Dental Journal
Majalah Kedokteran Gigi

Dental Journal
(Majalah Kedokteran Gigi)
2016 September; 49(3): 137–142

Research Report

# Effect of Cassave leaf flavonoid extract on TNF-α expressions in rat models suffering from periodontitis

**Zahara Meilawaty** and **Banun Kusumawardani**
Department of Biomedical Science
Faculty of Dentistry, Universitas Jember
Jember-Indonesia

***ABSTRACT***

***Background:*** *Bacteria playing a role in periodontitis are Gram-negative anaerobic bacteria that can release endotoxin or lipopolysaccharide (LPS). LPS acts as a stimulus to a variety of host cells that can stimulate expression of pro-inflammatory cytokines in periodontal disease, such as IL-1α, IL-1β, and TNF-α. Increased TNF-α then can lead to periodontal tissue destruction. Furthermore, cassava leaves have many health benefits due to flavonoid organic compound contained, known to possess anti-inflammatory activity are used as a medicine.* ***Purpose:*** *This study aimed to determine the effect of cassava leaf flavonoid extract as a basic ingredient of anti-inflammatory gel on TNF-α expression in Wistar rats suffering from periodontitis induced with Escherichia coli (E. coli) LPS.* ***Method:*** *This study used 24 male Wistar rats. Those rats were divided into six groups. Group 1 consisted of four rats induced with E. coli LPS for 2 weeks, and then decapitation was performed on day 3. Group 2 consisted of four rats induced with E. coli LPS for 2 weeks, and then decapitation was conducted on day 7. Group 3 consisted of four rats induced with E. coli LPS for 2 weeks, treated with the topical cassava leaf flavonoid extract gel (Manihot esculenta) at a concentration of 25%, and then decapitation was performed on day 3. Group 4 consisted of four rats induced with E. coli LPS for 2 weeks, treated with the topical cassava leaf flavonoid extract gel (Manihot esculenta) at a concentration of 25%, and then decapitation was conducted on day 7. Group 5 consisted of four rats induced with E. coli LPS for 2 weeks, treated with the topical cassava leaf flavonoid extract gel (Manihot esculenta) at a concentration of 50%, and then decapitation was performed on day 3. And, group 6 consisted of four rats induced with E. coli LPS for 2 weeks, treated with the topical Cassava leaf flavonoid extract gel (Manihot esculenta) at a concentration of 50%, and then decapitation was conducted on day 7. The topical Cassava leaf flavonoid extract gel was inserted into gingival sulcus on the first right molar of their lower jaw by using a blunted syringe needle. The gel was given two times a day for 7 days.* ***Result:*** *The expression of TNF-α in the control group was more than that in the treatment groups given the Cassava leaf flavonoid extract gel at the concentrations of 25% and 50%. The expression of TNF-α in the treatment groups given the cassava leaf flavonoid extract gel at the concentration of 50% was lower than that in the treatment groups given the cassava leaf flavonoid extract gel at the concentration of 25%.* ***Conclusion:*** *Cassava leaf flavonoid extract gel could be used as an anti-inflammatory gel characterized by a decrease in TNF-α expression in rat models suffering from periodontitis.*

***Keywords:*** *periodontitis; flavonoids of cassava leaves; TNF-α*

*Correspondence*: Zahara Meilawaty, Department of Biomedical Science, Faculty of Dentistry, Universitas Jember. Jl. Kalimantan No. 37 Jember 68121, Indonesia. E-mail: zhr_mel@yahoo.com

## INTRODUCTION

Periodontal disease is an oral health problem with a fairly high prevalence in all age groups in Indonesia, approximately about 96.58%.[1] Periodontal disease is a disease attacking tissues supporting teeth in children, adults, and parents. Periodontal disease sometimes can only attack gingival tissue, or attack periodontal tissue entirely, namely gingiva, periodontal ligament, cementum, and alveolar bone, called as periodontitis. Periodontitis is mainly caused by plaque bacteria.[2,3]

Dental Journal (Majalah Kedokteran Gigi) p-ISSN: 1978-3728; e-ISSN: 2442-9740. Accredited No. 56/DIKTI/Kep./2012.

