Kafkas Univ Vet Fak Derg
25 (4): 539-544, 2019
DOI: 10.9775/kvfd.2018.21284

Kafkas Universitesi Veteriner Fakultesi Dergisi
ISSN: 1300-6045 e-ISSN: 1309-2251
Journal Home-Page: http://vetdergikafkas.org
Online Submission: http://submit.vetdergikafkas.org

Research Article

The Ability of Electrolyzed Reduced Water to Act as an Antioxidant and Anti-Inflammatory Agent in Chronic Periodontitis Wistar Rats *(Rattus novergicus)*

Rini Devijanti RIDWAN [1,a] Tantiana TANTIANA [1,b] Darmawan SETIJANTO [2,c]
Astika Kinant KUSUMA [3,d] Ananda Firman PUTRANTO [4,e]

[1] Oral Biology Department, Faculty of Dental Medicine, Universitas Airlangga, Oral Biology, Surabaya, INDONESIA
[2] Dental Public Health, Fculty of Dental Medicine, Universitas Airlangga, Public Health, Surabaya, INDONESIA
[3] Faculty of Dental Medicine, Universitas Airlangga, Surabaya, INDONESIA
[4] Orthodontics Department, Faculty of Dental Medicine, Universitas Airlangga, Ortodontics, Surabaya, INDONESIA
[a] ORCID: 0000-0002-0090-9746; [b] ORCID: 0000-0001-5185-1647; [c] ORCID: 0000-0001-7182-2712; [d] ORCID: 0000-0001-7488-9517
[e] ORCID: 0000-0002-0960-8739

Article ID: KVFD-2018-21284 Received: 01.11.2018 Accepted: 25.02.2019 Published Online: 01.03.2019

How to Cite This Article

Ridwan RD, Tantiana T, Setijanto D, Kusuma AK, Putranto AF: The ability of electrolyzed reduced water to act as an antioxidant and anti-inflammatory agent in chronic periodontitis Wistar rats *(Rattus novergicus). Kafkas Univ Vet Fak Derg,* 25 (4): 539-544, 2019.
DOI: 10.9775/kvfd.2018.21284

Abstract

The chronic periodontitis was inflammation characterized by macrophage activation which releases various metabolites such as ROS that, in turn, produces malondialdehida (MDA) a biological biomarker of lipid peroxidation. The aim of this research is to analyze the effect of Electrolyzed Reduced Water (ERW) on the levels of malondialdehyde, macrophage and lymphocytes cells in Wistar rats suffering from chronic periodontitis. It constitutes an experimental laboratory study incorporating a random sampling method. Twenty-one Wistar rats were induced with up to 10^6 *Porphyromonas gingivalis* in the proximal area of the mandibular molar and divided into three groups which were administered orally on a daily basis as follows: a control negative group (distilled water); a control positive group (a dose of vitamin C at 1.08 mg/200 g Body Weight/day) and a treatment group (20 mL ERW) and observed between Day 1 and Day 14. Samples of gingival tissue were taken from the subjects for analysis with malondialdehyde and the conducting of macrophage and lymphocyte cell counts on Day 14. Data analysis comprised a One-Way Analysis of Variance (ANOVA) and a Mann Whitney test ($P<0.05$) based on a Saphiro-Wilk normality test and a Levene Test for Equality of Variances or a Kruskal Wallis test ($P>0.05$). ERW can decrease the level of malondialdehyde (4.3±0.7), the number of macrophage cells (19.4±8.6) and the number of lymphocyte cells (8.5±0.8) in the treatment group. The ERW mechanism can, therefore, be said to suppress the occurrence of further tissue damage triggered reactive oxygen species (ROS) in Chronic Periodontitis.

