Antioxidant Effect of Red Dragon Fruit Peel (*Hylocereus polyrhizus*) Extract in Chronic Periodontitis Rats

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Abstract

The aim of this study is to prove the effect of red dragon fruit (*Hylocereus polyrhizus*) peel extract on decrease malondyaldehyde level of gingival tissue in chronic periodontitis rats. The experiment was held by post test only control group design. Wistar rats divided into 9 groups. K- is control group of healthy rats, K+ is control group of periodontitis rats given Sodium Carboxymethyl Cellulose (CMC-Na) and P is treatment group of periodontitis rats given gel of red dragon fruit extract for 3 days and 7 days with concentration P1 (1 mg/mL/day), P2 (2 mg/mL/day), and P3 (4 mg/mL/day). After treatment, the rats were sacrificed and gingival tissues malondyaldehyde level of each group was measured by thiobarbituric acid (MDA-TBA) method. The result of data were analyzed using one way ANOVA ($p<0,05$). The study showed significant decrease on MDA level by 4 mg/mL concentration at 3 days ($p<0,05$) and significant decrease on MDA levels by 1 mg/mL, 2 mg/mL, and 4 mg/mL concentration at 7 days ($p<0,05$). The conclusion that the red dragon fruit peel extract can decrease malondialdehyde levels of gingival tissues in chronic periodontitis rats.

Experimental article (J Int Dent Med Res 2019; 12(4): 1363-1367)

Keywords: Hylocereus polyrhizus, malondialdehyde, chronic periodontitis.

Received date: 05 November 2018 **Accept date:** 12 May 2019

Introduction

Chronic periodontitis is a chronic oral disease common in adults and due to plaque accumulation, pathogenic-bacterial interactions with host immune response, systemic factors, and environmental factors resulting in the destruction of periodontal tissue.[1,2] The incidence of chronic periodontitis was reported to be high in Indonesia, and prevalence was increasing on systemic disease.[3] Study conducted by Mahalakshmi indicated that the bacteria Porphyromonas gingivalis is the most dominant bacteria in chronic periodontitis with the prevalence of about 80,5%.[4] Several recent studies have linked reactive oxygen species (ROS), antioxidant activity, and oxidative stress to the pathogenesis of periodontitis. In chronic inflammatory conditions such as chronic periodontitis, there is an increase in the amount of ROS produced by neutrophils in response to pathogenic bacteria, but excessive ROS production will cause oxidative stress and will result to the destruction of periodontal tissue and cell death.[5,6] Frequently-used criterions in determining oxidative damage associated with chronic periodontitis are lipid peroxidation and malondialdehyde (MDA) which are the end products of lipid peroxidation and is often used as an indicator of oxidative stress.[2,7]

Antioxidant therapy is now widely used as one of the treatment of chronic disease. The use of natural antioxidants is more desirable because of more compatible, cheaper, and less harmful effects to the body. Natural antioxidant compounds found in plant parts such as peel, stems, and leaves.[8] One of the plants that contain antioxidants is a red *H. polyrhizus* which is commonly known as dragon fruit. It is found in abundance in Indonesia and has many benefits to health, despite of lacking utilization of its epidermis part.[9] The peel of red *H. polyrhizus* has a greater antioxidant capacity because the total of its phenolic content is higher than its fruit flesh. Phenol compounds affect the oxidation

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process by reacting with free radicals, binding metal ions, and capturing radical oxygen.[10] The purpose of this study was to prove the effectiveness of the peel extract of red *H. polyrhizus* on the decrease in malondialdehyde levels in chronic periodontitis in rats.