DOI: 10.20473/j.djmkg.v49.i3.p137-142

Bacteria playing a role in periodontitis are Gram-negative anaerobic bacteria, secreting a variety of products including biologically active endotoxin or lipopolysaccharide (LPS).[4] LPS is one of factors triggering periodontal disorders. LPS can stimulate biological activity causing inflammation. The inflammatory response caused by LPS is the first part of the immune system against pathogens. LPS acts as a stimulus to a variety of host cells which will ultimately result in expression of pro-inflammatory cytokines in periodontal disease, such as interleukin (IL)-1α, IL-1β, and tumor necrosis factor-α (TNF-α).[5,6]

In the last few years, many researchers in Indonesia have developed many studies on medicinal plants that are useful as alternative drugs subtituting to chemical drugs on markets, including cassava leaves (*Manihot esculata*). Cassava leaves have many health benefits since they have a high level of vitamin C and some organic compounds, such as flavonoids, triterpenoids, tannins, and saponins. Flavonoids are known to have anti-inflammatory activity. Flavonoids are polyphenolic compounds that occur ubiquitously in plants having avariety of biological effects both in vitro an in vivo. They have been found to have antimicrobial, antiviral, anti-ulcerogenic, cytotoxic, antineoplastic, mutagenic, antioxidant, antihepatotoxic, antihypertensive, hypolipidemic, antiplatelet ant anti-inflammatory activities. Flavonoids also have biochemical effects, which inhibit a number of enzymes such as aldose reductase, xanthine oxidase, phosphodiesterase, Ca+2-ATPase, lypoxygenase, cycloxygenase.[7]

Flavonoids are assumed to suppress TNF-α expression released during inflammation. Similarly, a research conducted by Peluso shows that flavonoids can reduce TNF-α.[8] Some previous in vitro studies, futhermore, have shown that cassava leaf extract at concentrations of 12.5% and 25% can reduce COX-2 expression, an enzyme that plays a role during inflammation, and also can improve the viability of monocytes exposed by *Escherichia coli (E. coli) LPS*. [9] For those reasons, this research aimed to determine the effectiveness of the cassava leaf *flavonoid* extract as a basic ingredient of anti-inflammatory gel on TNF-α expression in wistar rats suffering from periodontitis induced with *E. coli LPS*.

## MATERIALS AND METHOD

All procedures in this research were approved by the Ethics and Advocacy Commetee of Faculty of Dentistry, University of Gajah Mada (No. 00 366/ KKEP/ FKG UGM/ EC/ 2015). Cassava leaves were identified at the Indonesian Institute of Sciences in Plant Conservation Center in Purwodadi. Cassava leaves as much as 450 grams were washed, cut into small pieces, and dried using aerated technique for 2 days in a room at a room temperature without being exposed to direct sunlight. They were dried in an oven for 24 hours at a temperature of $40^0$ C. After being dried in the oven, the weight of the dried cassava leaves became into 238.54 grams. The dried cassava leaves were smashed in a blender, and then sieved with a sieve of 80 maze to obtain as much as 207.25 grams of fine powder. The powder was macerated with 96% ethanol for 3 days, and stirred every 24 hours. The solution was concentrated by rotary evaporator at a temperature of $50^0$ C and a speed of 90 rpm/ rotation in order to become crude cassava leaf extract at a concentration of 100%, as much as 20 grams.