Keywords: *Anti-inflammatory, Reactive oxygen species, Electrolyzed reduced water, Malondialdehyde, Periodontitis*

Kronik Periodontitli Wistar Sıçan *(Rattus Novergicus)*'larda Elektrolize İndirgenmiş Suyun Antioksidan ve Antiinflamatuvar Etkisi

Öz

Kronik periodontitis, ROS gibi çeşitli metabolitleri salan ve böylece lipit peroksidasyonun biyolojik bir biyobelirteci olan malondialdehit (MDA)'in üremesine neden olan makrofajların aktivasyonuyla karakterize bir yangıdır. Bu çalışmanın amacı; kronik periodontitli Wistar sıçanlarda elektrolize indirgenmiş su (EİS)'yun malondialdehit seviyesi ile makrofaj ve lenfosit sayılarına etkilerini araştırmaktır. Çalışma deneysel olup rastgele örnekleme metodu kullanılmıştır. Yirmi bir Wistar sıçanın mandibular molar dişlerinin proksimal bölgelerine 10^6 *Porphyromonas gingivalis* uygulandı ve günlük ağız yoluyla belirtilen uygulamaları gerçekleştirmek için üç gruba ayrıldı; Kontrol negatif grubu (distile su), Kontrol pozitif grubu (1.08 mg/200 g vücut ağırlığı/gün Vitamin C) ve Uygulama grubu (20 mL EİS). Gruplar 1. günden 14. güne kadar gözlemlendi. Deneklerden gingival doku örnekleri 14. günde alınarak malondialdehit analizi ile makrofaj ve lenfosit sayımları yapıldı. Verilerin analizi Saphiro-Wilk normalite testi ve Varyansların eşitliği için Levene Testi veya Kruskal Wallis testine ($P>0.05$) dayanarak Tek yönlü varyans analizi (ANOVA) ve Mann Whitney testi ($P<0.05$) ile yapıldı. EİS uygulaması malondialdehit seviyesi (4.3±0.7) ile makrofaj (19.4±8.6) ve lenfosit (8.5±0.8) sayılarında düşmeye neden oldu. EİS'nin kronik periodontitte reaktif oksijen türlerinden kaynaklanan doku hasarını baskılayarak etki ettiği söylenebilir.

Anahtar sözcükler: *Antiinflamatuvar, Reaktif oksijen türleri, elektrolize indirgenmiş su, Malondialdehit, Periodontitis*

İletişim (Correspondence)
+62 31 5992785
rini-d-r@fkg.unair.ac.id

INTRODUCTION

Chronic periodontitis is a periodontal tissue inflammation characterized by migration of junctional epithelium to the apical, periodontal ligament attachment and alveolar bone loss [1,2]. The chronic inflammation is characterized by macrophage activation which releases various metabolites such as Reactive Oxygen Species (ROS) that, in turn, produces Malondialdehida (MDA) a biological biomarker of lipid peroxidation [3].

The administering of vitamin C to patients suffering from periodontitis represents an adjuvant therapy to accelerate the healing process by decreasing ROS levels in the inflammed area. Nevertheless, excessive amounts of vitamin C can prove harmful to the body and potentially become free radicals [4].

Based on previous research, chronic inflammation stimulates ROS production which must be suppressed. Electrolyzed Reduced Water (ERW) at a pH value of 9.8 has been used as chronic disease therapy for conditions such as gastrointestinal conditions, hypertension, diabetes and cancer [5]. ERW at a pH range between 8.5 and 9, may accelerate the healing process in cases of chronic inflammation [6].

Electrolyzed Reduced Water, which is produced by an electrolysis machine that renders the water pH alkaline, also plays a role in inducing detoxification, antioxidation and hydration, regulating blood pressure, promoting metabolism in addition to maintaining bone health [7]. It reduces chronic inflammation by donating active hydrogen atoms to unpaired electron bonds derived from ROS in the tissues. Consequently, the number of free electrons which are present in the tissues decreases [4].

The aim of this research is to analyze the mechanism effect of ERW on the MDA level and the number of macrophage and lymphocyte cells in Wistar rats suffering from chronic periodontitis.

MATERIAL and METHODS

Ethical Clearance and Study Design

This study received ethical clearance approval, number 174/KKEPK.FKG/VIII/2016, relating to animal subjects from the Ethics Research Committee, Faculty of Dental Medicine, Universitas Airlangga. The research constituted analytical observation involving the use of a laboratory experiment and a cross-sectional methodology.

