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The Effect Of Coenzim Q10 On The Number Of Lymfocite Cells In Male Wistar Rats With Periodontitis

Tara Yurian Nadhifa[1*], Aprilia Yuanita Anwaristi[2], Nur Ariska Nugrahani[3], Dwi Kurniawati[4]
[1,2,3,4] Department of Dentistry, Faculty of Dentistry, Universitas Muhammadiyah Surakarta, Jawa Tengah, Indonesia

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ABSTRACT

Periodontal disease is a dental and oral health problem in adult society. One of the periodontal diseases is periodontitis which is an inflammation of the periodontal tissue caused by accumulation of dental plaque which can lead to impaired masticatory function, tooth loss, alveolar bone damage, periodontal ligament damage, pocket formation, and gingival recession. Coenzyme Q10 acts as an antioxidant, the reduced form is ready to give electrons to deactivate ROS or block free radicals which can prevent oxidative stress thereby protecting lymphocyte cells and reducing the immune response which can lead to a reduction in inflammation in the periodontal tissues. The purpose of this study was to determine the effect of coenzyme Q10 on the number of lymphocyte cells in the gingiva of male wistar rats with periodontitis. The research method used is true experimental laboratory *quantitative* research with a research design that is a posttest only control group design with observation or observing the control group and the treatment group. The number of samples used was 24 rats. Observation of lymphocyte cells was carried out using a light microscope with a magnification of 200x and a magnification of 400x and then counted manually by the observer. The results showed that the significance value obtained in the *One-Way ANOVA test* was 0.000 (<0.05). Then, based on the results of the *Post Hoc Least Significant Difference* (LSD) *test*, it was found that the biggest difference in effect was in the K(-)5 group and the P5 group, where the difference was 59.00 with a significance value of 0.000 (<0.05). It can be concluded that there is an effect of coenzyme Q10 administration on the number of lymphocyte cells in the gingiva of male Wistar rats with periodontitis.

Email : j520190024@student.ums.ac.id

1. INTRODUCTION

Periodontal disease is a dental and oral health problem in adult society [1]. Periodontal disease is experienced by humans almost all over the world and covers 50% of the adult population. The prevalence and intensity of periodontal disease in Africa and Asia is higher than in America, Australia and Europe [2]. Periodontal disease in Indonesia has a fairly high prevalence. This is evidenced by the 2018 Riskesdas National Survey of dental and oral health problems in Indonesia of 57.8% and the prevalence of oral health problems experienced by the majority of the Indonesian population is periodontal disease of 96.58%. Data from Basic Health Research in 2018 the prevalence of Indonesian people suffering from periodontitis is 74.1% [3].

One of the periodontal diseases is periodontitis which is inflammation of the periodontal tissue caused by accumulation of dental plaque which can cause masticatory function disorders, tooth loss, alveolar bone damage, periodontal ligament damage, pocket formation, and gingival recession [4]. The clinical picture of periodontitis is characterized by inflammation of the periodontal tissue with apical migration of the junctional epithelium, loss of bone attachment, resorption of alveolar bone which causes tooth mobility, and shifting of teeth leading to tooth loss. Clinical examination in periodontitis shows increased probing depth, bleeding on slow probing, and changes in physiological contours such as redness and swelling of the gingiva [5]. Periodontal tissue damage can be caused directly by the presence of bacteria through the proteolytic enzyme lipopolysaccharide (LPS) or indirectly as a host

response to the presence of bacteria which are considered as foreign bodies through an inflammatory process [6]. The host response that interacts with microbes in periodontitis is carried out by removing various kinds of inflammatory cells, one of which is lymphocyte cells which are chronic inflammatory cells that are specific as a host immune response to injury when chronic inflammation occurs with the aim of protecting the periodontal tissue from bacterial activity [6].

Bacterial products such as lipopolysaccharide (LPS) can increase the secretion of proinflammatory cytokines resulting in an increase in the number of macrophages and neutrophils. Lymphocytes and macrophages stimulate each other persistently to be able to eliminate antigen triggering agents [7]. Lymphocytes play a role in specific immune responses, both humoral responses carried out by B lymphocytes and cellular ones carried out by T lymphocytes [6]. The use of a material or preparation to be applied to humans needs to pass several tests. One of them is pre-clinical trials conducted on test animals [8]. Experimental animals or laboratory animals are animals that are deliberately kept to be used as test animals to study and develop various fields of science on a research scale [9]. Wistar rats are one of the experimental animals that are often used in research because it allows researchers to investigate the etiology of disease. Researchers used male Wistar rats as experimental animals because male Wistar rats were not affected by the estrus cycle and pregnancy so they could provide more stable research results than female Wistar rats [10]. Based on the explanation above and there is still a lack of information regarding the effect of coenzyme Q10 as an effort to cure periodontitis.