Material and Methods

This study was approved by The Health Research Ethical Clearance Commission of the Faculty of Dental Medicine, Universitas Airlangga, Indonesia 217/HRECC.FODM/X/2017 and applied analytical-experimental study approach. Experimental study used 45 Wistar male rats, aged 5-6 months, 250-300 g in weight obtained from Biochemistry Laboratory, Faculty of Medicine - Universitas Airlangga, Surabaya. Seven days adaptation time provided to experimental animals. The rats were anesthetized intramuscularly using Ketamine 10% (Ketalar) and Xylazine 2% (Rompun) as much as 0.12 mL/100 g body weight, and were being induced to periodontitis with P. gingivalis (ATCC 33277) with concentration of 2 x 10^6 CFU/mL as much as 0.03 mL on the right gingival incisive sulcus and on left mandibular. Induction was carried out every three days for two weeks. The rats were grouped into nine groups consisting of a negative control group that consisted of healthy rats, a positive control group of chronic periodontitis that consisted of rats with zero provision of peel extract of red *H. polyrhizus* and provided only with CMC-NA (Oxchem) gel and standard feeding, and the experimental group the rats provided with standard feeding as well as peel extract of red *H. polyrhizus* with the following concentration; 1 mg/mL, 2 mg/mL, and 4 mg/mL, one time per day topically in the gingival mucosa as much as 0.1 mL for 3 days and 7 days. Furthermore, the entire control and treatment groups were euthanized using Ketamine in lethal doses. The gingival mucosal tissue of rats taken and processed and MDA-TBA method was used for counting MDA levels. The gingival tissue weighed for 500mg, then added with 4.5 mL of PBS solution in colder temperature and then dissolved using homogenizer. After a fine sample obtained, the sample was centrifuged with 5000 g for 15 minutes. Then 4 mL of supernatant was supplemented and added with 1 mL of 15% TCA solution to the supernatant. A further 0.37% TBA solution was provided with 0.25 N HCl and heated 80 ˚C in a waterbath for 15 minutes. The sample left cooling at room temperature for 60 minutes, then centrifuged at 3000 g for 15 minutes. The supernatant underwent absorbance measurement at 532 wavelength using a spectrophotometer (Shimadzu UV-1201) and MDA value calculated using regression equation of standard curve of MDA solution.

Results and Discussion

The rats induced by *P.gingivalis* experienced signs of inflammation like as the gingiva turned into redness, oedema and bleeding. After 2 weeks, clinical appearance showed gingival recession, tooth loosened with existence of pus. Clinical features of the oral cavity of the rats after induction of P.gingivalis bacteria observed (Figure 1).

The standard curve created after measuring the samples' absorbance to determine its absorbance range, which further to determine the concentration used whose value could include the sample's absorbance value. The standard curve obtained was y = 114.66x - 0.4263 with R2 = 0.9998 (figure 2).

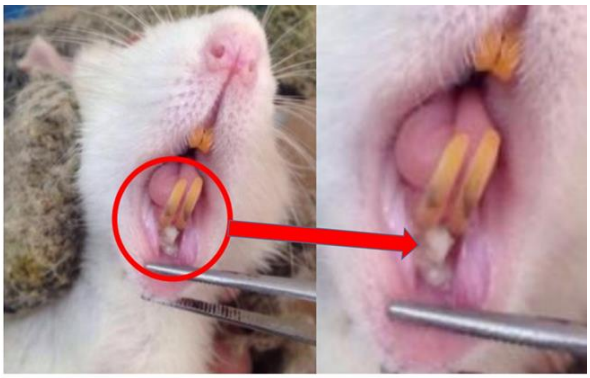

Figure 1. Clinical Examination of Chronic Periodontitis in Wistar Rat.

The result of the measurement indicated that there was an increase of MDA levels of chronic periodontitis in rats compared with healthy rats and after provision of peel extract of red *H. polyrhizus* for both 3 days and 7 days, the decrease of MDA levels in treatment group compared with positive control group (table 1). According to the HSD test, the data indicated that there was a significant difference between the negative control group and the positive control group and between 3-days or 7-days

treatment groups (p < 0.05). The positive control group of MDA level compared to the treatment group with concentration of 1 mg/mL and 2 mg/mL, with gel administration for 3 days showed no significant difference (p > 0.05).

