophils play a role in phagocytizing foreign bodies when acute inflammation occurs. Then on the 3rd, 5th, to 7th day, there was a decrease in the number of PMN neutrophils. This means that the inflammation was already in the next stage (prolonged inflammation), in which PMN neutrophils become apoptotic and the number of PMN neutrophils decreases. PMN neutrophils are replaced by monocytes which will mature into macrophages that play a role in cleaning debris and tissues including apoptotic PMN neutrophils.[11,12]

The number of groups that had a significant difference ($p < 0.05$) in neutrophils, macrophages, and angiogenesis was greater than those that had no significant difference ($p > 0.05$). Tables 4, 5, and 6 show the groups without any significant difference. The mean of PMN neutrophils on each treatment was significantly different ($p < 0.05$). This is because the leaves of *Jasminum sambac* (L.) *Aiton* have been reported to posses anti inflamatory activitydue to the flavonoids and chitosan contained in the Jasmine (*Jasminum sambac* (L.) Aiton leaf nano extract.[13] Flavonoids

Table 5. Summary of LSD test on the number of macrophages (p > 0.05/ not significantly different)

A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T
D1C-	D1T1	D1T2	D1T3	D1C+	D3C-	D3T1	D3T2	D3T3	D3C+	D5C-	D5T1	D5T2	D5T2	D5T3	D5C+	D7C-	D7T1	D7T2	D7C+
	A	A	A	A													A	A	
		B	B	B													B	B	B
			C	C													C	C	C
				D													D	D	D
																	E	E	E
						F	F				F	F	F		F	F			
							G	G			G	G	G	G	G	G			
								H	H			H	H	H	H	H			
									I			I	I	I	I	I			
													J	J	J	J			
											K								
												L							
													M	M	M	M			
														N	N	N			
															O	O			
																P			
																		R	R

D1/D3/D5/D7 : day1st/day3th/day5th/day7th
C- : Negative control group
C+ : Positive control group
T11/T2/T3 : Treatment (40%/45%/50%) group

have an anti-inflammatory effect by inhibiting COX-2, lipoxygenase, and tyrosine kinase. Flavonoids can make the number of inflammatory cells. That is why, the inflammatory reaction will last shorter and the proliferation process will take place immediately.[14] Chitosan has an antibacterial effect that can eliminate the etiology of gingivitis, which is bacterial plaque. In addition, chitosan has a positive energy, while the bacterial cell membrane has a negative energy. The interaction between these two kinds of energy will loosen up the tight junction of the epithelium in the bacterial cell membrane, causing the loss of the intracellular components of the bacteria.[15] In addition, chitosan can convert the particles of the extract into nanoparticles and cause the active ingredients to be more absorbable.[16]

The results of the Post Hoc LSD test showed that there was a significant effect between the negative control group and the positive control group, also with the 50% group on the 1st, 3rd, 5th, and 7th days ($p < 0.05$). This means that jasmine (*Jasminum sambac* (L.) *Aiton*) leaf nanospray and 0.12% chlorhexidine had a different effect with distilled water. However, on the 7th day, between the 50% group and the positive control group, there was no significant effect ($p > 0.05$). This means the jasmine (*Jasminum sambac* (L.) Aiton) leaf nanospray at a concentration of 50% had the same effect as 0.12% chlorhexidine as a treatment agent on gingivitis.

The highest number of macrophages based on the data was found on the 5th day in the negative control group while the lowest was found on the 7th day in the positive control group. The mean number of macrophages in all the groups increased on the 3rd and 5th days and decreased on the 7th day. This is in accordance with the result

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Table 6. Summary of LSD test on the number of angiogenesis (p > 0.05/ not significantly different)

A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T
D1C-	D1T1	D1T2	D1T3	D1C+	D3C-	D3T1	D3T2	D3T3	D3C+	D5C-	D5T1	D5T2	D5T3	D5C+	D7C-	D7T1	D7T2	D7T3	D7C+
										A						A	A	A	
				B		B		B											
					C		C	C				C	C						
						D													
							E	E				E	E						
								F											
											G	G	G		G				
												H							
											I			I	I				I
														J		J	J	J	
													K		K				K
													L						
															M				
																	N	N	N
																			O
																	P	P	
																		Q	

D1/D3/D5/D7 : day1st/day3th/day5th/day7th
C- : Negative control group
C+ : Positive control group
T11/T2/T3 : Treatment (40%/45%/50%) group

of the two-way ANOVA test in Table 2, showing the effect of observation time and concentration of *Jasminum sambac* (L.) *Aiton* leaf nano-extract on the number of macrophages when used as a spray treatment on gingivitis.