2. METHOD

The research method used is true experimental laboratory *quantitative* research with a research design that is a posttest only control group design with observation or observing the control group and the treatment group. The total number of samples used for the six groups was 24 male wistar white rats with 4 individuals in each group. The treatment group was given coenzyme Q10 0.1 ml which was applied using a micropipet 2 times a day for 7 days, namely in the morning and evening with a time difference of 7 hours starting at 09.00 and 16.00 WIB. Termination of the test animals was carried out on days 3, 5 and 7 with a total of 4 rats per day in each group then histology preparations were made, while the control group was not given coenzyme Q10. Observation of lymphocyte cells was carried out manually by observers using a light microscope using 200x magnification and 400x magnification with 5 fields of view on tissue preparations that had been stained using HE staining. Lymphocyte cell observations were carried out at the Anatomical Pathology Laboratory, Universitas Muhammadiyah Surakarta in April-July 2023. Data analysis is used by carrying out the *normality test* (*Shapiro-Wilk test*). The homogeneity test in this study was carried out using the *Levene Test*. Then the *One-Way ANOVA* and *Post Hoc Least Significant Difference* (LSD) *tests* were performed.

3. RESULTS AND DISCUSSION

This study used experimental animals in the form of male Wistar rats which were then divided into 6 groups, namely the negative control group on day 3 K(-)3, the negative control group on day 5 K(-)5, the negative control group on day 7 K(-)7, treatment group on the 3rd day P3, treatment group on the 5th day P5, treatment group on the 7th day P7. The negative control group K(-) was a group that was induced by periodontitis for 7 days without given treatment. The treatment group was the group that was induced by periodontitis and then 0.1 ml of coenzyme Q10 was applied. The treatment was carried out on the 7th day of periodontitis incubation because periodontitis had occurred. Clinical symptoms of the gingiva include changes such as redness of the gingiva, gingival recession, and changes in the gingival contour.

Samples were obtained by taking the gingival tissue of male Wistar rats who had periodontitis decapitated on the third, fifth, seventh day after periodontitis induction and then preparations were made with Haematoxylin-Eosin staining. Observation and calculation of the number of lymphocyte cells was carried out using a light microscope with a magnification of 200x and a magnification of 400x, a purple image of lymphocytes was obtained. Lymphocytes in HE staining will appear purple in the form of a large, round nucleus with a single nucleus, their slightly visible cytoplasm surrounds the nucleus as a light blue ring, the size of the lymphocytes found varies from medium to large. Counting the number of

lymphocytes was done manually as many as 5 fields of view then averaged. The results of counting the number of lymphocyte cells in male Wistar rats in the negative control and treatment groups can be seen in table 1 and figure 4.