Animal Model

This research was conducted at the Microbiology Laboratory, Faculty of Dental Medicine, Universitas Airlangga and the Biomedic and Parasitology Laboratory, Faculty of Medicine, Brawijaya University. The samples, consisting of 21 male Wistar rats selected by random sampling and weighing between 150 and 200 grams, were assigned to one of three groups: a control negative group, a control positive group and a treatment group. Each of these three groups was itself further sub-divided into three groups which were fed orally and subjected to observation on a daily basis between day 1 and day 14. The seven members of each group were fed from a small container twice a day, once in the morning and again in the afternoon. They drank up to 20 mL (4x5 mL) per day via a small pipe attached to a bottle of water. In order to induce chronic periodontitis in members of the treatment group, they were inoculated with 10^6 CFU of *P. gingivalis* ATCC 33277 bacteria in 30 mL (0.03) of PBS. The bacteria were administered to the proximal area of the mandibular molars once every three days over a period of two weeks by means of a disposable 0.5 cc syringe. The symptoms of chronic periodontitis induced by *P. gingivalis* ATCC 33277 included inflammation of the gingival, gingival hyperplasia, pocket formation and periodontal attachment loss within the affected area. The negative control group was provided with 20 mL (4x5mL) of distilled water per day, while the positive control group received a daily 1.08 mg/200 g BW/dose of vitamin C solution. The treatment group received a daily dose of 20 mL (4x5 mL) of ERW at a pH of 9.8.

Histopathology Examination

Hematoxylin Eosin (HE) staining was performed to determine the number of macrophages and lymphocytes present. In addition, gingival tissue from the left side of the jaws of the subjects was reduced to a powder. MDA levels were subsequently examined by homogenizing the tissues with cold phosphate buffer (50 mM) before inserting them into the assay tubes and adding 10 µL of 31 Probucol. 200 µL of the existing standard samples were also introduced into the assay tubes. R1 reagents of MDA-586 kit (MDA BIOXYTECH 586) were added before being vortexed. 150 µL of R2 reagent was subsequently added to each tube prior to re-vortexing.

Measurement of Malondialdehyde

The next stage consisted of incubating all control and treatment group tubes at a temperature of 45ºC for 60 min after which the samples were centrifuged at 10.000x for ten min to separate the supernatants. These were subsequently inserted into a cuvette in order to permit reading of the absorbance values at a wavelength of 532 nm. The MDA levels were observed and compared using a spectrophotometer at the same wavelength. The absorbance data derived was then calculated using alogarithm formula.

Statistical Analysis

Data was analyzed using Statisitical Package for Social Science (SPSS) version 17.00 (IBM SPSS, Chicago, USA). Analysis of Variance was performed ($P<0.05$) based on

Levene's variance of homogeneity test and Saphiro-Wilk normality test result (P>0.05).

RESULTS

The quantitative data obtained confirmed the MDA levels in each group. ERW reduced MDA levels *(Fig. 1)* and, therefore, also the number of macrophages and lymphocytes *(Fig. 2; Fig. 3)*. The results of Histopathlogy examination in terms of the number of macrophage and lymphocytes can be seen in *Fig. 4*. While there were significant differences in macrophage and lymphocyte cell numbers between the groups P=0.000 (P<0.05), those in the MDA level P=0.670 (P>0.05) were insignificant.

DISCUSSION

Based on the statistical test results, there was no significant difference in the MDA levels between groups possibly due to not all of the samples having been placed in the assay

