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Effect Of Topical Application Of Ozonated Olive Oil On The Number Of Lymfocite Cells In Male Wistar Rats With Periodontitis

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ABSTRACT

Periodontitis is an inflammation of the supporting tissues of the teeth caused by specific microorganisms or specific groups of microorganism which results in progressive destruction of the periodontal connective tissue and alveolar bone by forming pockets, recession or both. When there is inflammatory periodontitis, the lymphocyte cells increase. Lymphocytes are specific chronic inflammatory cells that become an immune response to the presence of an injury during inflammation. One of the treatments that support wound healing in periodontitis is Ozonated Olive Oil. Ozonated Olive Oil has a disinfectant ability that can suppress the growth of bacteria such as those contained in periodontitis. This study aims to determine the effect of topical application of Ozonated Olive Oil on the number of lymphocyte cells in male Wistar rats with periodontitis. The number of male wistar rats used was 24. Observation of lymphocyte cells was carried out using a light microscope with a magnification of 200x and counted manually by the observer.The results showed that the mean number of lymphocytes in the experimental group was 24±3.26599 and the negative control group was 48±5.65685. The result of the *One-Way ANOVA* data analysis is 0.000 (0<0.05). The conclusion of this study was that the use of 0.1 ml of Ozonated Olive Oil had an effect on the number of lymphocyte cells in male Wistar rats who had periodontitis.

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1. INTRODUCTION

Periodontal tissue is the tissue that surrounds the teeth and has a function as a support for the teeth. Periodontal tissue consists of gingiva, cementum, periodontal connective tissue and alveolar bone. There are several types of diseases of the periodontal tissues, one of which is periodontitis [1]. Periodontitis is inflammation of the supporting tissues of the teeth caused by specific microorganisms or specific groups of microorganisms which results in progressive destruction of the periodontal connective tissue and alveolar bone by forming pockets, recession or both [2]. Periodontitis arises due to accumulation of plaque and bacteria. Microorganisms that colonize the plaque can produce lipopolysaccharide (LPS) which can damage the periodontal tissue. In addition, periodontitis will also experience an increase caused by several factors such as local factors, systemic factors, and environmental factors that can affect plaque accumulation. One of the pathogenic bacteria as the causative agent of periodontitis is the proteolytic bacterium Porphyromonas gingivalis. These bacteria can cause damage to the periodontal tissue through their proteolytic enzymes (LPS) as a host response to the presence of bacteria which are considered foreign bodies, then the host response interacts with microbes in periodontitis to secrete inflammatory cells, one of which is lymphocytes [3].

Ozone (O3) is a gas molecule that functions as a periodontitis therapy. In addition, ozone is also a natural gas molecule that causes lysis of bacterial cell membranes which causes dental problems due to its oxidant and oxidizing properties. Ozone therapy has recently been widely used for the treatment of dental problems because it is biologically based as well as atraumatic [4]. Mice are one of the animals that can be used in trials of ozone therapy. Mice are used for trials of food and nutritional deficiency in all types of animals including humans. Ozonated Olive Oil (O3) is pure olive oil which has been ozonized with a stream of ozone oxygen in a ratio of 5:95% until the olive oil changes from a greenish liquid to a white gel which can increase the number of osteoblasts of alveolar bone and blood vessels

in the periodontitis healing process. 4]. In addition, ozone in ozonized olive oil is more stable and longer lasting than others, is widely applied as a therapeutic agent for wound healing and contains ozonide molecules that can trigger tissue regeneration [4]. In this study Ozonated Olive Oil PurO3 was used in a gel form which has been widely used as a wound healing agent consisting of a mixture of pure olive oil and ozone without chemicals. Ozonated Olive Oil has the ability as a disinfectant which can suppress the growth of bacteria, such as bacteria contained in periodontitis [4].