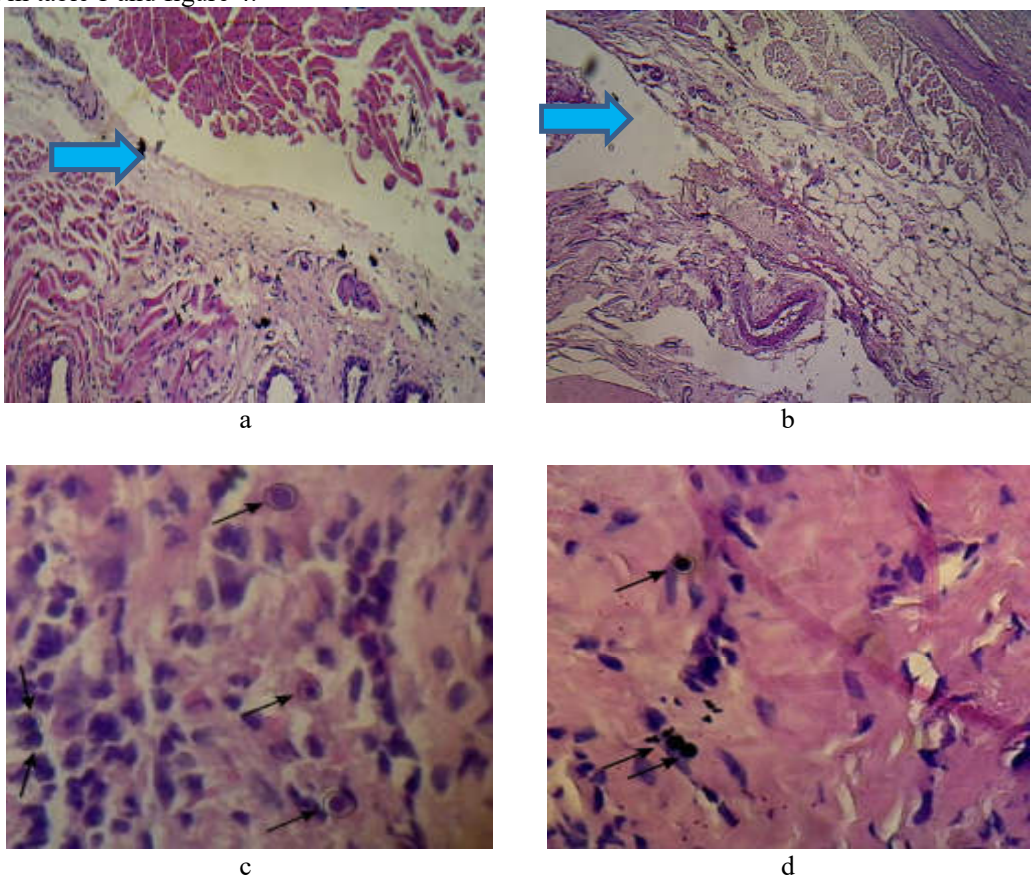

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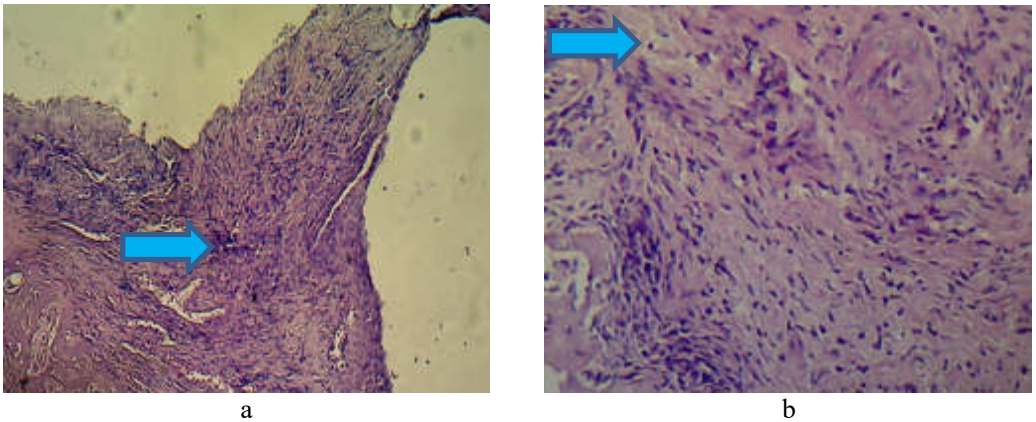
Figure 1. Lymphocytes on the 3rd day preparations with HE staining at 200x magnification (a) Negative control group (b) Treatment group and 400x magnification (c) Negative control group (d) Treatment group

Based on (Figure 1) above it was found that the gingival tissue of male Wistar rats who experienced periodontitis in the negative control group on D+3 showed a picture of a high number of lymphocyte cells and the treatment group on D+3 showed a picture of a low number of lymphocyte cells.



