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Research Report

# Effect of electrolyzed reduced water on Wistar rats with chronic periodontitis on malondialdehyde levels

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***ABSTRACT***

***Background:*** *Periodontal disease is a progressive destructive change that causes loss of bone and periodontal ligaments around the teeth that can eventually lead to its loss. The main bacteria in chronic periodontitis is Porphyromonas gingivalis. Aggregatibacter actinomycetemcomitans, a pathogen associated with aggressive periodontitis, initiates a proinflammatory response that causes tissue destruction of periodontal, alveolar bone resorption and subsequent tooth loss. Electrolyzed reduced water (ERW) is an alkaline water, ERW not only has a high pH and low oxidation reduction potential (ORP), but also contains several magnesium ions. Magnesium ions proven effective for the prevention of various diseases.* ***Purpose:*** *To analyze the level of malondialdehyde (MDA) in Wistar rats with cases of chronic and aggressive periodontitis that consumed ERW.* ***Method:*** *Wistar rats were divided into four groups, each group with 10 rats. The first and second group were Wistar rat with chronic periodontitis and consume drinking water and ERW. The third and fourth group were Wistar rat with aggressive periodontitis and consume drinking water and ERW. This experiment is done by calculating the levels of MDA. The calculation of the levels of MDA is done with spectrophotometric assay for MDA.* ***Result:*** *The results of this experiment show that the level of MDA in serum in group that consume ERW had decreased significantly different with thegroup that consume drinking water with the statistical test.* ***Conclusion:*** *It can be concluded that ERW can decrease the MDA level in Wistar rat with chronic and aggressive periodontitis case.*

***Keywords:*** *chronic periodontitis; aggressive periodontitis; electrolyzed reduced water; malondialdehyde level; Wistar rat*

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## INTRODUCTION

Electrolysis of water produces reduced water at the cathode and oxidized water at the anode. Electrolyzed-reduced water (ERW) has a very negative oxidation-reduction potential. ERW is also called alkali electrolysis water, alkaline-ionic water, alkaline cathode water, and alkaline ionized water, based on its physicochemical and physiological aspects. ERW indicates an alkaline pH, rich in hydrogen molecules, and has a negative oxidation reduction potential (ORP) scavenging activity and reactive oxygen species (ROS) scavenging[1]. ERW with high pH and significant negative redox potential (RP) was shown to have superoxide dismutase (SOD)-like activity and catalase-like activity, and thus, scavenge active oxygen species and protect DNA from damage by oxygen radicals in vitro.[2]

Bioactivity of ERW is its antioxidant activity. ERW mimics the activity of antioxidant enzymes, such as SOD and catalase (CAT) by scavenging ROS. Cellular oxidative damage to DNA, RNA, and protein molecules caused by ROS can be markedly opposed by ERW. Additionally, ERW has a therapeutic effect on various diseases, including diabetes, tumors, and renal disease. Reduced water shows high pH, low dissolved oxygen (DO), very high molecular dissolved hydrogen (DH), and highly redox potential (RP) values. Reduce water, as well as catalase and ascorbic acid, can directly scavenge H2O2. Reducing water suppresses the damage of single stranded DNA by the active oxygen

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species produced by the oxidation of ascorbic acid Cu (II) by dose dependent, indicating that reduced water can scavenge not only $O_2$ and $H_2O_2$, but also $O_2$ and OH.[1,2]