The crude extract of cassava leaves as much as 20 grams was added with 100 ml of absolute ethanol, and then was processed ultrasonically for 10 minutes. It was added with 10 ml of 5% $H_3PO_4$, heated at a temperature of $80^0$ C for 30 minutes, and then settled for 8 hours. The top layer formed was taken, and then *vaccum* filtration was conducted. The filtrate was extracted with 10 ml of petroleum ether (repeated 3 times). The extract result was roasted at a temperature of $60^0$ C. To reduce the amount of ethanol, it was added with water to a volume of 5 ml. 20 ml of acetonitrile was added, and sonication process was conducted for 5 minutes. It was centrifuged at 4000 rpm for 5 minutes. The upper layer formed was taken and dried in order to obtain cassava leaf flavonoid extract. The cassava leaf flavonoid extract then was tested using liquid chromatography-tandem mass spectrometry (LC-MS/ MS) to determine the level of flavonoid. The procedure was based on a modification of two different protocols proposed by Docheva *et al*. and Muhammad *et al*.[10,11]

The extract was altered into gel at the Laboratory of Pharmaceutical Faculty of Pharmacy, Universitas Jember. The manufacturing process of gel base was started with carbopol developed in hot water in a mortar, and then stirred until homogeneous before added with triethanolamine (TEA) in small increments until gel mass was formed. The cassava leaf flavonoid extract was mixed with propylene glycol until homogeneous. The mixtures of the extract and propylene glycol were then mixed into the gel base, and stirred until homogeneous. The distilled water remained was added to the gel in small increments until homogeneous. The procedure of making the gel was based on a modification of protocol proposed by Ahmed *et al*.[12] Propylene glycol is a solvent that can dissolve a variety of materials, such as corticosteroids, phenol, sulfa drugs, barbiturates, vitamins A and D, alkaloids, and many local anesthesia.[13]

This study used 24 male Wistar rats divided into 6 groups. Group 1 consisted of four rats induced with *E. coli* LPS for 2 weeks, and then decapitation was performed on day 3. Group 2 consisted of four rats induced with *E. coli LPS* for 2 weeks, and then decapitation was conducted on day 7. Group 3 consisted of four rats induced with *E. coli* LPS for 2 weeks, treated with the topical cassava leaf flavonoid extract gel at a concentration of 25%, and then decapitation was performed on day 3. Group 4 consisted of four rats induced with *E. coli* LPS for 2 weeks, treated with the topical cassava leaf flavonoid extract gel at a concentration of 25%, and then decapitation was conducted on day 7. Group 5 consisted of four rats induced with *E.*

Dental Journal (Majalah Kedokteran Gigi) p-ISSN: 1978-3728; e-ISSN: 2442-9740. Accredited No. 56/DIKTI/Kep./2012.

DOI: 10.20473/j.djmkg.v49.i3.p137-142

*coli LPS* for 2 weeks, treated with the topical cassava leaf flavonoid extract gel at a concentration of 50%, and then decapitation was performed on day 3. Group 6 consisted of four rats induced with *E. coli* LPS for 2 weeks, treated with the topical cassava leaf flavonoid extract gel at a concentration of 50%, and then decapitation was conducted on day 7.

Afterwards, in the early stage, those Wistar rats were anaesthetized using ketamine (KTM 100) at a dose of 0.5 ml/ kg BM,[14] injected into their quadriceps muscle/ triceps muscle of their right rear-foot. *E. coli* LPS (Sigma) at a concentration of 1 mg/ ml in PBS was injected at the gingival sulcus of their first right mandibular molar, as much as 5 mL into lingual part and 5 mL into buccal part. It was injected every three days for two weeks using a tuberculin syringe with a 30 gauge syringe to trigger periodontitis. This method was based on the modification of a method proposed by Buduneli *et al*.[15]

After the rats suffering from periodontitis, their gingiva enlarged and became reddish. There were also pockets. Radiographically, their alveolar bone declined (Figure 1 and 2). The provision of the cassava leaf flavonoid extract gel with the concentrations of 25% and 50%[9] was applied to the gingival sulcus of their first right mandibular first molar using the blunted syringe needle. The gel was applied twice a day for 7 days. This procedure was based on a procedure proposed by Sato *et al*.[16] The exceess gel on the gingival sulcus then was cleaned with a cotton pellet.

Decapitation was performed on those rats on days 3 and 7 after the administration of Cassava leaf flavonoid extract gel. Decapitation on day 3 was considered as inflammatory phase. It was then followed with proliferative phase considered as wound healing process on day 7.[17] This treatment was performed in Biomedical Laboratory of Faculty of Dentistry, Universitas Jember.