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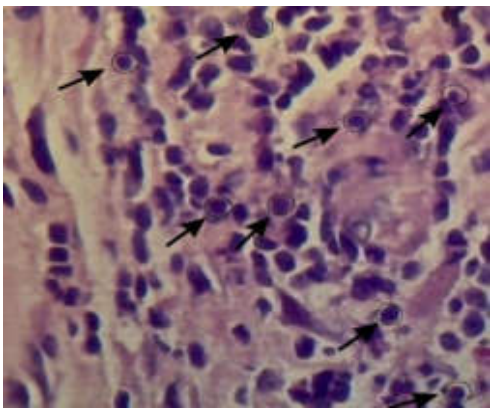
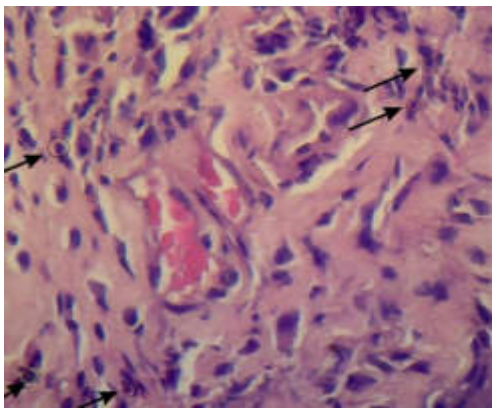

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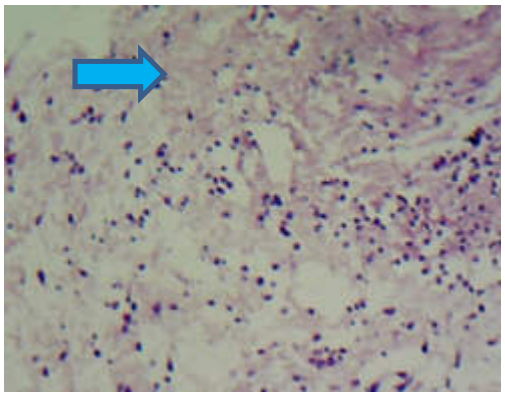
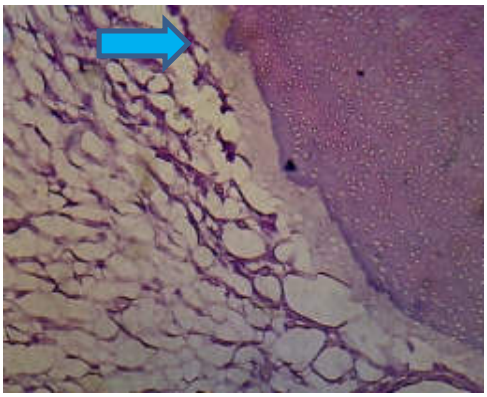
Figure 2. Lymphocytes on the 5th day preparations with HE staining at 200x magnification (a) Negative control group (b) Treatment group and 400x magnification (c) Negative control group (d) Treatment group

Based on (Figure 2.) above, it was found that the gingival tissue of male Wistar rats who experienced periodontitis in the negative control group on D+5 showed a picture of a high number of lymphocyte cells and the treatment group on D+5 showed a picture of a low number of lymphocyte cells.

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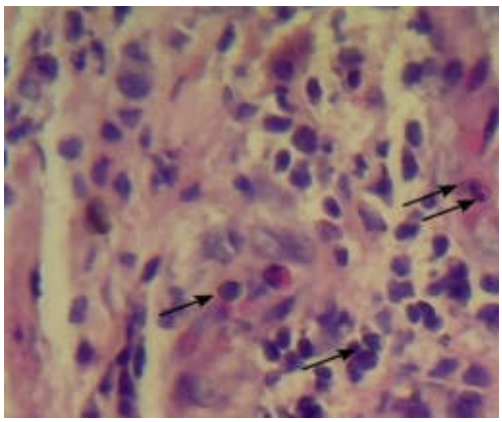
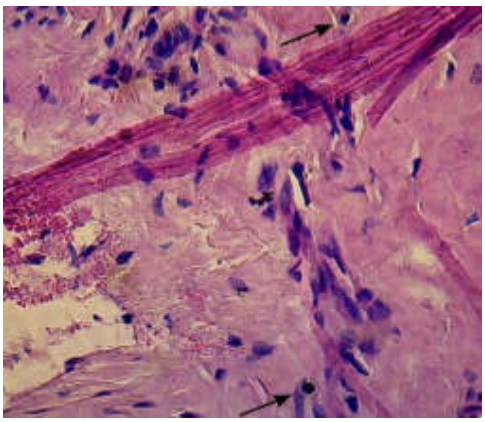

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Figure 3. Lymphocytes on the 7th day preparations with HE staining at 200x magnification (a) Negative control group (b) Treatment group and 400x magnification (c) Negative control group (d) Treatment group