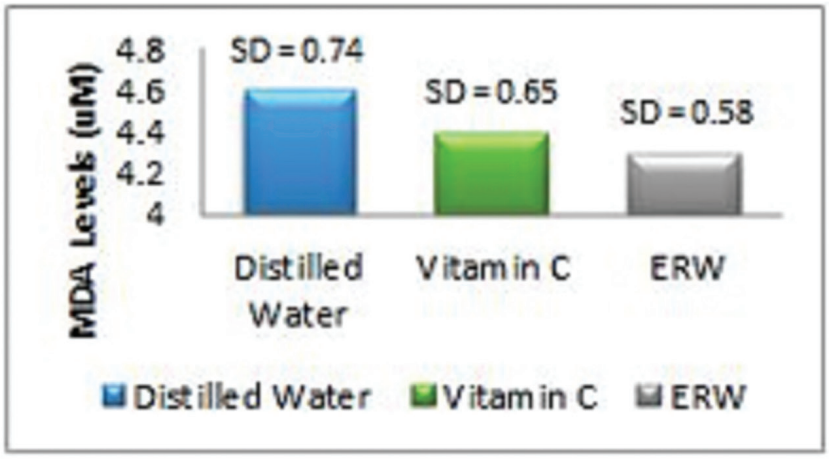

Fig 1. Mean MDA levels in each group

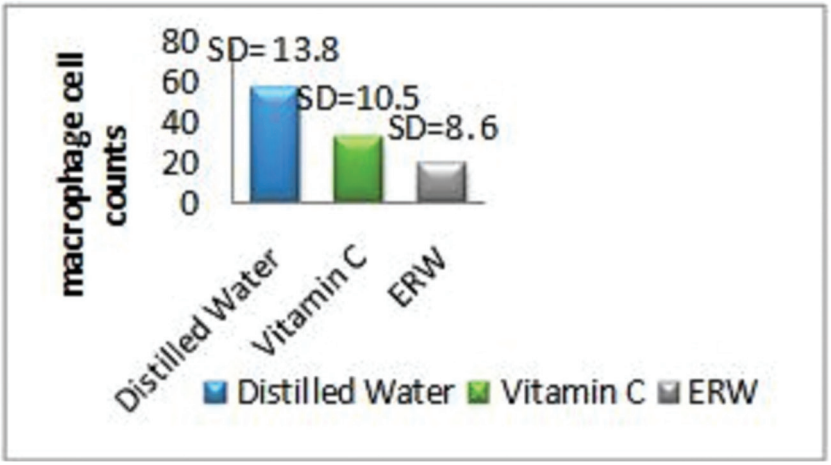

Fig 2. Mean number of macrophage cells in each group

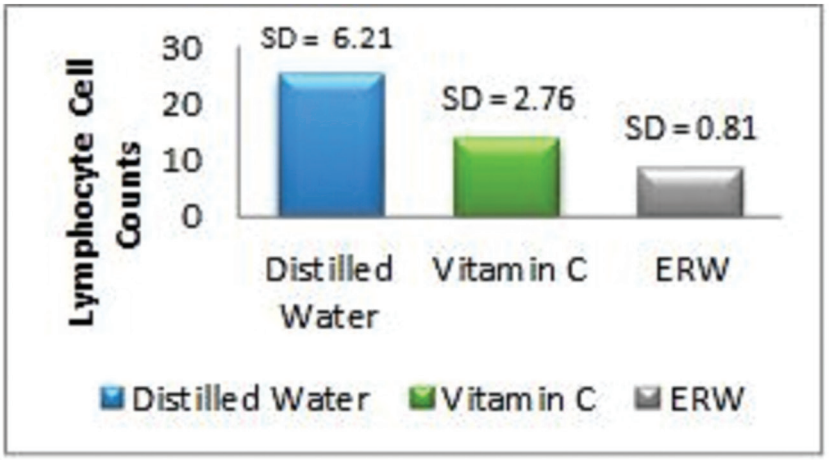

Fig 3. Mean number of lymphocyte cells in each group

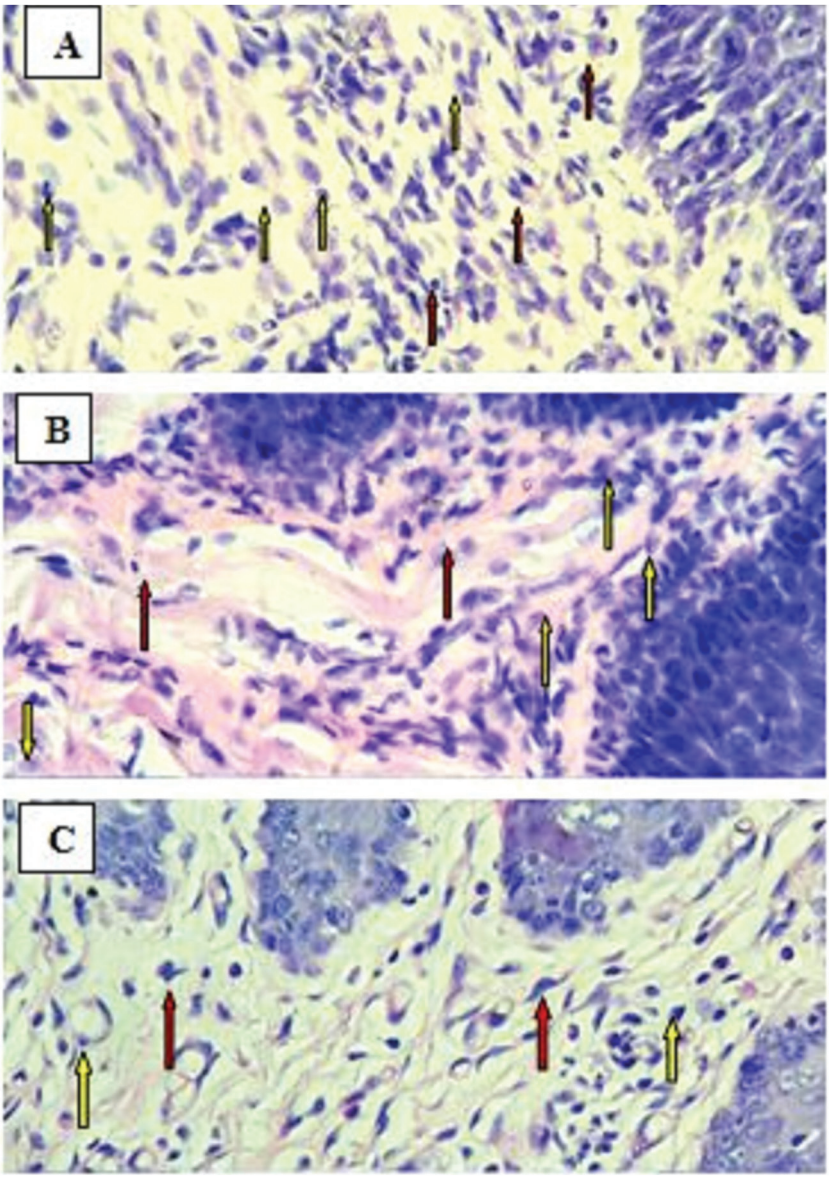

Fig 4. HPA examination results in the gingival tissues at 400x magnification for Macrophage *(Yellow arrows);* Lymphocyte *(Red arrows);* (A) Negative control group; (B). Positive control group; (C) and the Treatment group

tubes. The presence of certain samples differing from the standard color meant that the data obtained was not significant. This insignificant result could have occurred due to a decrease in ERW antioxidant activity. A previous study by Henry and Chambron argued that ERW is affected by heat and, if not consumed immediately, can affect the stability of active hydrogen atoms [4]. Significantly, during the research reported here, ERW antioxidant activity decreased. The data obtained also indicated differences in the mean MDA level. However, in general, the results showed that ERW produced anti-ROS antioxidant activities in cases of chronic periodontitis affecting Wistar rats. These findings concurred with those of research conducted by Freeman [7] which posited that ERW exerts an antioxidant effect.

Shirahata et al.[8], state that ERW, as an antioxidant, contains NaOH with high levels of hydrogen molecules and abundant Pt nanoparticles. At the time that Pt nanoparticles are synthesized, they produce a compound of O_2, OH and H_2O. Pt nanoparticles will activate the hydrogen molecules through catalytic processes and can also demonstrate both antioxidant activity, for example, that of superoxide anions which is identical to the function of superoxide enzymes, and antioxidant enzyme activity such as that of catalases. Moreover, Pt nanoparticles can stimulate the reducibility of antioxidants, although they demonstrate weak auto-oxidant activities [7,9].