After the decapitation, their lower jaw was taken, and then fixed in a buffered formalin solution. The process of decalcification was conducted using EDTA for ±4 weeks. After their tissues softened, the tissues were excised. The tissues were then cleaned and washed with running water for 60 minutes. The softened and cleaned tissues were soaked in alcohol at concentrations of 70%, 80%, and 90%, as well as absolute alcohol (100%) I, absolute alcohol (100%) II, and absolute alcohol (100%) III to remove the water in the tissues. The tissues were successively soaked at each concentration for 60 minutes. The tissues then were soaked in solutions of xylol, xylol II, and xylol III respectively for 60 minutes. Paraffin infiltration process was gradually carried out in an oven at a temperature of $60^0$ C. The preparations then were put into pure paraffin I, pure paraffin II, and pure paraffin III respectively for 60 minutes. After that, embedding and labeling processes were performed. Cutting then was conducted using a microtome. Heating was performed at a temperature of $40^0$ C to dry on a hot plate. All of these treatments were conducted in Biomedical Laboratory of Faculty of Dentistry, Universitas Jember. The procedure was a modification of two different procedures conducted by Leitao[18] and Jimson.[19]

Immunohistochemical staining was performed in accordance with a staining procedure was a modification by Schiessl[20] and Olsen.[21] Deparaffinization of the tissues was carried out using xylol III for 2 minutes, xylol II for 2 minutes, xylol I for 2 minutes, absolute alcohol III for 2 minutes, absolute alcohol II for 2 minutes, absolute alcohol I for 2 minutes, 90% alcohol for 2 minutes, 80% alcohol for 2 minutes, and 70% alcohol for 2 minutes. Those tissues then were washed three times with PBS each for 5 minutes. The tissues were put in a solution of 0.3% $H_2O_2$ in methanol for 20 minutes. Those tissues were washed again with running water for 10 minutes, washed with distilled water for 3-5 minutes, and then washed three times with PBS, each for 5 minutes. They were incubated in antigen retrieval (citrate buffer) using a microwave for 10 minutes, and then cooled at a room temperature for 30 minutes. They were washed three times with PBS, each for 5 minutes.

Ultra V block was applied, and then incubation was conducted for 5 minutes at a room temperature. The tissues were washed three times with PBS, each for 5 minutes. Primary antibody (polyclonal antibody TNF-α) in a ratio 1: 100 (10µ antibody : 1cc PBSA) was given, and then

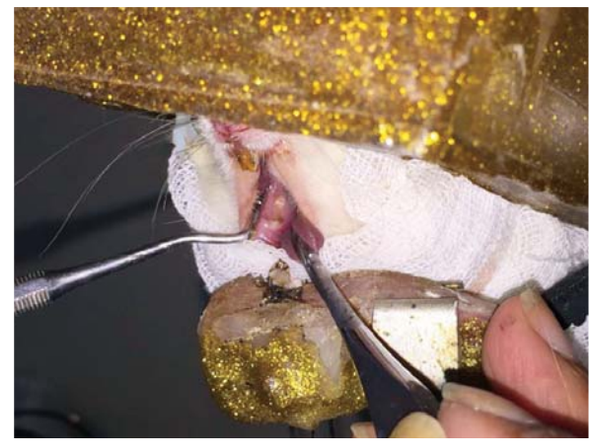

**Figure 1.** A swollen and reddish rat gingiva.

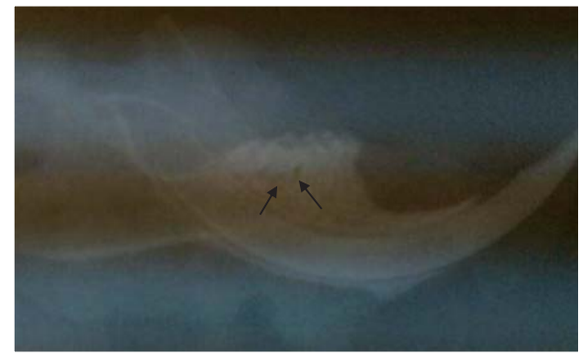

**Figure 2.** A rat dental radiography picture (arrows indicating radiolucent area where alveolar bone decreased).

Dental Journal (Majalah Kedokteran Gigi) p-ISSN: 1978-3728; e-ISSN: 2442-9740. Accredited No. 56/DIKTI/Kep./2012.