Periodontitis can be further classified as chronic or aggressive. Chronic periodontitis usually has a slow to moderate rate of progression, with local and systemic factors such as plaque, calculus, smoking, and diabetes often contributing to the disease. *Porphyromonas gingivalis*, a Gram-negative anaerobe bacteria, has been frequently isolated from lesions in chronic periodontitis patients and is considered an etiological agent of the disease.[3] Aggressive periodontitis is a type of periodontitis in which periodontal ligament destruction and rapid alveolar bone occur in healthy systemic individuals generally in younger age groups but patients may be older.[4] Although the prevalence of aggressive periodontitis is much lower than for chronic periodontitis, management of aggressive periodontitis is more challenging than chronic periodontitis due to its strong genetic predisposition as an unmodifiable risk factor. Although the prevalence has been reported to be much smaller than for chronic periodontitis, this may result in loss of teeth in affected people if not diagnosed at an early stage and treated appropriately.[5] Aggressive periodontitis can be distinguished from chronic periodontitis by age onset, rapid disease progression rate, and related subgingival microflora composition, changes in host immune response, and family aggregation of diseased individuals.[6]

The presence of inflammation of chronic periodontitis have resulted in an influx of immune cells use a lot of oxygen, causing excess reactive oxygen species (ROS) production.[7] Oxidative stress causes oxidative damage to lipids that can be detected by elevated levels of malondialdehyde (MDA) in the cells.[8] In normal circumstances in cell there is a balance between ROS generation and antioxidant activity.[9,10] If there is interference on the balance it will cause oxidative stress that can damage cell components. This research aims to study the role of ERW to MDA level which is one biomarker of their oksidative stress on Wistar rats with chronic periodontitis.

## MATERIALS AND METHODS

This study using Wistar rats as animals model. Fourty Wistar rats divided into four groups, ten Wistar rat as the first group was Wistar rats with chronic periodontitis that induced with *Porphyromonas gingivalis* bacteria and consume ERW (pH8.5). Second group was ten Wistar rats with chronic periodontitis and consume with drinking water. The other group were Wistar rat that induced with *Aggregatibacter actinomycetemcomitans* as a aggressive periodontitis models. Twenty Wistar rat with aggressive periodontitis consume ERW and drinking water. MDA samples for examination were taken from Wistar rat blood. As much as 5 cc of blood drawn by using a syringe inserted into tubes that had contained EDTA, then centrifuged at 3500 rpm for 5 minutes. Liquid blood plasma that has been separated from the solid part of blood was transferred to MDA microplate for examination. MDA level measurement is done using ELISA method with a kit MDA- 586 BIOXYTECH on spectrophotometry.

## RESULT

In chronic periodontitis case, Kolmogorof Smirnov test showed that the data were normally distributed ($p<0.05$) and the value Levene test shows that the data homogeneous ($p>0.05$) so that it can proceed with different test One-way ANOVA. One-way ANOVA test results show the value of $P<0.05$ thus concluded that there are significant differences between all groups. Then followed by Tukey HSD post hoc test to see differences in each group. There are significant differences ($p<0.05$) between the control group 7 days with treatment group seven days, as well as the control group and the treatment group 14 days to 14 days. The results showed a decrease in MDA levels of a group of Wistar rats with chronic periodontitis by ERW for 7 and 14 days than in the group given drinking water (Table 1). In table 1 shows

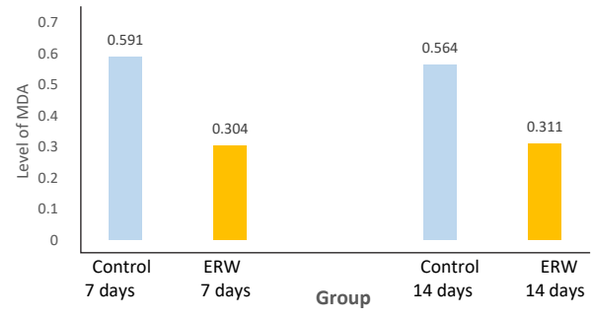


**Figure 1.** Mean number of MDA levels in Wistar rat.

**Table 1.** Mean and standart deviation of MDA levels in Wistar rat with chronic periodontitis that consume ERW and drinking water

| Group | Level of MDA (μM) | SD | Kolmogorof Smirnov | ANOVA | p |
|---|---|---|---|---|---|
| Control group (7 days) | 0.591 | 0.032 | 0.644 | 0.193 | 0.000 |
| ERW (7 days) | 0.304 | 0.026 | 0.991 | | |
| Control group (14 days) | 0.564 | 0.057 | 0.991 | | |
| ERW (14 days) | 0.311 | 0.033 | 0.999 | | |

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significantly differences between the groups of Wistar rats with chronic periodontitis with ERW administration for 7 days and 14 days compared to the group with drinking water same at Figure 1.

