

## VITAMIN E PREVENTS OXIDATIVE STRESS AND INFLAMMATION CONDITIONS IN PERIODONTITIS WISTAR RATS

### VITAMIN E MENCEGAH STRES OKSIDATIF DAN KONDISI PERADANGAN PADA TIKUS WISTAR PERIODONTITIS

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#### ABSTRAK

Periodontitis terutama disebabkan bakteri patogen periodontal yang dapat memicu pembentukan radikal bebas yang berlebihan, menyebabkan stres oksidatif sehingga terjadi kerusakan jaringan. Hal ini mempunyai pengaruh bermakna terhadap peningkatan produksi Reactive Oxygen Species (ROS) dalam tubuh dan kerusakan sel pada jaringan alveolar gigi. Penelitian ini bertujuan untuk mengetahui pengaruh vitamin E dalam mengatasi stres oksidatif pada tikus putih strain Wistar yang mengalami periodontitis. Penelitian ini merupakan penelitian eksperimental dengan post test only control group design. Tiga puluh ekor tikus dikelompokkan menjadi 5 kelompok yaitu kontrol negatif (tanpa perlakuan), kontrol positif (diberi silk ligature 14 hari) dan perlakuan 1, 2, dan 3 (diberi silk ligature selama 14 hari, dilanjutkan dengan pemberian vitamin E dengan dosis 20, 40, dan 60 IU selama 10 hari). Pada hari terakhir penelitian dilakukan pengambilan darah untuk pemeriksaan kadar Malondialdehyde (MDA) dan penilaian inflamasi gingiva melalui skor indeks gingiva. Hasil penelitian menunjukkan bahwa rerata kadar MDA pada kelompok kontrol positif yang diberikan silk ligature saja mempunyai kadar MDA tertinggi dibandingkan dengan kelompok kontrol negatif dan semua kelompok perlakuan. Pemberian vitamin E mampu menurunkan kadar MDA serum secara bermakna pada semua kelompok perlakuan ( $p < 0,05$ ). Vitamin E juga mampu menurunkan skor indeks gingiva dibandingkan dengan kelompok kontrol positif pada semua kelompok. Simpulan: Pemberian vitamin E mampu mengatasi stres oksidatif dan inflamasi pada periodontitis yang ditandai dengan penurunan kadar MDA dan skor indeks gingiva.

**Kata Kunci:** Vitamin E, Stress Oksidatif, Inflamasi, Periodontitis

#### ABSTRACT

Periodontitis is mainly caused by periodontal pathogenic bacteria, which can trigger excessive free radical formation, causing oxidative stress and tissue damage. This has a significant effect on increasing the production of Reactive Oxygen Species (ROS) in the body and cell damage in the alveolar tissue of the teeth. This study aimed to determine the effect of vitamin E in overcoming oxidative stress in Wistar strain white rats with periodontitis. This research is an experimental study with a post-test-only control group design. Thirty rats were grouped into five groups, namely negative control (without treatment), positive control (given silk ligature 14 days), and treatments 1, 2, and 3 (given silk ligature for 14 days, followed by administration of vitamin E with doses 20, 40, and 60 IU for ten days). On the last day of the study, blood was collected to check the levels of Malondialdehyde (MDA) and gingival inflammation through the gingival index score. The results showed that the mean MDA levels in the positive control group given silk ligature alone had the highest MDA levels compared to the negative control group and all treatment groups. Administration of vitamin E significantly reduced serum MDA levels in all treatment groups ( $p < 0.05$ ). Vitamin E also reduced the gingival index score compared to the positive control group in all groups. Conclusion: The administration of vitamin E can overcome oxidative stress and inflammation in periodontitis, characterized by a decrease in MDA levels and gingival score index.