DOI: 10.20473/j.djmkg.v49.i3.p137-142

settled for 24 hours at $4^0$ C. They were washed again three times with PBS, each for 5 minutes. Biotinylated goat anti-polyvalent (secondary antibody) was applied, and then incubation was conducted for 5 minutes at a room temperature. They were washed three times with PBS, each for 5 minutes. Streptavidin peroxidase was applied, and then incubation was performed for 5 minutes at a room temperature. They were washed three times with PBS, each for 5 minutes. Incubation then was carried out in DAB chromogen dye (1,3-diamino benzidine), and settled for 10-20 minutes. They were washed again three times with PBS, each for 5 minutes. They then were washed with running water for 10-15 minutes. Counterstain with Mayer's hematoxylin was performed for 1-5 seconds. Dehydration (as opposed to deparaffinization) was carried out. Mounting then was performed using Canada balsam and then covered with a coverslip.

TNF-α expressions on mesial gingival fibroblasts which cell cytoplasm was brown were observed. Observation was performed under a microscope with a magnification of 400 times. The research data obtained were the mean number of TNF-α expressions calculated per three visual fields.

The data then were tested using a normality test, Shapiro-Wilk test. The results of the Shapiro-Wilk test showed that the data had normal distribution. As a result, a parametric statistical test was performed using one way Anova test to determine differences in TNF-α expression in all groups. LSD test was carried out to compare TNF-α expressions in between the treatment groups.

## RESULTS

Microscopically, the color of cells expressing TNF-α was brownish, whereas the color of cells not expressing TNF-α was purplish blue. More details can be seen in Figure 3. The mean and standard deviation of TNF-α expressions in each treatment group can be seen in the following Table 1.

In the control group, the highest mean number of TNF-α expressions on the $7^{th}$ day was 13.83, whereas in the treatment groups given the Cassava leaf flavonoid extract gel at the concentration of 50% on the $7^{th}$ day, the lowest mean number was 8.22. Results of the one way Anova test showed that there were significant differences in TNF-α expressions in between the treatment groups.

## DISCUSSION

The results of this research showed that the mean number of TNF-α expressions in the control group was higher than that in the groups given the Cassava leaf flavonoid extract gel at the concentrations of 25% and 50% as anti-inflammatory. TNF-α expressions in the control group increased on the $7^{th}$ day, while TNF-α expressions in the groups given the Cassava leaf flavonoid extract gel at the concentrations of 25% and 50% decreased on the $7^{th}$ day compared to on the 3rd day. Thus, it can be said that the provision of the Cassava leaf flavonoid extract gel at the concentrations of 25% and 50% could decrease TNF-α expressions.

TNF-α is produced primarily by activated monocytes and macrophages. However, it can also be produced by B cells, T cells, and fibroblasts, playing a role in inflammation.[21] TNF-α is also considered as a powerful immune response modulator mediating induction of adhesion molecules and other cytokines, as well as activation of neutrophils. Nevertheless, excessive TNF-α can damage endothelial cells, causing vascular occlusion and improving endothelium permeability.[22]

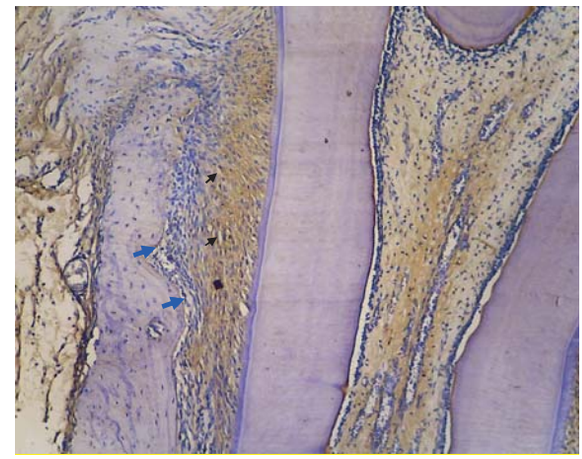

**Figure 3.** The brown cell nuclei indicated TNF-α expressions (black arrows). Meanwhile, the blue cell nuclei blue did not show any TNF-α expressions (blue arrows).

