THE EFFECT OF POTATO (Solanum tuberosum L.) SKIN EXTRACT ON ALKALINE PHOSPHATASE LEVEL IN PERIODONTITIS

C C Prihastuti1, W Ratnasari1, Hernayanti2

1Dental Medicine Study Program, Faculty of Medicine, Jenderal Soedirman University, Purwokerto, Indonesia; christianaprihastuti@unsoed.ac.id
2Ecotoxicology Department, Faculty of Biology, Jenderal Soedirman University, Purwokerto, Indonesia; hernayanti.hernayanti@unsoed.ac.id

Email: christiana.prihastuti@unsoed.ac.id.

Abstract. Periodontitis is a chronic inflammation on tooth supporting tissues leads to alveolar bone resorption and tooth loss. Increase of alkaline phosphatase (ALP) level is found in periodontitis condition. Potato skin extract is known for its antioxidant and antiinflammation compounds therefore presumably potential as adjuvant therapy for periodontitis. This study aim was to know the effect of oral intake of potato skin extract on alveolar bone ALP and serum ALP level in model periodontitis Sprague dawley rats. Type of the study was experimental with posttest-only control group design. Thirty-five male Sprague dawley rats were divided into five groups: healthy control, negative control (aquadest-treated periodontitis), and potato skin extract-treated periodontitis in three different concentrations (25%, 50%, 75%). After 14-day treatments, ALP level on alveolar bone and blood serum samples from each group were measured using UV-Vis spectrophotometer. The results showed both alveolar bone ALP and serum ALP level were lower in potato skin extract-treated periodontitis groups in comparison to negative control group. Statistic analysis using One-way Anova showed significant difference of ALP level amongst groups (p<0.05). Post-hoc LSD test showed significant differences of ALP level between each treated and control groups (p<0.05). This study concluded potato skin extract decreased ALP level, with concentration 75% showed lowest both ALP levels of alveolar bone and serum samples, indicated alveolar bone remodeling. Moreover, it is suggested that ALP level in serum can represent ALP level on alveolar bone tissues in periodontitis condition.

Keywords: potato skin extract; ALP level; periodontitis

1. Introduction
Periodontitis is chronic inflammation on periodontal tissue caused by plaque bacteria colonies, characterized by gingival attachment loss and alveolar bone loss [1,2]. Cytotoxic agents of predominant bacteria causing periodontitis, for example lipopolysaccharides (LPS) of Porphyromonas gingivalis induce migration of proinflammatory cytokines, e.g. Tumor Necrosis Factor (TNF) α, Interleukin (IL)-1α and β, IL-6, also polymorphonuclear neutrophils from blood circulation into damaged tissue. Deposition of cytokines and neutrophils in infected periodontal tissue causes secretion of Receptor Activator of NF-κB Ligand (RANKL) by osteoblasts or stromal cells which will activate osteoclasts and alveolar bone resorption [3].

Alveolar bone resorption induces bone regeneration initiated with alkaline phosphatase (ALP) secretion by osteoblasts. ALP plays role in calcium and phosphate deposition in bone matrix. ALP is found in several tissues throughout the body, for examples liver, bone, plasenta, renal proximal tubule, and intestines. ALP level of liver and bone are high in blood serum therefore have been used as a biomarker of bone metabolism and remodeling following bone disorders. Elevation of ALP level
occurs during bone remodeling and decreases when osteoid mineralization finished [4, 5, 6]. Previous study revealed elevation of ALP level in gingival crevicular fluid (GCF) sample from periodontal tissue with periodontitis condition in comparison to from healthy or gingivitis area of the same patients [7].

Periodontitis causes oxidative stress imbalance in periodontal tissues. Host Modulation Therapy as an adjuvant treatment for periodontitis can be approached by administration of exogenous antioxidant. Potato peel has been studied and known for its high antioxidant content which are phenolic compounds [8, 9].

Previous study showed that phenolic compounds in potato peel, such as chlorogenic acid and caffeic acid, decreases free radical 1,1-diphenyl-2-picrylhydrazyl (DPPH). Potato peel is more effective than potato flesh in DPPH inhibition. Other study depicted 10% potato peel extract has therapeutic effect to decrease of blood sugar level also liver and renal malondialdehyde level in rat model of diabetes mellitus. In contrast, the 10% potato peel extract increases antioxidant activities for Glutathione-S-transferase (GST), Superoxide dismutase (SOD), dan Glutathione peroxidase (GPX) level [9].