Mice are similar to humans in their reproductive system, nervous system, disease (cancer and diabetes), and anxiety. This happens because of the similarity of DNA organization and gene expression where 98% of human genes have genes comparable to mouse genes [5]. Research conducted by previous researchers found that ozonated olive oil can significantly increase the number of osteoblasts of alveolar bone and blood vessels in the healing process of periodontitis, which means that there is a significant difference in the number of osteoblasts between the treatment group and the control group [4]. Induction of periodontitis in rats was carried out using silk-ligature by tying it to the subgingival area around the mandibular incisors [4]. Based on the description above and the results of research that varied from other studies where ozonated olive oil functioned as an inflammatory response due to periodontitis, the researchers were interested in conducting research on the Effect of Ozonated Olive Oil on Lymphocyte Cell Count in the Gingiva of Male Wistar Rats Experiencing Periodontitis which was different from previous research on the cells to be examined. This study aims to determine the effect of topical application of Ozonated Olive Oil on the number of lymphocyte cells in male Wistar rats with periodontitis.

2. METHOD

This research is a quantitative research which is a pure laboratory experiment (true experimental laboratory) with a post-test only randomized control group design by making observations or observations on the control group and the treatment group. The total number of samples used for the four groups was 30 male Wistar white rats with 5 individuals in each group. Then termination of the test animals was carried out on the 10th, 12th and 14th day after administration of the active ingredient Ozonated olive oil to 1 rat from each group. The treatment group was given Ozonated Olive Oil topically using a microbrush twice a day for 14 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 10, 12 and 14 with a total of 3 rats per day in each group then histology preparations were made, while the control group was not given Ozonated Olive Oil. Observation of lymphocyte cells was carried out manually by observers using a light microscope using 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 research was conducted at the Pharmacology Laboratory, Anatomical Pathology Laboratory, Faculty of Medicine, Universitas Muhammadiyah Surakarta in April - July 2023. This type of research is a pure experimental laboratory (true experimental laboratory) with a post-test only randomized control group research design. The sample in this study were 24 male wistar white rats which were divided into 6 groups, where each group consisted of 4 samples. Ozonated Olive Oil in this study was administered topically using a microbrush twice a day, morning and evening. The data obtained from further research was carried out using descriptive statistical tests to determine the number of lymphocyte cells.

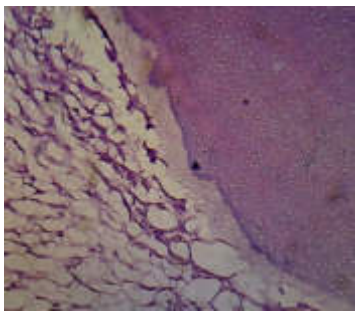
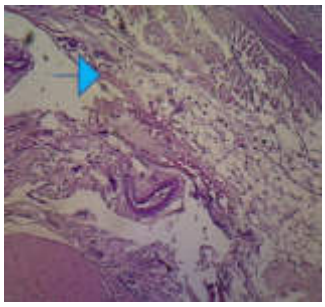

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Figure 1. Lymphocytes with 200x magnification

The results of the descriptive statistical analysis in this study can be seen in the following table:

Table 1. Descriptive Statistical Test Results

Group	N	(Mean ± SD)
K(-) 3	4	48 ± 5,65685
K(-) 5	4	32 ± 7,30297
K(-) 7	4	26 ± 6,92820
OZO 3	4	39 ± 3,82971
OZO 5	4	31 ± 3,82971
OZO 7	4	24 ± 3,26599

Information:

N : Number of samples
Mean : Average value
SD : Deviation standard
K (-) 3 : Control group 3 days after periodontitis induction
K (-) 5 : Control group 5 days after periodontitis induction
K (-) 7 : Control group 7 days after periodontitis induction
OZO 3 : Treatment group 3 days after periodontitis induction
OZO 5 : Treatment group 5 days after periodontitis induction
OZO 7 : Treatment group 7 days after periodontitis induction

Based on the table above, it is known that the highest average value is in the control group K (-) 3 or the group induced by periodontitis but not given the active ingredient (3 days after periodontitis induction) with an average value of 48 with a standard deviation of 5.65685. Meanwhile, the lowest average value was in the OZO 7 group or the treatment group which was induced by periodontitis and 0.1 ml of Ozonated olive oil was applied (7 days after periodontitis induction), with an average value obtained of 24 with a standard deviation of 3.26599. Furthermore, to determine the normality of the data used in this study, a data normality test was carried out. The data normality test in this study was carried out using the Shapiro-Wilk test. The results of the data normality test using the Shapiro-Wilk test are as follows:

Table 2. Data *Normality Test* Results

Group	df	Sig.
K(-)3	4	0,161
K(-)5	4	0,714
K(-)7	4	0,195
OZO 3	4	0,272
OZO 5	4	0,272
OZO 7	4	0,683

Information:

Sig. : Significance value
df : Number of samples

K (-) : Control group
OZO : Treatment group

Based on the results of the data *normality test* using the *Shapiro-Wilk test*, it is known that the significance value (*sig.)* for each sample group has a value greater than 0.05 (P>0.05). So it can be concluded that the data used in all groups has a normal distribution. Furthermore, to see the variance of the data, a *homogeneity test* was carried out. The *homogeneity test* in this study was carried out using the Levene Test. The results of the homogeneity test using the *Levene Test* are as follows:

Table 3. *Homogeneity Test* Results

Levene Statistic	df 1	df 2	Sig.
1,258	5	18	0,324

Based on the results of the *homogeneity test* using the *Levene test*, it is known that the significant value (*sig*.) obtained in the *homogeneity* test using the *Levene test* is 0.324 (> 0.05). So it can be concluded that the data used in this study have the same or homogeneous variance. Then, to determine whether there is a difference in the effect of Ozonated olive oil on the number of lymphocyte cells in the gingiva of male Wistar rats with periodontitis, a *One-Way ANOVA* test was performed. The results of the *One-Way ANOVA* test in this study are as follows:

Table 4. Resutlh *One-Way ANOVA*

Mean Square	F	Sig.
316,267	10,948	0,000

Based on the results of the *One-Way ANOVA* test in the table above it is known that the significance value (*sig*.) obtained in the *One-Way ANOVA* test is 0.000 (<0.05). So it can be concluded that there is an effect of Ozonated olive oil on the number of lymphocyte cells in the gingiva of male Wistar rats who experience periodontitis. Furthermore, to determine which effect caused a decrease in the number of lymphocyte cells in the gingiva of male Wistar rats with periodontitis, a *Post Hoc Least* Significant Difference (LSD) test was performed. The results of the *Post Hoc Least* Significant Difference (LSD) test in this study are as follows:

Table 5. Test results LSD

Group		*Mean Difference*	Sig.	Information
K(-)3	K(-)5	16,00000*	0,001	Significant
	K(-)7	22,00000*	0,000	Significant
	OZO 3	9,00000*	0,029	Significant
	OZO 5	17,00000*	0,000	Significant
	OZO 7	24,00000*	0,000	Significant
K(-)5	K(-)3	-16,00000*	0,001	Significant
	K(-)7	6,00000	0,132	Not significant
	OZO 3	-7,00000	0,082	Not significant
	OZO 5	1,00000	0,795	Not significant
	OZO 7	8,00000*	0,050	Significant
K(-)7	K(-)3	-22,00000*	0,000	Significant
	K(-)5	-6,00000	0,132	Not significant
	OZO 3	-13,00000*	0,003	Significant
	OZO 5	-5,00000	0,205	Not significant
	OZO 7	2,00000	0,605	Not significant
OZO 3	K(-)3	-9,00000*	0,029	Significant
	K(-)5	7,00000	0,082	Not significant
	K(-)7	13,00000*	0,003	Significant
	OZO 5	8,00000*	0,050	Significant
	OZO 7	15,00000*	0,001	Significant
OZO 5	K(-)3	-17,00000*	0,000	Significant
	K(-)5	-1,00000	0,795	Not significant
	K(-)7	5,00000	0,205	Not significant

	OZO 3	-8,00000*	0,050	Significant
	OZO 7	7,00000	0,082	Not significant
OZO 7	K(-)3	-24,00000*	0,000	Significant
	K(-)5	-8,00000*	0,050	Significant
	K(-)7	-2,00000	0,605	Not significant
	OZO 3	-15,00000*	0,001	Significant
	OZO 5	-7,00000	0,082	Not significant

Based on the results of the *Post Hoc Least* Significant Difference (LSD) test, it was found that the highest mean difference was found in group K(-)3 or the group induced by periodontitis but not given the active ingredient (3 days after induction of periodontitis) with the OZO 7 group or the treatment group which was induced by periodontitis and applied 0.1 ml of Ozonated olive oil (7 days after induction of periodontitis), with an average difference of 24. in the K(-)3 group or the periodontitis-induced group but not given the active ingredient (3 days after periodontitis induction) with the OZO 3 group or the treatment group which was induced periodontitis and 0.1 ml of Ozonated olive oil was applied (7 days after periodontitis induction), with a mean difference of 9.