The ability of ERW to release hydrogen atoms is reinforced in line with a study by Choi [10] confirming that ERW possesses a hexagonal structure. As a result, ERW can easily provide hydrogen atoms as active components by penetrating the cell components in the tissue. As a compound, ROS has a free electron bond which will stabilize when ERW is applied to the inflamed tissue. Therefore, it can be said that the ERW mechanism is capable of preventing the occurrence of further tissue damage triggered by ROS [8,9].

In this study, the chronic inflammatory cells examined were macrophages and lymphocytes. The statistical test results showed there to be a significant difference in the mean numbers of macrophages and lymphocytes between the groups of Wistar rats suffering from chronic periodontitis. This result indicates that ERW plays an anti-inflammatory function as explained in a previous study conducted by Lee et al.[11] This proves that ERW has a positive effect on immune response through a preventative process against cellular protein breakdown in lymphocytes as inflammatory cells. Similarly, a study conducted by Dovi states that ERW may inhibit COX-2 expression by decreasing proinflammatory cytokines, namely; IL-1 and TNF-α. Decreasing or inhibiting IL-1 and TNF-α synthesis can lead to reduced stimulation of cell membrane phospholipids with the result that arachidonic acid cannot be released from the cell membrane phospholipids by phospholipase activation. This condition triggers both reduced COX-2 protein synthesis and prostaglandin biosynthesis culminating in a decreased inflammatory response [12,13].

Based on the results of the MDA level measurement, the number of macrophages and lymphocytes in the control group was used as a standard to determine more specifically the antioxidant effects of ERW. Vitamin C, which has been used as adjuvant therapy for chronic periodontitis treatment, was employed as another antioxidant source. The blood plasma of patients suffering from chronic periodontitis contained lower vitamin C levels than those under normal conditions [13]. Vitamin C was considered an antioxidant because it can be donated electron thus, prevent the formation of other compounds through the oxidation process by releasing the carbon chain. After donating electrons to the free radicals, vitamin C will be oxidized to semi-dehydroascorbut acid or ascorbyl radicals that are relatively stable, rendering it an antioxidant. In other words, ascorbic acid can react with free radicals, reducing reactive free radicals to non-reactive free radicals. The free radicals experiencing a reduction from reactive ones to non-reactive ones are called scavengers or sequencers [14].

The results of this study showed that ERW had antioxidant and anti-inflammatory capabilities superior to vitamin C, since the pH of ester C used as the third generation of vitamin C is extremely similar to that of water, while ERW has an alkaline pH. A study conducted by Arifah [6] showed an increase in the number of macrophages and lymphocytes treated with ERW because of the balance between the acidity of the tissue and the alkalinity of the drinking water. Inflamed tissue possesses a more acidic pH than normal tissue due to an anaerobic metabolism process which is a response to chronic inflammation involving an increased demand for the energy necessary for homeostasis [5,15,16].

Light visible spectrophotometry (UV-Vis) at a wavelength of 532 nm was employed to observe the binding activity of α-diphenyl-β-picrylhydrazyl (DPPH) as an antioxidant marker. The working principle of UV-VIS spectrophotometry is that light will be absorbed by the solution in the cuvette, although some rays are also transmitted [15]. The light absorbance values produced contained heterogeneous numbers confirming that the statistical test results were not significant. Several factors can influence spectrophotometer measurement results such as poor control during sample processing prior to calculation of the visible spectrophotometer readings. This results in heterogeneous sample colors despite the treatment of the samples being consistent. This error could occur due to differences in sample volumes in the assay tubes before MDA levels were measured by spectrophotometer. ERW can act as anti-inflammatory agent with reduced antioxidant activity in Wistar rats suffering from chronic periodontitis. The ERW mechanism can suppress the occurrence of further tissue damage triggered by ROS. However, further study is required to confirm this conclusion.

Acknowledgement

The research was self-funded by the authors who would like to thank the Oral Biology Department, Faculty of Dental Medicine, Universitas Airlangga for its support.

Conflict of Interest

The authors confirm that there is no conflict of interest in this study.