Study about potential effect of antioxidant contents in potato peel to inhibit oxidative stress and alveolar bone turnover in periodontitis condition has not been conducted. Therefore, this study aim was to investigate the effect of potato skin extract intake as a natural exogenous antioxidant compounds toward ALP level of alveolar bone and blood serum in rat model of periodontitis.

2. Materials And Methods
The type of conducted research was laboratory experimental research with posttest-only control group design. Ethical clearance was obtained from the Ethics Commission, Medical Faculty of Sebelas Maret University, Dr. Moewardi, Surakarta (Letter number 379/IV/HREC/2017).

2.1. Potato Skin Extract Preparation
Potatoes (Solanum tuberosum Granula variety) harvested at day 90 from "Cupu Manik Astagina" potato farmer group in Wadas Putih Village, Parikesit Village, Kejajar District, Wonosobo (1360 meters above sea level). Potato skins were peeled, dried for 3-5 days, and processed to be powder. The potato skin extraction was done by maceration method with ethanol solvent.

2.2. Samples Grouping
The samples were 35 male Sprague dawley rats aged 2-3 months with 200-250 grams in weight. The rats were divided into 5 groups with simple random sampling distribution. The groups were HC (healthy control group), NC (negative control group, periodontitis condition treated with aquadest), T1 (periodontitis treated with potato skin extract concentration 25%), T2 (periodontitis treated with potato skin extract concentration 50%), and T3 (periodontitis treated with potato skin extract concentration 75%).

2.3. Periodontitis Induction with LPS
Rat model of periodontitis in the NC, T1, T2, and T3 groups were induced by LPS injection in buccal gingiva of the right mandibular molars at a dose of 5 µg/ 0.05 ml PBS, once per day for 8 days. At day 9, one rat from each periodontitis model group (NC, T1, T2, and T3) and also healthy control group (HC) were sacrificed by ketamine injection and decapitated. Clinical and radiography examination were conducted and revealed edematous and erythematous gingiva and lower jaw alveolar crest resorption in rats from periodontitis model groups.

2.4. Experimental Treatment
Potato skin extract or aquadest was administered orally by gavage technique once per day for 14 days. In treated groups, potato skin extract was given in dose of 25% (250 mg/ kgBB), 50% (500 mg/
kgBB), and 75% (750 mg/ kgBB) for T1, T2, and T3 groups respectively. On the other hands, aquadest was given for control groups (HC and TC).

2.5. Sample Collection
At day 15, rats were euthanized by ketamine injection and decapitated. Mandibular alveolar bone samples were collected by excision using scalpel. The alveolar bone were chopped until soft and suspended in PBS solution (1:10) then centrifuged at 200 g for 10 minutes. Each 20 µL suspension solution was mixed with 1000 µL of R1 reagent (Alkaline phosphatase FS IFCC mod. 37°C; DiaSys) and incubated for 1 minute. Then, the solution was mixed with 250 µL R2 reagent (Alkaline phosphatase FS IFCC mod. 37°C; DiaSys).

Blood samples (1.5 ml) were collected from canthus orbitalis. The blood samples were centrifuged at 200 g for 10 minutes to separate serum from blood cells. Each 20 µL blood serum samples was mixed with 1000 µL of R1 reagent (Alkaline phosphatase FS IFCC mod. 37°C; DiaSys) and incubated for 1 minute. Then, the solution was mixed with 250 µL R2 reagent (Alkaline phosphatase FS IFCC mod. 37°C; DiaSys).

2.6. Measurement of ALP levels
ALP levels were measured using Spectrophotometry UV-Vis Test at 405 nm wavelength and the absorbance values was read at first, second, and third minute. ALP levels calculated using the Kinetic Method.

2.7. Data Analysis
Data in each sample group was analyzed using One-way ANOVA T-Test with 95% confidence level (P-value≤0.05) and continued with the Post-hoc Least Significance Difference (LSD) test with 95% confidence level (P-value≤0.05).

3. Results And Discussion
The results showed that ALP level of alveolar bone in the treatment groups were decrease as the concentration of potato skin extract intake increase. The lowest alveolar bone ALP level was in T3-treated group with potato skin extract concentration 75% (14.42 U/L). On the other hand, the highest alveolar bone ALP level was found in the negative control group (23.62 U/L) (Table 1).

| Table 1. Mean and Standard Deviation (SD) of Alveolar Bone ALP Level |
|------------------------|--------|---------------|------------------|
| Groups | n | Mean ± SD | Alveolar Bone ALP Level (U/L) |
|--------|---|-----------|------------------|
| HC | 6 | 13.00 ± 0.76 |
| NC | 6 | 23.62 ± 0.60 |
| T1 | 6 | 18.98 ± 1.02 |
| T2 | 6 | 16.09 ± 0.67 |
| T3 | 6 | 14.42 ± 0.40 |

HC= healthy control; NC= negative control; T1= potato skin extract concentration 25%; T2= potato skin extract concentration 50%; T3= potato skin extract concentration 75%.