**Table 1.** Mean, standard deviation, and one way Anova test results of TNF-α expressions in the treatment groups based on decapitation time

| Group | TNF-α expressions | | | Sig. |
|---|---|---|---|---|
| | $\bar{X}$ | ± | SD | |
| On day -3 | | | | |
| Control | 11.33 | ± | 0.44 | 0.02 |
| 25% GEFDS | 9.67 | ± | 0.28 | |
| 50% GEFDS | 8.56 | ± | 0.68 | |
| On day -7 | | | | |
| Control | 12.23 | ± | 1.05 | 0.01 |
| 25% GEFDS | 8.56 | ± | 0.41 | |
| 50% GEFDS | 8.17 | ± | 0.31 | |

Note: 25% GEFDS: Cassava leaf flavonoid extract gel at a concentration of 25%; 50% GEFDS: Cassava leaf flavonoid extract gel at a concentration of 50%; $\bar{X}$ : Mean; SD: Standard Deviation; Sig: Significance of one way Anova test results

Dental Journal (Majalah Kedokteran Gigi) p-ISSN: 1978-3728; e-ISSN: 2442-9740. Accredited No. 56/DIKTI/Kep./2012.

DOI: 10.20473/j.djmkg.v49.i3.p137-142

Cassava leaves, known as a medicinal plant for mild diseases, can be used as analgesics and anti-inflammatory.[23,24] Cassava leaves contain some organic compounds, such as flavonoids, saponins, tannins, and triterpenoids. Cassava leaves play a role in suppressing inflammatory process. Flavonoids are potential to suppress the inflammation by blocking the cycle path of cyclooxygenase (COX) and lipoxygenase. As a result, inflammatory cells that migrate are limited, and clinical signs of inflammation are reduced. Inhibition of COX and lipoxygenase pathways also directly causes inhibition of the biosynthesis of eicosanoid. [25]

The results of this research, moreover, also showed that the highest number of TNF-α expressions was found in the control group (LPS). LPS is a potent stimulus to secrete TNF since LPS can induce inflammation. Lipopolysaccharide, a product of microorganisms, can involve TLR4 system that can activate NF-κB and generate genes encoding the protein components of the nonspecific immune responses, including inflammatory cytokines (TNFα, IL-1 and IL-12).[26] In other words, the inflammatory response is triggered by an immune reaction in the cellular level, so other pro-inflammatory cytokines, such as TNF-α, can be improved.[27]

Furthermore, the number of TNF-α expressions in the groups given the Cassava leaf flavonoid extract gel at the concentrations of 25% and 50% as anti-inflammatory was less than that in the control group. This is presumably due to anti-inflammatory effects of flavonoids. The anti-inflammatory effects of flavonoids may be due to their action in inhibiting the accumulation of leukocytes at inflammation sites. During inflammation, many endothelial derived mediators and complement factors may lead to adhesion of leukocytes to endothelial wall, as a result, the leukocytes become immobilized and stimulate neutrophil degranulation.[28] Therefore, it can be said that the provision of flavonoids can reduce both the number of immobilized leukocytes and the activation of complements resulting in lower adhesion of leukocytes to the endothelium and can decrease body inflammatory response.[25]

In addition, flavonoids play a role as an anti-inflammatory by blocking IκB kinase. Consequently, degradation of IκB preventing activation of NF-kβ will not occur. Thus, TNF-α levels will not increase. This condition may be due to quercetin contained. Quercetin flavonoids then can trigger a decrease in TNF-α levels through inhibition of Nuclear Factor Kappa B (NF-κB). NF-κB plays a role in controlling expressions of genes encoding proinflammatory cytokines and chemokines, such as TNF-α, IL-1β, IL-6, and other proteins.[29] It can be concluded that the administration of Cassava leaf flavonoid extract gel as anti-inflammatory can reduce TNF-α expressions in rat models suffering periodontitis.