Based on (Figure 3.) above, it was found that the gingival tissue of male Wistar rats who experienced periodontitis in the negative control group on D+7 showed a picture of a high number of lymphocyte cells and the treatment group on D+7 showed a picture of a low number of lymphocyte cells. Research data processing was carried out with the help of the SPSS 25.0 program to obtain an overview of the distribution and summary of the data in order to clarify the presentation of the results, which then obtained the mean and standard deviation of the number of lymphocyte cells in each treatment group and control group, as follows:

Table 1. The Results of The Mean and Standard Deviation of The Number Of Lymphocytes In The Gingiva of Male Wistar Rats With Periodontitis.

Kelompok		Mean	±Std. Deviation
Hari ke-3	K(-)3	59.00	± 3.830
	P3	42.00	± 4.000
Hari ke-5	K(-)5	88.00	± 10.832
	P5	58.00	± 9.522
Hari ke-7	K(-)7	50.00	± 7.659
	P7	29.00	± 3.830

K(-)3 : Negative control group without treatment for 3 days after periodontitis was induced and then underwent surgery on the 3rd day

P3 : The treatment group was given coenzyme Q10 0.1 ml which was applied 2 times a day for 3 days after periodontitis was induced and then underwent surgery on the 3rd day

K(-)5 : Negative control group without treatment for 5 days after periodontitis was induced and then underwent surgery on the 5th day

P5 : The treatment group was given coenzyme Q10 0.1 ml which was applied 2 times a day for 5 days after periodontitis was induced and then underwent surgery on the 5th day

K(-)7 : Negative control group without treatment for 7 days after periodontitis was induced and then underwent surgery on the 7th day

P7 : The treatment group was given coenzyme Q10 0.1 ml which was applied 2 times a day for 7 days after periodontitis was induced and then underwent surgery on the 7th day

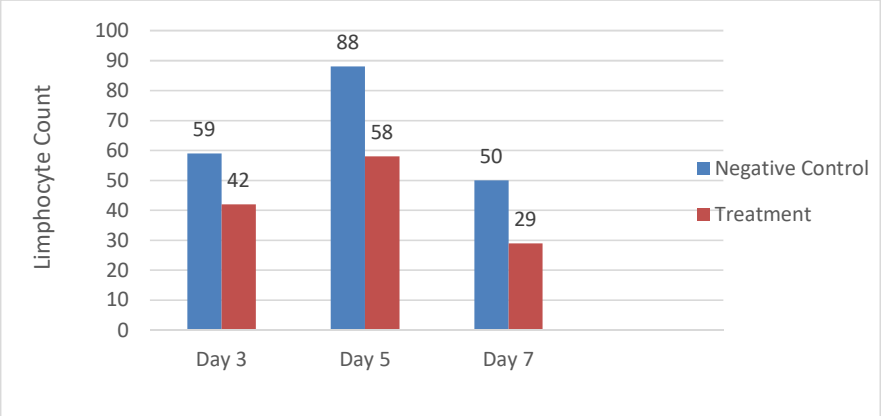

Figure 4. Diagram of the mean number of lymphocytes in the gingiva of male Wistar rats with periodontitis in each experimental group (mmol/L)

Based on table 1. and figure 4. it shows that on day 3 the negative control group had an average number of lymphocytes higher, namely 59.00 compared to the treatment group, which had an average number of lower lymphocytes, namely 42.00. Day 5 the negative control group had an average lymphocyte count higher, namely 88.00, compared to the treatment group, which had a lower average lymphocyte count, namely 58.00. Day 7 the negative control group had an average lymphocyte count higher, namely 50.00, compared to the treatment group, which had a lower average lymphocyte count, namely 29.00. In addition, from the diagram above it is found that the negative control group experienced an increase in the number of lymphocytes on day 3 and day 5 and decreased on day 7; the

treatment group experienced an increase in the number of lymphocytes on the 3rd and 5th day and decreased on the 7th day. The first statistical test is to determine the normality of the data using the *Shapiro-Wilk test*, where a data is said to have a normal distribution if p > 0.05. The *normality test* results were obtained as follows.