**Keywords:** Vitamin E, Oxidative Stress, Inflammation, Periodontitis

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## INTRODUCTION

Periodontal disease is an infectious disease caused by the accumulation of bacteria that can cause inflammation around the teeth supporting tissues, including the gingiva, periodontal ligament, and alveolar bone (Genco and Williams, 2010). If periodontal disease has been going on for a long time and is severe, it can cause pain, discomfort, masticatory disorders, and ultimately tooth loss. Periodontal disease affects humans almost all over the world and reaches 50% of the adult population. Studies have shown that periodontal disease affects the health of the world's population, which is the most significant cause of tooth loss (Glascoe L et al., 2015). Predisposing factors for periodontal disease are stress, nutritional deficiency, systemic disease, alcohol and tobacco consumption, genetics, and plaque accumulation (Quamilla, 2016). The diagnosis of periodontitis is conventionally carried out by measuring periodontal tissue damage, such as examination of clinical attachment level and probing depth (Hong et al., 2019). One of the causes of the increasing prevalence of dental and oral problems in Indonesia is the lack of public awareness in maintaining dental and oral health. Studies of the etiology of the prevention and

treatment of periodontal disease show that this disease can be prevented by maintaining good oral hygiene (Santoso, 2019).

Oxidative stress has been linked as a major contributor to more than 100 disorders and more recently in periodontists (Ayala et al., 2014). The body's antioxidant defense system plays an important role in fighting chronic disease. Inflammation (local or systemic) has been directly associated with periodontal disease (Liu et al., 2014). Measurement of oxidative damage can provide a direct assessment of oxidative stress. Some markers of oxidative stress are Malondialdehyde (MDA), Superoxide dismutase (SOD), Nitric Oxide (NO), Glutathione Peroxidase (GPx), Glutathione Transferase (GST) (Monisha and Savitha, 2016). Malondialdehyde (MDA) is one of the parameters to measure the state of oxidative stress in the body. The higher the plasma MDA level, the higher the oxidative stress that occurs in the cells (Khoubnasabjafari et al., 2015). Malondialdehyde (MDA) is the primary and most studied product of polyunsaturated fatty acid peroxidation, indicating an increase in oxidative stress. Malondialdehyde (MDA) is the main product of polyunsaturated fatty acid peroxidation, which increases the level of

reactive thiobarbituric acid in peripheral blood in chronic periodontitis patients (Ayala et al., 2014).

Excessive oxidative stress conditions in the body are highly reactive and damaging to cell structures in the form of carbohydrates, nucleic acids, lipids, and proteins. In addition, free radicals also change cell function and even cause cell death (Liu et al., 2014). If the endogenous antioxidant system is insufficient, the body needs antioxidants from outside (exogenous) such as vitamins A, C, E, and flavonoid compounds (Kurutas, 2016). Vitamins A, C, and E are compounds that contain antioxidants because they have antibacterial, antiviral, and cell apoptosis-inducing or anti-tumor activities (Birben et al., 2012). Vitamin E is fat-soluble and consists of two isomers: tocopherols and tocotrienols (Mohd Zaffarin et al., 2020). Vitamin E as an exogenous antioxidant can break radical chain reactions (Stolzenberg-Solomon et al., 2009) so that it can inhibit ROS and oxidative stress. (Rodriguez,2019). This study aims to determine the effect of vitamin E on malondialdehyde (MDA) levels and gingival inflammation in Wistar periodontitis strain white rats.

## METHODS

This study used a sample of 30 white male rats of Wistar strain (n= 30), with body weights ranging from 200-250 grams from the Laboratory of the Faculty of Pharmacy, Andalas University. Rats were acclimatized for one week. Food and drinks are available ad libitum. The sample was divided into five groups, and each group consisted of six rats: negative control group, which did not receive any treatment, a positive control group was given a silk ligature to the rat's lower central incisor to induce periodontitis, treatment group 1 (T1), treatment 2 (T2), and treatment 3 (T3), namely rats with periodontitis and given vitamin E with three different doses, namely 20, 40, and 60 IU for ten days. This research has passed the ethics of the Faculty of Medicine, Andalas University with Number 364/KEP/FK/2019.

### *Periodontitis Induction*

The rats were anesthetized using ether, then induced periodontitis by binding a silk ligature to the rat's lower central incisor. The excess wire was tucked at the mesial margin for 14 days, as previously reported (Ionel et al., 2015).

### *Assessment of inflammation with Gingival Index Score*

The gingival index is used to assess the severity of inflammation. Measurements were made on the gingiva on four sides of the examined teeth, namely the distovestibular papilla, the vestibular gingival margin, the mesiovestibular papilla, and the oral gingival margin.