The number of macrophages between the treatment groups showed significant differences ($p < 0.05$) due to the role of flavonoids, saponins, and chitosan contained in the jasmine leaf nano-extract.[17] The role of flavonoids on inflammation process has been described previously. Saponins along with flavonoids help healing processes because they can strengthen connective tissues by reducing lipid peroxidation and increasing vascularity, thereby increasing the availability of oxygen in the healing area. Chitosan has antimicrobial components and helps wound healing processes through its ability to depolymerize, thus releasing N-acetyl-d-glucosamine which can stimulate macrophages.[15,18,19,20]

The differences in the number of macrophages between observations time were influenced by the role of macrophages. These cells replace the role of neutrophils and begin to appear on day 3 to day 7 and reach the peak on day 5. Macrophages eliminate cellular debris, foreign bodies, and bacteria in injured tissues. They also play a role in cleaning neutrophils by phagocytosing apoptotic neutrophils. The number of macrophages will decrease after reaching the peak along with the healing process.[21,22,23]

The results of this study showed that the highest mean number of blood vessels was found on day 1 in the negative and positive control groups. On the other hand, the lowest mean number of blood vessels was found on day 1 in

the 50% treatment group. Based on the results of the two-way ANOVA test, there was an effect of observation time and concentration of *Jasminum sambac* (L.) *Aiton* leaf nano-extract on the number of blood vessels.

Based on the results of the LSD test, the difference was not significant on day 1 in the 40%, 45%, and 50% treatment groups with the positive control group, indicating that the nano-extract concentrations of 40%, 45%, and 50% had almost the same effect as 0.12% chlorhexidine. On the 3rd day, the difference was not significant in the 45% treatment group with the negative control group, indicating that the nano-extract concentration of 45% gave almost the same effect as distilled water. On the 5th day, the differences were not significant in the 45% treatment group with the positive control group, indicating that the nano-extract concentration of 45% had almost the same effect as 0.12% chlorhexidine. On the 7th day, the difference was not significant in the 50% treatment group with the positive control group, indicating that the nano-extract concentration of 50% had almost the same effect as 0.12% chlorhexidine.

The difference in the number of blood vessels between groups was caused by the content of flavonoids, saponins, and tannins. Flavonoids can induce vascular endothelial growth factor (VEGF) which plays a role in the angiogenesis process. Saponins play a role in stimulating angiogenesis by increasing VEGF production. Tannins accelerate wound healing by several cellular mechanisms by chelating free radicals and reactivation of oxygen, increasing the formation of blood vessels and fibroblasts. The difference in the mean number of blood vessels between treatment days was due to the process of blood vessel formation (angiogenesis), originating from previously formed capillaries and playing a role in maintaining the continuity of tissue function. In addition, blood vessels have an important role in tissue repair to provide nutritional intake for the regenerating tissue. Four important things in regeneration in wound healing is the adequacy of cells, blood vessels, growth, and scaffolding.[24,25,26,27,28] This study concluded that there was an effect of observation time and concentration of *Jasminum sambac* (L.) *Aiton* on the number of neutrophils, macrophages, and angiogenesis as a spray treatment on gingivitis-induced *Sprague Dawley* rats.

Based on this research, it is important to carry out a series of in vivo tests, especially the biocompatibility of Jasmine leaf nano-extract spray, so in the end it can be carried out clinically. In further development, nanospray of jasmine leaves expectedly can be produced as an herb for the treatment of gingivitis, so as to reduce the use of chemicals and minimize the possibility of allergies.

CONCLUSION

Observation time and concentrations of spray containing *Jasminum sambac* (L.) Aiton leaf nano-extract affect the number of neutrophils, macrophage, and angiogenesis in the treatment of gingivitis-induced *Sprague Dawley* rat ($p < 0.05$).

CONFLICT OF INTEREST

The authors declare no conflict of interest with the data contained in the manuscript.

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