Correction Number	Original Text	Correction Text	Changes done on page number and line number
1	p<0,05	p<0.05 (Please change comma to period)	Abstract
2	80,5%	80.5% (Please change comma to period)	Introduction
3	the samples' absorbance	absorbance of the sample (please changed the text)	Results and Discussion
4	figure 2	Figure 2 (Please change to capital letter)	Results and Discussion
5	table 1	Our table is missing (Please add the table)	Results and Discussion
6	3-days or 7-days	3 days or 7 days (Please delete "-")	Results and Discussion
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Group	n sample	$\bar{X} \pm SD$ (nmol/mg)	
		3rd day	7th day
Control -	5	1.01 ± 0.08^{a}	
Control +	10	10.37 ± 0.39^{b}	6.38 ± 0.46^{b}
P1(1 mg/mL/day)	10	10.08 ± 0.89^{bc}	4.87 ± 0.80^{c}
P2(2 mg/mL/day)	10	9.21 ± 0.95^{bc}	4.76 ± 0.25^{c}
P3(4 mg/mL/day)	10	6.75 ± 0.59^{d}	3.72 ± 0.36^{d}

Table 1.The mean value of MDA level in each group.

The values with different superscript letters in a column are significantly different (p<0.05).

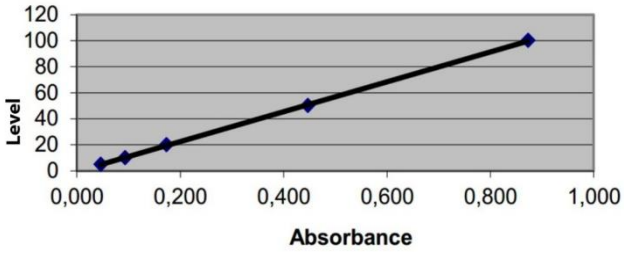

Figure 2. Standard Curve of MDA.

In the treatment group, group with concentration of 1 mg/mL and group with concentration of 2 mg/mL also showed no significant difference (p > 0,05). Meanwhile, the treatment group with concentration of 4 mg/mL showed a significant difference (p <0.05) compared to the control group and both treatment groups with concentration of 1mg/mL, and 2 mg/mL. MDA levels in positive control group compared to all treatments; 1 mg/mL, 2 mg/mL, and 4 mg/mL concentrations with gel administration for 7 days showed significant difference (p < 0.05). Similarly, treatment with concentration 1 mg/mL, 2 mg/mL compared to treatment with concentration 4 mg/mL also showed significant differences (p < 0.05). However, there was no significant difference between treatment concentrations of 1 mg/mL and 2 mg/mL (p > 0.05).

Experimental rats induced by P. gingivalis with interval 3 days for 2 weeks experienced clinical chronic periodontitis, characterized by inflammation of the gingiva that turned into redness, oedema, bleeding, gingival recession, teeth loosening, and the presence of pus. This might be due to virulence factors of P.gingivalis bacteria such as LPS that could damage the periodontal tissues, and could induce host immune response to produce cytokines pro-inflammatory.[11]

There was an increase in MDA levels in the positive control group (group with chronic periodontitis) compared with the negative control group (healthy rats). Increased MDA levels in chronic periodontitis are caused by an increase in the amount of excessive ROS produced as a body's defense mechanism against microorganisms. This situation causes oxidative stress and triggers lipid peroxidation. Reactive oxygen species (ROS) are highly reactive and not easy to detect, so one way to measure the presence of free radical activity and oxidative damage in the body as performed in this study is to measure the final product of lipid peroxidation, malondialdehyde.[12]

The results of the study indicated that the 3rd day of positive control group had higher MDA levels compared with the positive control group on day 7. At the onset of periodontitis, there was infiltration of neutrophils to the tissues caused by chemical mediators released by inflamed tissues. The presence of high neutrophil activity led to more free radical produced and when excessive free radicals formed by the body, the body was unable to compensate which further led to high levels of MDA on day 3. However, neutrophils have a short lifespan (2-4 days in tissue). According to study conducted by Prasetya there was a decrease in infiltration of 7th day neutrophils compared with day 3 in rat periodontitis. The decrease of neutrophil infiltration also influenced the amount of free radical produced hence the decrease of MDA level in positive control group on day 7.[6,13]