Statistic analysis using Saphiro-Wilk and Levene test showed that data of ALP level of alveolar bone was distributed normal (P-value≥0.05) (Table 2) and homogenous (P-value=0.065) respectively. One-way ANOVA statistic test depicted a significant difference among groups (P-value=0.000). Post-hoc LSD test result showed there were significant differences in alveolar bone ALP levels between each groups (P-value≤0.05; Table 3). The statistic analysis indicated significant effect of various concentration of potato skin extract oral administration on alveolar bone ALP level in periodontitis condition.
Table 2. Saphiro-Wilk Normality Test Result of Alveolar Bone ALP Level

| Groups | n  | Sig. |
|--------|----|------|
| HC     | 6  | 0.495|
| NC     | 6  | 0.817|
| T1     | 6  | 0.313|
| T2     | 6  | 0.253|
| T3     | 6  | 0.203|

HC= healthy control; NC= negative control; T1= potato skin extract concentration 25%; T2= potato skin extract concentration 50%; T3= potato skin extract concentration 75%.

Table 3. Post-hoc LSD Test Result of Alveolar Bone ALP Level

| Groups | KS | KN | P1 | P2 | P3 |
|--------|----|----|----|----|----|
| HC     |    |    | 0.000* | 0.000* | 0.000* |
| NC     | 0.000* |    | 0.000* | 0.000* | 0.000* |
| T1     | 0.000* | 0.000* |    | 0.000* | 0.000* |
| T2     | 0.000* | 0.000* | 0.000* |    | 0.000* |
| T3     | 0.001* | 0.000* | 0.000* | 0.000* |    |

* P-values≤0.05

HC= healthy control; NC= negative control; T1= potato skin extract concentration 25%; T2= potato skin extract concentration 50%; T3= potato skin extract concentration 75%.

Furthermore, measurement of blood serum samples showed higher ALP level in comparison to alveolar bone samples. However, the pattern of the ALP levels in control and treated groups were similar in both samples. The ALP level in blood serum was decline in relation to concentration increase of potato skin extract. The lowest serum ALP level was in group T3, periodontitis condition treated with potato skin extract with 75% concentration (51.46 U/L) whereas the highest ALP levels was in negative control group (NC; 84.32 U/L) (Table 4).

Table 4. Mean and Standard Deviation (SD) of Serum ALP Level

| Groups | n  | Mean ± SD Alveolar Bone ALP Level (U/L) |
|--------|----|--------------------------------------|
| HC     | 6  | 46.41 ± 2.71 |
| NC     | 6  | 84.32 ± 2.03 |
| T1     | 5  | 69.20 ± 1.15 |
| T2     | 6  | 57.44 ± 2.42 |
| T3     | 6  | 51.46 ± 1.42 |

HC= healthy control; NC= negative control; T1= potato skin extract concentration 25%; T2= potato skin extract concentration 50%; T3= potato skin extract concentration 75%.

The serum ALP level data was normally distributed and homogenous as indicated by Saphiro-Wilk test (P-value≥0.05; Table 5) and Levene test (P-value=0.076). One-way ANOVA test showed a significant difference of serum ALP level among groups (P-value=0.000). Moreover, Post-hoc LSD test depicted the significant differences were found between each treated and control groups (P-value≤0.05; Table 6). The results were interpreted as significantly different effect of various concentration of potato skin extract oral intake towards ALP level in serum.
Table 5. Saphiro-Wilk Normality Test Result of Serum ALP Level

| Groups | n  | Sig.  |
|--------|----|-------|
| HC     | 6  | 0.557 |
| NC     | 6  | 0.804 |
| T1     | 5  | 0.314 |
| T2     | 6  | 0.059 |
| T3     | 6  | 0.469 |

HC= healthy control; NC= negative control; T1= potato skin extract concentration 25%; T2= potato skin extract concentration 50%; T3= potato skin extract concentration 75%.