Discussion

Based on the results of the One-Way ANOVA test, it is known that the significance value obtained is 0.000 (<0.05). So it can be concluded that there is an effect of Ozonated olive oil on the number of lymphocyte cells in male Wistar rats who have periodontitis. Then, based on the results of the study it was also known that the highest mean difference was in the K(-)3 group and the OZO 7 group, where the average difference was 24. This shows that the highest mean difference was in the K(-)3 group or the group induced periodontitis but not given the active ingredient (3 days after periodontitis induction) with the OZO 7 group induced periodontitis and 0.1 ml of Ozonated olive oil was applied (7 days after induction) periodontitis) had a lower number of lymphocyte cells compared to the control group that was not given Ozonated olive oil.

Lymphocytes are chronic inflammatory cells that are specific as a host immune response to the presence of an injury during chronic inflammation [6]. Lymphocytes play an important role in the body's immune system because they have an influence on the immune response, such as the presence of infecting microorganisms and other foreign bodies that cause inflammation [7]. Long-term inflammation will produce an adaptation or specific immune response. Host responses that interact with microbes in periodontitis are carried out by removing inflammatory cells, one of which is lymphocyte cells [3].

Lymphocytes will activate the receptor activator of nuclear factor-kB ligand (RANKL) which will bind to the receptor activator of nuclear factor-kB (RANK). The binding of RANKL to RANK causes differentiation and activation of osteoclasts so that the number of osteoclasts increases and bone resorption occurs [8]. The number of lymphocytes during inflammation will increase and cause damage to collagen, fibronectin, and laminin which contributes to the local destruction of gingival tissue [6].

Ozonated olive oil or olive oil that has been ozonized and applied topically can increase the number of alveolar osteoblasts and blood vessels in the recovery process. Ozonated olive oil can stimulate the release of growth factors such as TGF-B, PDGF, IL-8, and TBX2. These various growth factors will speed up the process of wound recovery and tooth growth [4]. The ozone compound in olive oil has the ability as an antimicrobial which works as a suppression of bacterial growth. The mechanism of ozone oxidation can be involved in a direct reaction of free radical destruction [9].

Ozonized olive oil also functions to reduce infections caused by oral microorganisms found in dental plaque. In addition, ozone has a stronger ability to deactivate microorganisms in periodontal tissue and is good enough to fight microorganisms compared to chlorhexidine. The application of Ozonated olive oil has a strong antibacterial function against periodontal microorganisms and has a potential effect as an additional application after Scaling and root planning (SRP) in the periodontitis healing process which will also reduce the number of lymphocyte cells [10].

4. CONCLUSION

It can be concluded that there is an effect of Ozonated olive oil on the number of lymphocyte cells in male Wistar rats who have periodontitis. The highest mean difference was in the K(-)3 group and the OZO 7 group, where the average difference was 24. This shows that the highest average difference was in the K(-)3 group or the group that induced periodontitis but was not given the active ingredient (3 days after periodontitis induction) with the OZO 7 group that was induced periodontitis and applied 0.1 ml of Ozonated olive oil (7 days after periodontitis induction) had the number of cells fewer lymphocytes compared to the control group that was not given Ozonated olive oil. In addition, there is also a need for further research taking into account the number of samples, the volume of Ozonated olive oil administration, a more diverse length of exposure, so that more complete research results can be obtained regarding the effect of Ozonated olive oil administration on the number of lymphocyte cells in male Wistar rats experiencing periodontitis. There is a need for further research taking into account the number of samples, the volume of Ozonated olive oil administration, a more diverse duration of exposure, so that more complete research results can be obtained regarding the effect of Ozonated olive oil administration on the number of lymphocyte cells in male Wistar rats experiencing periodontitis.

ETHICAL CLEARANCE

This research has received approval from RSUD Dr. Moerwadi Surakarta number 742/V/HREC/2023.

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