REFERENCES

1. Hamrun M, Nurlindah H, Hatta M: Vitamin D gene receptor polymorphism on chronic periodontitis patient. *MSc Thesis,* Department of Oral Biology, Faculty of Dentistry, Hasanuddin University, Makassar, 165-172, 2011.

2. Mane AK, Karmakar AP, Bharadwaj RS: Anaerobic bacteria in subjects with chronic periodontitis and in periodontal health. *J Oral Health Comm Dent*, 3 (3): 49-51, 2009.

3. Susanti A, Yuniastuti Y, Iswari R: Reactive oxygen species activity and makrofag after aloe vera gel stimulation in *Salmonella typhimurium* infection. *J Math Nat Sci,* 35 (1): 2-20, 2012.

4. Purwantaka P: Validation of deoxyribose methods as a radical test of arrest hydroxyl free by vitamin C *in-vitro. MSc Thesis,* Fakultas Farmasi Universitas Sanata Dharma Yogyakarta, 23-30, 2005.

5. Chambron J: Physico-chemical, biological and therapeutic characteristics of electrolyzed reduced alkaline water (ERAW). *Water*, 5, 2094-2115, 2013. DOI: 10.3390/w5042094

6. Arifah RA: The effect of alkaline drinking water to increase the number of plasma cells in chronic inflammation. *Undergradutate Thesis,* Fakultas Kedokteran Gigi. Universitas Airlangga Surabaya, 35-47, 2015.

7. Freeman KJ: The alkaline lifestyle approach to healing diabetes. **In**, *Carlsbad, Alkaline People Publishing*. 1st ed., 37-40, Hawking Book, Corona, California, United, 2014.

8. Shirahata S: Advanced research on the healt benefit of reduce water. *MSc Thesis,* Department of Bioscience and Biotechnology, Faculty of Agriculture, Kyushu University, Japan, 125-127, 2012.

9. Kasuyama K, Tomofuji T, Ekuni D, Tamaki N, Azuma T, Irie K, Endo Y, Morita M: Hydrogen-rich water attenuates experimental periodontitis in rat model. *J Clin Periodontol*, 38 (12): 1085-1090, 2011. DOI: 10.1111/j.1600-051X.2011.01801.x

10. Choi W, Hee-Souk K, Hyeon YL: Enhancement of anti-skin inflammatory activities of scutellaria baicalensis using an alkaline reduced water extraction process. *MSc Thesis,* Department of Medical Biomaterials Engineering, Kangwon National University, Chuncheon, Gangwon, Korea, 200-701, 2014.

11. Lee MY, Kim YK, Ryoo KK, Lee YB, Park EJ: Electrolyzed-reduced water protects agains oxidative damage to DNA, RNA, and protein. *Appl Biochem Biotechnol*, 135 (2): 133-144, 2006.

12. Ohsawa I, Ishikawa M, Takahashi K, Watanabe M, Nishimaki K, Yamagata K, Katsura K, Katayama Y, Asoh S, Ohta S: Hydrogen acts as a therapeutic antioxidant by selectively reducing cytotoxic oxygen radicals. *Nat Med*, 13, 688-694, 2007. DOI: 10.1038/nm1577

13. Dovi JV, Szpaderska AM, DiPietro LA: Neutrofil function in the healing wound: adding insult to injury, burn and shock trauma institute. Illinois, USA. *MSc Thesis,* Departement of Surgery, Loyoda University Medical Center Maywood, 275-280, 2006.

14. Staudte H, Sigusch BW, Glockmann E: Grapefruit consumption improves vitamin C status in periodontitis patients. *Br Dent J*, 199, 213-217, 2005. DOI: 10.1038/sj.bdj.4812613

15. Muhammad I: Vitamin C antioxidant effects on male wistar rats *(Rattus norvegicus* L) affected by smoking smoke. Postgraduate School. *MSc Thesis*, Institute Pertanian Bogor, 20-25, 2009.

16. Aghazy AZZ: The antioxidant effect of green tea extract *(Camellia Sinensis* L.) chronic periodontitis patient's saliva. *Undergradutate Thesis,* Fakultas Kedokteran Gigi. Universitas Airlangga Surabaya Thesis, 50-65, 2015.