Table 6. Post-hoc LSD Test Result of Serum ALP Level

| Groups | KS | KN | P1 | P2 | P3 |
|--------|----|----|----|----|----|
| HC     |    |    |    |    |    |
| NC     | 0.000*| 0.000*| 0.000*| 0.000*| 0.000*|
| T1     | 0.000*| 0.000*| 0.000*| 0.000*| 0.000*|
| T2     | 0.000*| 0.000*| 0.000*| 0.000*| 0.000*|
| T3     | 0.000*| 0.000*| 0.000*| 0.000*| 0.000*|

* P-values≤0.05

HC= healthy control; NC= negative control; T1= potato skin extract concentration 25%; T2= potato skin extract concentration 50%; T3= potato skin extract concentration 75%.

This study in both alveolar bone and serum samples suggested the highest level of ALP were found in the negative control groups which mimicking the inflammatory condition of periodontal tissue without therapy. These results are consistent with previous studies which stated that there is an increase in ALP level in patients with periodontitis [7]. In a periodontitis condition, there is an imbalance in alveolar bone remodeling due to increase of migration of pro-inflammatory cytokines (TNF α, IL-1α & β, IL-6) and macrophage cells to the damaged tissue. Activation of macrophages triggers inducible Nitric Oxide Synthase (iNOS) to produce nitric oxide (NO). Excessive NO products will bind with O₂⁻ free radical in sequence to form peroxynitrite (ONOO⁻) which affects a decrease of endothelial NOS (eNOS) activity. This cascade induces vasoconstriction which then inhibits tissue healing process [15]. Therefore, the increase of oxidative stress in periodontal tissue inflammation causes alveolar bone resorption which is characterized by increase of osteoclasts that exceeding the number of osteoblasts. This condition will then trigger abnormal osteoblastic activity in the form of proliferation and maturation of immature osteoblasts to compensate bone damage. At this stage, ALP level increases significantly [10, 11, 12].

In this study, periodontitis therapy with potato skin extract in various concentrations for 14 days depicted lower ALP level in both alveolar bone and serum in comparison to aquadest as negative control. It was previously postulated by [13] that final stage of bone remodeling was characterized by mineralization of the extracellular bone matrix and return of osteoblast activity, marked by decrease in ALP secretion by osteoblasts. Therefore this study results indicated alveolar bone healing following oral administration of potato skin extract.

Intake of potato skin extract in various concentrations for 14 days depicted lower ALP level in both alveolar bone and serum in comparison to aquadest as negative control. It was previously postulated by [13] that final stage of bone remodeling was characterized by mineralization of the extracellular bone matrix and return of osteoblast activity, marked by decrease in ALP secretion by osteoblasts. Therefore this study results indicated alveolar bone healing following oral administration of potato skin extract.

Intake of potato skin extract with 25%, 50%, and 75% concentrations were significantly decreased the ALP levels of both alveolar bone and serum as the extract concentration increase. Furthermore, potato skin extract concentration 75% demonstrated the lowest ALP level approaching the ALP level in healthy control groups even though remains different statistically (P-value≤0.05). Decrease of the ALP level following periodontitis therapy using potato skin extract is presumably caused by the active contents in potato skin extract in form of phenolic compounds, flavonoids, and α-tocopherol. Phenolic compounds have known to play important role in alveolar bone remodeling in periodontitis rat. Phenolic are antioxidant compounds thus can inhibit oxidative stress through inhibition of ONOO⁻. Phenolic compounds will donate one of the electrons to free radicals which then form a bond that inhibits the activity of oxidant compounds and oxidative stress [13]. In addition, flavonoids
content in potato skin inhibits LPS as well as to prevent bone resorption and increase bone mass in diabetic rats [8]. Moreover, vitamin E content, α-tocopherol, in potato skin extract have also been reported to inhibit NO activity and superoxide production by macrophages and neutrophils through inhibition of p47 phosphorylation during NADPH activity [14]. Further investigation is needed regarding mechanism and therapeutic potential of potato skin extract on alveolar bone remodeling.

Examination of ALP biomarker in the alveolar bone as the local site of periodontitis as well as in the blood serum demonstrated a similar pattern following therapy with potato skin extract. ALP levels in bone and liver are reported found in the same concentration [10]. This study demonstrated that ALP level in systemic blood circulation is higher than in alveolar tissue and can be a parameter of healing process in the alveolar bone. Moreover, blood sample collection offers easy procedure in comparison to biopsy of the affected alveolar bone. Therefore the ALP level from blood serum can be a biomarker for osteoblast activity and remodeling process in alveolar bone.