Two experienced researchers assessed inflammation with the Gingival Index Score. The results of the examination of the two researchers were statistically analyzed. It was found that there was no significant difference in the assessment of the Gingival Index Score ( $p > 0.05$ ) between the two researchers.

The criteria for determining the gingival index score are as follows: 0: normal gingiva; 1: mild inflammation of the gingiva characterized by a change in color slight edema; on palpation, there is no bleeding; 2: moderate gingival inflammation, red, edematous, and shiny gingiva on palpation, bleeding occurs ;and 3: Gingival inflammation is severe; the gingiva is bright red, edematous, ulcerated, the gingiva tends to bleed spontaneously.

Formula to calculate Gingival Index Score:

$$\text{Gingival Index Score} = \frac{\text{Total score of all teeth} / 4}{\text{Number of teeth examined}}$$

### *Malondialdehyde Level Measurement*

The malondialdehyde levels were examined using the Thiobarbituric acid reactive substance (TBARS) method. The materials used are serum sample, trichloroacetic acid (TCA) 5%, Na Thio Barbituric Acid, and MDA Standard. In the serum MDA examination, three tubes were provided, namely blanks (0.5 ml of distilled water or aquadest), standard (0.5 ml of standard MDA), and samples (0.5 ml of serum). In each tube, 2.5 ml of 5% TCA was added, then mixed with a vortex, centrifuged for 10 minutes (2000 rpm), repeated centrifugation, then 1.5 ml of filtrate was taken from each tube and put into a new tube. After that, 1.5 ml of Na Thio Barbituric Acid was added to each tube, heated in a water bath for 30 minutes, cooled, and read with a spectrophotometer with a wavelength of 550 nm. The MDA level of the sample was calculated using the formula for the absorbance of the sample multiplied by the standard MDA concentration. (Rita et al., 2009).

### *Statistical Analysis*

Data on MDA levels and gingival index scores were tested for normality. MDA data was tested by Oneway Anova and followed by Post-Hoc Bonferroni, while the gingival index score was tested by Kruskal Wallis and followed by

Post-Hoc Mann Whitney, because the data is not normally distributed. The data is declared meaningful if the p-value < 0.05.

## RESULTS AND DISCUSSION

### *Vitamin E can reduce serum malondialdehyde levels in rats with periodontitis*

Periodontal disease caused by the attachment of ligatures to the gingival sulcus causes an increase in serum malondialdehyde levels. Administration of vitamin E with three different doses (20, 40, and 60 IU) was able to significantly reduce malondialdehyde levels with a p-value of 0.001 (Table.1)

**Table 1. Average Serum Malondialdehyde Levels After Administration Of Vitamin E In Periodontitis Rats**

Groups	Mean ± SD (nmol/ml)	
Negative Control	2.69 ± 4.18	
Positive Control	4.86 ± 4.10	
Treatment 1	3.62 ± 0.73	*p-value < 0.05
Treatment 2	3.46 ± 0.75	
Treatment 3	3.31 ± 0.55	

\*Anova Test Between Group

The results of the Post Hoc Test showed that there was a significant difference in the mean levels of Malondialdehyde between the negative control group and the positive control group, and the positive control group and all

treatment groups (T1, T2, and T3, p-value < 0.05)

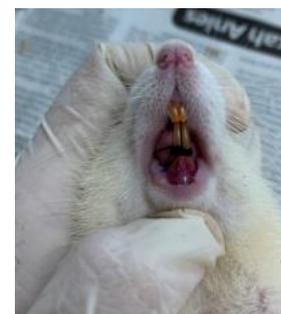
### *Vitamin E reduced the gingival index score in White Rats (Rattus norvegicus) with Periodontitis*

**Table 2. Mean Gingival Index Score After Administration of Vitamin E in Periodontitis Rats**

Group	Median	Min	Max
Negative Control	0	0	0.5
Positive Control	3	2.5	3
Treatment 1	2	2	2
Treatment 2	1.5	1.5	2
Treatment 3	1.25	1	1.5

\*Kruskal Wallis Test Between Group (\*p<0.05)

The Mann-Whitney Post Hoc Test results showed a significant difference in gingival index score and periodontitis between negative control group vs. positive control group, negative control vs. treatment 1, negative control vs. treatment 2, negative control vs. treatment 3, positive control vs. treatment 1, positive control vs. treatment 2, positive control vs. treatment 3, treatment 1 vs. treatment 2, treatment 1 vs. treatment 3, and treatment 2 vs. treatment 3 (p-value < 0.05).