Table 2. *Shapiro-Wilk Normality Test* Results

Group	df	*Sig.*
K(-)3	4	0.272
P3	4	0.625
K(-)5	4	0.062
P5	4	0.488
K(-)7	4	0.272
P7	4	0.275

Based on the table above, it shows that the significance value for all sample groups has a value greater than 0.05 (p> 0.05). It can be concluded that the data used in all groups has a normal distribution. After knowing that the data is normally distributed, then to determine the variance of the lymphocyte count data that has a different variant or does not carry out a *homogeneity test* using the *Levene Test,* is as follows.

Table 3. *Levene Test Homogeneity Test* Results

Levene Statistic	df1	df2	*Sig.*
1.416	5	18	.247

Based on the table above, a *statistical Levene* coefficient of 1.416 is obtained with a significance value of 0.247. If the significance value is compared to p=0.05, it can be concluded that the significance value is greater than 0.05. So, from this test it can be seen that the homogeneity test of variance has been fulfilled. After the two tests that underlie the *One Way ANOVA test* have been fulfilled, then the *One Way ANOVA test* is carried out, which aims to evaluate the difference in the value of the number of lymphocytes between groups, whether there is an effect of differences in treatment of the test animals. Based on this statistical test, it can be seen whether there is a significant difference in the number of lymphocytes between groups. The difference in the average lymphocytes was considered significant if the p value <0.05. The following is the calculation result of the *One Way ANOVA test.*

Table 4. *One-Way ANOVA Test* Results

Mean Square	F	*Sig.*
1626.267	30.620	.000

Based on the table above, a significance of 0.00 is obtained. The significance value obtained from the calculation process is smaller than 0.05 (<0.05). So from this test it can be seen that there is a significant difference in the number of lymphocytes between groups. Analysis of the difference in the number of lymphocytes from the six groups can be seen in the *Post Hoc* Multiple Comparison test. The *Post Hoc* method used is *Least Significant Difference* (LSD). The *Post Hoc Least Significant Difference* (LSD) test is a test that aims to find out which treatment is significantly different if the null hypothesis is rejected. In the LSD *Post Hoc test*, a data is said to be significantly different if the significance value of p <0.05. The results of the *Post Hoc Least Significant Difference* (LSD) test in this study are as follows.

Table 5. Uji *Post Hoc Least Significant Difference* (LSD)

	K(-) 3	K(-) 5	K(-) 7	P 3	P 5	P 7
K(-) 3		0.000*	0.098	0.004*	0.848	0.000*
K(-) 5	0.000*		0.000*	0.000*	0.000*	0.000*
K(-) 7	0.098	0.000*		0.138	0.138	0.000*
P 3	0.043*	0.000*	0.431		0.006*	0.014*
P 5	0.998	0.011*	0.999	0.006*		0.000*
P 7	0.004*	0.000*	0.069	0.014*	0.000*	

In this test, a data is said to be significantly different if the significance value is p <0.05 and at a 95% confidence interval. There is a difference that is not significant if p => 0.05. The table above compares each treatment group. The results of the LDS Post Hoc test showed that there were significant differences between groups K(-)3 and K(-)5, K(-)3 and P3, K(-)3 and P7, K(-)5 and K(-)7, K(-)5 and P3, K(-)5 and P5, K(-)5 and P7, K(-)7 and P7, P3 and P5, P3 and P7, P5 and P7. The significant difference was due to the value (P < 0.5). Insignificant differences were shown by groups K(-)3 with K(-)7, K(-)3 with P5, K(-)7 with K(-)3, K(-)7 with P3, K(-)7 with P5, P3 with P5, P3 with P7. The insignificant difference was due to the P value > 0.05.

Discussion

Observations in this study were carried out on the 3rd, 5th and 7th day after all experimental animals had experienced periodontitis. The process of periodontitis in this study was made by using a silk ligature measuring 0.3 mm tied to the subgingiva in the cervical lower incisor teeth with ties forming a figure of eight. Then, Porphyromonas Gingivalis bacteria were applied using an irrigated syringe to a silk ligature that had been attached to the subgingiva of the mandibular incisor teeth of rats for 2 days. After the occurrence of periodontitis on the 7th day the silk ligature was removed because it had shown a clinical picture of periodontitis. Clinical symptoms of the gingiva include changes such as redness of the gingiva, gingival recession, and changes in the gingival contour. This shows that the installation of silk ligatures on the subgingiva of the mandibular incisors for 7 days can cause periodontitis [11]. On the 7th day of periodontitis incubating, before given treatment, scaling and root planning was carried out first as the first step in periodontitis treatment which aims to eliminate local factors that cause chronic inflammation, then acute inflammation occurs due to epithelial injury [12].