In the event of oxidative stress, the body will perform homeostasis by producing endogenous antioxidants. However, there are

many factors that affects the velocity and amount of endogenous antioxidants produced therefore it needs to be assisted with the addition of exogenous antioxidants.[14] In this study, chronic periodontitis in rats were treated with peel extract of red *H. polyrhizus* on the gingival mucosa that served as antioxidants. The result of this study showed that *H. polyrhizus*, with gel concentration 1 mg/mL and 2 mg/mL did not show any significant difference compared with positive control group in 3 days provision. This could be due to shorter treatment time and inadequate concentration. However, gel administration with concentration of 4mg/mL showed significant difference compared with positive control group which could be caused by the increase of concentration used, antioxidant activity was also higher so that it reduce free radicals formed and prevented lipid peroxidation.[15]

Meanwhile, provision of peel extract of red *H. polyrhizus* for 7 days showed significant difference between positive control group and treatment group 1 mg/mL, 2 mg/mL, and 4 mg/mL. These result indicated that the longer and the higher the administration time and the concentration given, the greater of the antioxidant activity. This study also proved the influence of dose relationship which indicated by the higher concentration given, the lower MDA content obtained. In the result of this study, experimental animals provided with peel extract of red *H. polyrhizus* with a concentration of 4 mg/mL had the lowest level of malondialdehyde.

Decreased MDA levels in this study could be caused by the active substances; antioxidants contained in peel extract of red *H. polyrhizus*. Previous study conducted by Septiana showed that phenolic content of peel extract of red *H. polyrhizus* lowers the levels of MDA in mice with dyslipidemia.[16] According Nurliyana & Wu, peel of red *H. polyrhizus* has a high content of phenolic compounds.[10,15] This compound is closely related to antioxidant activity, especially polyphenol groups such as flavonoids that are widely contained in the peel and seeds of plants. The mechanism of flavonoids as antioxidants is by acting as radical scavenger peroxyl (ROO*) which will be regenerated into ROOH, and act as hydroxyl radical scavenger (OH*) to be regenerated into H2O. The resultant compounds of peroxyl radical regeneration and hydroxyl radical are more stable, whereas the formed phenytoil radical (flavonoids-O *) become less reactive to perform propagative reactions.[17,18,19]

The betacyanin content in peel of red *H. polyrhizus* has potential as an antioxidant, since its nature as radical scavenger, hence reducing oxidative damage.[20] Betacyanin is a pigment in peel of red *H. polyrhizus* which also contributes to the total phenolic content since it has phenol structure in its molecules. In addition, ascorbic acid (vitamin C) contained in the peel of red *H. polyrhizus* is also an antioxidant by reacting directly with superoxide anions, hydroxyl radicals, oxygen singlet thus inhibiting the interaction between lipids with oxidants and preventing lipid peroxidation.[21,22]

However, decreasing levels of MDA at concentrations of 1mg/mL, 2 mg/mL, and 4 mg/mL obtained in this study were still not close enough to the average MDA levels of healthy rats. This might be due to the concentration provided and the insufficient provision time. For further study, it is suggested to increase the concentration of gel of peel extract and to lengthen period of administration so that the average MDA level could result to closer normal MDA levels.

Conclusions

This study can be inferred that provision peel extract of red *H. polyrhizus* can decrease the level of MDA in rats with chronic periodontitis. The concentration of 4mg/mL resulted to the highest decrease of MDA level. Consumption of dragon fruit in the community can improve the health of gums and periodontal tissue.

Conflict of Interest

The authors state that there were no conflicts of interest related to this study.

Acknowledgement

The authors are thankful to the Universitas Airlangga, Surabaya, Indonesia, for their invaluable support and for providing all the research facilities.

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