## ACKNOWLEDGEMENT

This research was funded by Universitas Jember DIPA (Daftar Isian Pelaksanaan Anggaran) year 2015 based on a decree no. DIPA-042.04.2.400073/ 2015.

## REFERENCES

1. Tampubolon NS. Dampak karies gigi dan penyakit periodontal terhadap kualitas hidup. http://library.usu.ac.id. 2005. Accessed December 15, 2016.
2. Kurniawati A. Hubungan kehamilan dan kesehatan periodontal. J Biomed 2005; 2(2): 43-51.
3. Fitria E. Kadar IL-1B dan IL-8 sebagai Penanda periodontitis, faktor resiko kelahiran prematur. J PDGI 2006; 56(2): 60-4.
4. Djais AI. Periodontitis sebagai faktor resiko jantung koroner aterosklorosis. J PDGI 2006; 56(2): 53-9.
5. Ren L, Leung K, Darveau RP, Jin L. The expression profile of lipopolysaccharide-binding protein, membrane-bound CD 14, and toll-like receptors 2 and 4 in chronic periodontitis. J Periodontol 2005; 76(11): 1950-9.
6. Indahyani D, Santoso AS, Utoro T, Marsetyawan HNE. Pengaruh induksi lipopolisakarida (LPS) terhadap osteopontin tulang alveolaris tikus pada masa erupsi gigi. Ind J Dent 2007; 14(1): 2-7.
7. Rathee P, Chaudhary H, Rathee S, Rathee D, Kumar V, Khli. Mechanism of Action of Flavonoids as Anti-inflammatory Agents: A Review. J Inflammation & Allergy 2009; 8(3): 229-35.
8. Peluso I, Raquzzini A, Serafini M. Effect of flavonoids on circulating levels of TNF-α and IL-6 in humans: a systematic review and meta-analysis. Mol Nutr Food Res 2013; 57(5): 784-801.
9. Meilawaty Z. Potensi ekstrak daun singkong (Manihot utilissima) dalam memodulasi COX-2 pada monosit yang dipapar LPS. Dental Journal (Majalah Kedokteran Gigi) 2013; 46(4): 212-7.
10. Docheva M, Dagnon S, Statkova-Abeghe S. Flavonoid content and radical scavenging potential of extracts prepared from tobacco culvitars and waste. Natural Product Research 2014; 28: 1328-34.
11. Muhammad AA, Pauzi NAS, Arulselvan P, Abas F, Fakurazi S. In vitro wound healing potential and identification of bioactive compounds from Moringa oleifera Ram. bioMed Research International 2013, 2013: 1-10.
12. Ahmed MG, Choudhari R, Acharya A. Formulation and evaluation of in situ gel of atorvastatin for the treatment of periodontitis. RGUHS J.Pharm Sci 2015; 5(2): 53-60.
13. Rowe RC, Sheskey PJ, Owen SC. Handbook of pharmaceutical excipients. 5th edition. London: Pharmaceutical; 2006. p. 111-3.
14. Duarte PM, Assis DR, Casati MZ, Sallum AW, Sallum EA, Nociti Jr, FH. Alendronate may protect againts increased periodontitis-related bone loss in estrogen-deficient rats. J Periodontol 2004; 75(9): 1196-202.
15. Buduneli E, Vardar S, Buduneli N, Berdeli AH, Turkoglu O, Baskesen A, Atilla G. Effect of combined systemic administration of low-dose doxycycline and alendronate on endotoxin-induced periodontitis in rats. J. Periodontol 2004; 75(11): 1516-23.
16. Sato S, Fonseca MJV, Ciampo JOD, Jabor JR, Pedrazzi V. Metronidazole-containing gel for the treatment of periodontitis: an in vivo evaluation. Braz Oral Res 2008; 22(2): 145-50.
17. Velnar T, Bailey T, Smrkolj V. The wound healingprocess: an overviewof the cellular and molecular mechanisms. J Int Med Res 2009; 37(5): 1528-42.
18. Leitao RFC, Rocha FAC, Chaves HV, Lima V. Locally applied isosorbide decreases bone resorption in experimental periodontitis in rats. J Periodontol 2004; 75(9): 1227-32.
19. Jimson S, Balachander N, Masthan KMK, Elumalai R. A comparative study in bone decalcification using different decalcifying agents. Int J of Sci and Res 2014; 3(8): 1226-9.