Kolmogorof Smirnov test in Aggressive periodontitis showed that the data were normally distributed ($p<0.05$) and the value levene test shows that the data are not homogeneous ($p<0.05$) and so can not proceed with different test One-way ANOVA. Different test done using Kruskal Wallis test. Kruskal Wallis test results show the value of $p<0.05$ thus concluded that there are significant differences between all groups. Then proceed with the test Mann-witney to see the difference in the two groups. There are significant differences ($p<0.05$) between the control group 7 days with treatment group and control 7 days to 14 days; the control group and the treatment group 14 days to 14 days; as well as the treatment group and control 7 days to 14 days (Table 2). The results showed a decrease in MDA levels of a group of Wistar rats with aggressive periodontitis by ERW for 7 and 14 days than in the group given drinking water (Table 2). In Table 2 shows significantly differences between the groups of Wistar rats with aggressive periodontitis with ERW administration for 7 days and 14 days compared to the group that consume drinking water.

## DISCUSSION

In the group of Wistar rats with chronic and aggressive periodontitis that administration by ERW decreased levels of MDA, it will show a decrease in ROS levels due in normal circumstances there is a balance between ROS and antioxidant activity in cell. If the balance is disrupted will cause oxidative stress which can causing damage to the cell components. One of the damages caused by the condition oxidative[5-7] produce a number of compounds such as epoxides, hydrocarbons and aldehydes. Between aldehyde compounds produced were MDA. Some antioxidants endogenous that acts to prevent the occurrence of oxidative damage is the MnSOD, catalase and reduced glutathione (GSH).

The decrease of MDA in groups of Wistar rats with chronic and aggressive periodontitis that administration by ERW at 7 and 14 days showed the role of ERW that have the potential of oxidation and reduction of low and high pH capable of eliminating reactive oxygen in cells and were able to cause damage to the plasmid DNA of bacteria, this situation is consistent with research on the Park *et al.* in 2012.[1,11] ERW produces reduced water at the cathode and oxidized water at the anode. ERW has an extremely negative oxidation-reduction potential. ERW scavenges cellular reactive oxygen species (ROS) and suppresses single-strand breaks of plasmid DNA in bacteria.[11]

Molecular hydrogen has ability as an effective antioxidant treatment,[12] based on its free radical scavenger properties, and has been successfully used in a variety of pathological conditions involving acute oxidative stress.[13,14] The main molecular target of molecular hydrogen is not clearly understood. The main mechanism of action is advised to rinse hydroxyl radicals (HO) and peroxynitrite (ONOO) in particular, thereby reducing oxidative damage to membrane lipids and DNA.[9] In addition, recent reports show a consistent effect on gene and protein expression and phosphorylation.[15,16] Based from these research it can be concluded that the ERW administration for 7 days and 14 days resulted in decreased levels of MDA in blood of Wistar rats with chronic and aggressive periodontitis.

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**Table 2**. Mean and standart deviation of MDA levels in Wistar rat with aggressive periodontitis that consume ERW and drinking water

| Group | Level of MDA (μM) | SD | Kolmogorof Smirnov | ANOVA | p |
|---|---|---|---|---|---|
| Control group (7 days) | 0.646 | 0.041 | 0.995 | 0.001 | 0.000 |
| ERW (7 days) | 0.357 | 0.118 | 0.749 | | |
| Control group (14 days) | 0.842 | 0.066 | 0.930 | | |
| ERW (14 days) | 0.212 | 0.053 | 0.991 | | |



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