Periodontal tissue damage can be caused directly by the presence of bacteria through the proteolytic enzyme lipopolysaccharide (LPS) or indirectly as a host response to the presence of bacteria which are considered as foreign bodies through an inflammatory process [6]. The host response that interacts with microbes in periodontitis is carried out by removing various kinds of inflammatory cells, one of which is lymphocyte cells which are inflammatory cells that are specific as host immune responses to injury when acute or chronic inflammation occurs with the aim of protecting the periodontal tissue from bacterial activity [6]. Bacterial products such as lipopolysaccharide (LPS) can increase the secretion of proinflammatory cytokines resulting in an increase in the number of macrophages and neutrophils. Lymphocytes and macrophages stimulate each other persistently to be able to eliminate antigen triggering agents [7]. Lymphocytes play a role in specific immune responses, both humoral responses carried out by B lymphocytes and cellular ones carried out by T lymphocytes [6]. Macrophage cells stimulate an increase in the production of chemical mediators, one of which is excessive reactive oxygen species (ROS), which acts as a second messenger to the surface of T cells to increase T cell activation, one of which is helper T cells to produce secretions in the form of cytokines that stimulate B cells to proliferate into memory cells and produce antibodies [13]. T cells and B cells activate the receptor activator of nuclear factor-kappaB signaling ligand (RANKL) which is involved in osteoclast activity. Lymphocytes release antibodies as a protective mechanism but also activate osteoclasts, which result in bone resorption. The increase in the number of lymphocyte cells causes damage to collagen and periodontal tissue cells [14]. Periodontitis disease in periodontal tissues has high levels of neutrophils and macrophages resulting in an increase in excessive ROS production and an excessive inflammatory atmosphere resulting in an increase in the number of lymphocyte cells in the area of inflammation [15].

The initial step in periodontitis treatment is scaling root planning first which aims to eliminate local factors that cause chronic inflammation, then acute inflammation occurs due to epithelial injury. Acute and chronic inflammation, both local and systemic, play a key role in lymphocyte differentiation in tissues [16]. Lymphocytes will migrate to the wound area on day 3, then the number of lymphocytes will peak on day 5, and on day 7 lymphocytes will decrease. This is because the acute and chronic inflammatory phase will experience a peak on the 5th day. At that time, ROS is generated in high levels to kill bacteria and trigger epithelial migration and proliferation [12]. The *one-way ANOVA test* that was carried out obtained a significance value of 0.000 (p <0.05) so that it can be concluded that there was a significant difference in the number of lymphocytes between the negative control group and the

treatment group. This shows that there is an effect of giving coenzyme Q10 on the number of lymphocytes in the healing process of periodontitis in the gingiva of male Wistar rats incubated with periodontitis. This is also supported by previous research to measure the speed of healing of white Wistar rats based on the number of neutrophils and lymphocytes using Coenzyme Q10 which results in a faster healing process compared to the untreated group, thereby accelerating the reduction of inflammation and faster healing process. compared to the untreated group [17]. Other studies have shown that the application of coenzyme Q10 in a rat model of acute pulpitis can reduce the activation of the number of inflammatory cells resulting in reduced inflammation and faster wound healing [18].

Based on the *Post Hoc* LSD (*Least Significance Differences*) test which was carried out as a follow-up to the *One Way ANOVA test*, it was shown that the group that was not treated with K(-) on the 3rd, 5th and 7th day experienced an increase from day 3 -3 to the 5th day, and decreased on the 7th day. The number of lymphocytes in the group that was not given K(-) treatment from day 3 to day 5 to day 7 had no significant difference. This can happen because the group did not apply active compounds that can accelerate the wound healing process, it is also a sign that inflammation is still occurring in this group. This is in accordance with previous research, in the group that was given aquadest there was no significant difference because there was no active compound content in the distilled water [19].