**Figure 1. Determination of Gingival Index Score**

In this study, rats with periodontitis had increased malondialdehyde (MDA) compared to the negative control group, which was not ligated. Administration of vitamin E with three different doses was able to reduce levels of malondialdehyde (MDA) in periodontitis white rats. There were significant differences in MDA levels in the negative control group with the positive control group and the positive control group with treatment 1 (T1), treatment 2 (T2), and treatment 3 (T3). This difference indicates that the higher the level of vitamin E administration, the higher the level of difference in MDA levels. The significant difference between the group that was given wire ligature, the group that was given 20, 40, and 60 IU of vitamin E, and the negative control was due to the presence of vitamin E as a free radical scavenger, thereby reducing oxidative stress as reflected in MDA levels. Vitamin E has a strong defense against ROS and works more effectively at the cellular membrane level and in protection against lipid peroxidation (Trivedi and Lal, 2017).

Vitamin E can neutralize free radicals by donating one electron to form stable compounds and end free radicals.. (Sharifi-Rad et al., 2020). The results of other studies show that there is an

effect of giving vitamin E on reducing MDA levels (Arajibani et al., 2010), and Carvalho's research concludes that vitamin E is able to prevent the formation of MDA (Carvalho et al., 2013). In addition, several studies have provided evidence that vitamin E offers advances in the treatment of various diseases caused by oxidative stress due to its ability to increase antioxidant levels and also found a negative relationship between vitamin E levels and oxidative stress (Nazrun et al., 2012; Tahan et al., 2011). In periodontitis, inflammation is caused by bacteria that destroy the connective tissue and support the alveolar bone of the teeth. Polymorphonuclear leukocytes (PMNs) act as significant mediators of the host response to proliferating periodontal pathogenic microorganisms. Activated PMNs generate large amounts of ROS and result in periodontal tissue destruction (Singh et al., 2017).

In this study, the results showed an effect of vitamin E on gingival inflammation in periodontitis rats. In the study, it can be seen that the groups that are significantly different are all groups, namely the negative control group with all other treatment groups, as well as the positive control group, treatment 1 (T1), treatment 2 (T2) and treatment 3 (T3) with all

other treatment groups. The decrease in the incidence of inflammation, especially in the group given vitamin E, is thought to be caused by the activity of vitamin E in reducing the level of inflammation. Vitamin E has the potential to help control chronic inflammation that can occur in periodontal disease by inhibiting the increase in the production of proinflammatory or inflammatory cytokines such as interleukin-6 (IL-6), Interleukin-8 (IL-8) or Tumor Necrosis Factor (TNF- $\alpha$ ), and by decreasing the production of these proinflammatory cytokines, it will be able to lower the level of inflammation. (Zing, 2018) From these results, it can be seen that the administration of a minimum dose of vitamin E in accordance with this study, namely 20 IU, which was given to rats with periodontitis conditions, would have an impact on gingival inflammation. However, the higher vitamin E administration of 40 and 60 IU resulted in more significant changes. Periodontitis is an inflammatory disease that affects the supporting structures of the teeth leading to loss of alveolar bone and teeth (Könönen et al., 2019).

The main causative factor is the microorganism that colonizes the subgingival dental plaque, which induces an inflammatory

host response. However, the inflammatory infection and the surrounding healthy tissue eventually lead to the destruction of the periodontium. Chronic inflammation in the tissues surrounding the teeth caused by an imbalance between the oral biofilm and the periodontal host response will lead to the loss of the supporting tissues of the teeth (Singh et al., 2017). The use of vitamin E as an adjunct to nonsurgical periodontal treatment has been shown to affect periodontal parameters positively (Behfarnia et al., 2021).

## CONCLUSION

Administration of vitamin E can overcome oxidative stress and inflammation in periodontitis conditions.

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