DOI: 10.20473/j.djmkg.v49.i3.p137-142

20. Schiessl B, Mylonas I, Hantschmann P, Kuhn C, Schulze S, Kunze S, Friese K, Jeschke U, Expression of endothelial NO synthase, induchible NO synthase, and estrogen receptors alpha and beta in placental tissue of normal, preeclmptic, and intrauterine growth-restricted pregnancies. J of Histochemistry & Cytochemistry 2005; 53(12): 1441-9.
21. Olesn T, Goll R, Cui G, Christiansen I, Florholmen J, TNF-alpha gene expression in clorectal mucosa as a predictor of remission after induction therapy with infliximab in ulcerative colitis. J Cytokine 2009; 46(2009): 222-7.
22. Brennan FM, McInnes IB. Evidence that cytokines play a role in rheumatoid arthritis. J Clin Invest. 2008; 118(11): 3537-45.
23. Abbas AK, Lichtman AH. Cytokines. In: Cellular and molecular immunology. 5th ed. Philadelpia: WB. Saunders Company; 2005. p. 243-54.
24. Fasuyi AO, Aletor VA. Protein replacement value of cassava (Manihot esculenta, Crantz) leaf protein concentrate (CLPC) in broiler starter: effect on performance, muscle growth, haematology and serum metabolites. International Journal of Poultry Science 2005; 4(5): 339-49.
25. Garcia D, Domingues MV, Rodrigues E. Ethnopharmacological survey among migrants living in the Southeast Atlantic Forest of Diadema, Sao Paulo, Brazil. J Ethnobiol Ethnomed 2010; 6: 29.
26. Nijveldt RJ, Van Nood DEC, Van Hoorn PG, Boelens K, Van Norren PAM, Van Leeuwen. Flavonoids: a review of probable mechanisms of action and potential applications. Am J Clin Nutr 2001; 74(4): 418-25.
27. Sargowo D, Sumarno IK, Muliartha D, Kamaruddin M. Peran lipopolisakarida helicobacter pylori terhadap aktivitas neutrofil pada penderita infark miokard akut melalui degradasi kolagen tipe IV. J Kardiol Ind 2007; 28: 327-37.
28. Ahmed E. Immune mechanisms in atherosclerosis. Dissertation. Konferensrummet, Centrum för Molekylär Medicin, Karolinska Sjukhuset; 2001.
29. Hidayati NA, Listyawati S, Setyawan AD. Kandungan kimia dan uji antiinflamasi ekstrak etanol Lantana camara L. pada tikus putih (Rattus norvegicus L.) jantan. J Bioteknologi 2008; 5(1): 10-7.
30. Nieman DC, Henson DA, Davis JM, Angela Murphy E, Jenkins DP, Gross SJ, Carmichael MD, Quindry JC, Dumke CL, Utter AC, McAnulty SR, McAnulty LS, Triplett NT, Mayer EP. Quercetin's influence on exercise-induced changes in plasma cytokines and muscle and leukocyte cytokine mRNA. J Appl Physiol 2007; 103(5): 1728-35.

Dental Journal (Majalah Kedokteran Gigi) p-ISSN: 1978-3728; e-ISSN: 2442-9740. Accredited No. 56/DIKTI/Kep./2012.

DOI: 10.20473/j.djmkg.v49.i3.p137-142