In the treatment group that was applied coenzyme Q10 as much as 0.1 ml on the 3rd, 5th and 7th day there was an increase from the 3rd to the 5th day and a decrease on the 7th day. The LSD Post Hoc test showed that the increase in the number of lymphocyte cells in the treatment group that was applied coenzyme Q10 as much as 0.1 ml from day 3 to day 5 to day 7 had a significant difference. This is because after curettage which aims to remove local factors that cause chronic inflammation, acute inflammation occurs due to epithelial injury. The inflammatory phase in acute conditions will peak on the 5th day. At that time, ROS was generated in high levels to kill bacteria and trigger cell migration and proliferation. After passing through the peak phase of inflammation, there are no excess ROS levels so that it does not interfere with the cell regeneration process. The number of lymphocyte cells on day 7 was lower indicating that excessive blocking of ROS by antioxidants during inflammation reduced ROS thereby preventing oxidative stress which causes excess inflammation and preventing cell damage and accelerating the healing phase [12].

The group that was not treated with K(-) and the group that was given coenzyme Q10 as much as 0.1 ml (P) on the 3rd, 5th and 7th day found that the group (P) had an average number of lymphocytes that were lower than the K(-) group. The LSD Post Hoc test showed that the K(-) group and (P) group on day 3, day 5 and day 7 had a significant difference. This was due to the administration of 0.1 ml of coenzyme Q10 in group (P). coenzyme Q10 contains antioxidants [18]. The antioxidant coenzyme Q10 is obtained from the ability of coenzyme Q10 to modulate the production of Nuclear Related Factor 2 (Nrf2). Nuclear Related Factor 2 will stimulate antioxidant responsive element (ARE) so that it will produce antioxidant enzymes. The antioxidant enzymes produced can suppress the production of ROS so that ROS decreases and becomes stable, thus preventing oxidative stress and inflammatory cells from decreasing [18]. ROS that is reduced and becomes stable prevents T cell activation and B cell stimulation so that the number of lymphocyte cells decreases [14]. The results of this study are in accordance with previous studies which stated that coenzyme Q10 administration after scaling and root planing treatment could reduce clinical parameters of chronic periodontitis in smokers [20].

In the negative control group that was not treated with K(-) on day 5 it showed that the average number of lymphocytes was the highest compared to day 5 in the treatment group. Whereas in the treatment group that was applied coenzyme Q10 as much as 0.1 ml P on the 7th day showed the lowest average number of lymphocytes compared to the 7th day in the negative control group. This is in accordance with previous studies that lymphocytes will migrate to the wound area on day 3, then their number will peak on day 5, and on day 7 lymphocytes will decrease [16]. The highest average number of lymphocyte cells occurred in the negative control group on day 5 because the negative control group was not given any treatment, thus indicating an ongoing infection that caused inflammation. At that time, ROS were produced in high levels to kill bacteria and trigger cell migration and proliferation resulting in oxidative stress and excessive inflammatory conditions [12]. Excessive ROS production acts as a second messenger for T cell receptor signals and activation of T cells can increase the induction of T cell proliferation which stimulates B cells, so that the number of lymphocytes increases [14]. The

inflammatory phase will last as long as there is excessive bacterial invasion (infection). Prolonged inflammation can occur and cause a delay in proliferation, thereby affecting the healing process and healing failure can occur [6]. The lowest average number of lymphocyte cells occurred in the 7th day treatment group because in the treatment group that was applied coenzyme Q10 as much as 0.1 ml showed that excessive blocking of ROS by the antioxidant coenzyme Q10 during inflammation can stabilize ROS, so ROS decreases and becomes stable so as not to cause oxidative stress which can cause cell damage and inhibit the healing phase. ROS that decreases and becomes stable prevents T-cell activation and B-cell stimulation so that the number of lymphocyte cells decreases [12].

4. CONCLUSION

This is evidenced by the large significance value obtained in the *One-Way ANOVA test* of 0.000 (<0.05). Then, based on the results of the *Post Hoc Least Significant Difference* (LSD) *test,* it was found that the biggest difference in effect was in the K(-) 5 group and the P5 group, where the difference was 59.00 with a significance value of 0.000 (<0.05). It can be concluded that there is an effect of coenzyme Q10 administration on the number of lymphocyte cells in the gingiva of male Wistar rats with periodontitis. In future studies it is suggested to conduct research on various doses of coenzyme Q10 as a comparison of the most effective doses for adjunctive therapy in the treatment of periodontitis.

ETHICAL CLEARANCE

This research has received approval from RSUD Dr. Moerwadi Surakarta number 735/V/HREC/2023.

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