4. Conclusion
Potato skin extract intake affected alveolar bone remodeling in periodontitis condition characterized by decrease in ALP level both in alveolar bone and serum. The potato skin extract concentration 75% demonstrated the most effective effect on the decrease of ALP levels, nearly approaching healthy condition. The serum ALP level can be a biochemical marker for detection and evaluation of periodontal disease progress.

References
[1] Petersen, P.E. and Ogawa, H. (2005). Strengthening The prevention of periodontal disease: the WHO approach. Journal of Periodontal. 76 (12): 2187-2193. https://doi.org/10.1902/jop.2005.76.12.2187
[2] Newman, M.G., H.H. Takei, P.R. Klokkevold and F.A. Carranza, (2012). Carranza’s Clinical Periodontology. 9th ed. Saunders Elseviers, Philadelphia. ISBN: 9780323228022, pp: 70-364.
[3] Gautam, P., Gupta, P., Sharma, P., and Dangwal, P. (2015). Virtous Aspects of Vicious Bacterial Toxins. Journal of Chemical and Pharmaceutical Research. 7(6): 280. ISSN: 0975-7384.
[4] Blake, G.M. and Fogelman, I. (1998). Application of Bone Densitometry for Osteoporosis. Endocrinology and Metabolism Clinics of North America. 27: 267-88. https://doi.org/10.1016/S0889-8529(05)70005-0.
[5] Yudaniayati, I.S. (2005). Aktivitas Alkaline Phosphatase Pada Proses Kesembuhan Patah Tulang Femur dengan Terapi CaCO3 Dosis Tinggi Pada Tikus Jantan (Sprague dawley). Media Kedokteran Hewan. 21(1):15-17.
[6] Goes, P., Melo, I.M., Dutra, C.S., Lima, A.P.S., and Lima, V. (2012). Effect of Alendronate on Bone-specific Alkaline Phosphatase on Periodontal Bone Loss in Rats. Archives of Oral Biology. 57: 1537. https://doi.org/10.1016/j.archoralbio.2012.07.007.
[7] Malhotra, R., Grover, V., Kapoor, A., and Kapur, R. (2010). Alkaline Phosphatase as A Periodontal Marker. Indian Journal of Dental Research. 21(4): 531-536. https://doi.org/10.4103/0970-9290.74209.
[8] Singh, N. and Rajini, P.R. (2004). Free Radical Scavenging Activity of an Aqueous Extract of Potato Peel. Food Chemistry. 85: 611-613. https://doi.org/10.1016/j.foodchem.2003.07.003
[9] Singh, N., Kamat, V., and Rajini, P.S. (2005). Attenuation of Hyperglycemia and Associated Biochemical Parameters in STZ-Induced Diabetic Rats by Dietary Supplementation of Potato Peel Powder. Clinica Chimica Acta. 353:165-175. https://doi.org/10.1016/j.cca.2004.10.016
[10] Rose, L.F., R.J. Genco, D.W. Cohen and B.L. Maelay, (2000). Periodontal Medicine. Hamilton: B.C. Decker Inc. PMID: 24130938, pp: 675-678.
[11] Chairani, S., Utami, S. and Suniarti, D.F. (2011). Effect of Chitosan on Protein Content in The Medium Culture of Osteoblasts Exposed to Oxidative Stress. Dentika Dental Journal. 16(1): 53-55.
[12] Przekora, A. and Ginalska, G. (2014). Enhanced Differentiation of Oseoblastic Cells on Novel Chitosan/ Beta-1,3 Glucan/ Bioceramic Scaffolds for Bone Tissue Regeneration. Biomedical Materials. 10(1): 1-13. https://doi.org/10.1088/1748-6041/10/1/015009.

[13] Chapple, I.L.C. and Matthews, J.B. (2000). The Role of Reactive Oxygen and Antioxidant Species in Periodontal Tissue Destruction. Journal Compilation. 43: 173-174. https://www.researchgate.net/publication/288231635.

[14] Singh, N. and Rajini, P.S. (2008). Antioxidant-Mediated Protective Effect of Potato Peel Extract in Erythrocytes Against Oxidative Damage. Chemico-Biological Interactions. 173: 100. https://doi.org/10.1016/j.cbi.2008.03.008.

[15] Kumar, V., A.K. Abbas and J.C. Aster, (2013). Robbin’s Basic Pathology 9th Ed. Elsevier Saunders. Canada. ISBN: 9780323286046, pp: 38-54.