ORIGINAL ARTICLE

Effect of Kecombrang Flower (*Etlingera elatior*) Ethanolic Extract on the Number of Macrophages in Periodontitis induced with Hyperglycemia

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ABSTRACT

Introduction: Chronic inflammation can mediate *diabetes mellitus* and periodontitis and will exacerbate these conditions through a bidirectional relationship. Periodontitis-induced inflammation triggers immune cell activation, one of which is macrophages. The study aimed to determine the effect of *kecombrang* flower (*Etlingera elatior*) ethanolic extract on macrophage number in periodontitis of Wistar rats induced with hyperglycemia. **Methods:** A 70% ethanolic extract of *kecombrang* flowers was prepared, with flavonoid content identified through Thin Layer Chromatography (TLC). Thirty-two Wistar rats induced by hyperglycaemia and periodontitis were divided into two groups of equal size, namely treatment and control group. Induction of hyperglycaemia was carried out by intraperitoneal injection of streptozotocin (40 mg/kg BW). Periodontitis was induced using silk ligature with size 3/0 in the subgingival area of the mandibular incisors for 7 days. The treatment group received intraperitoneal injections of the extract (100 mg/kg BW) while control administered saline, daily for seven days. Gingival tissue was collected on days 1, 3, 5, and 7 post-injections, processed histologically by Haematoxylin-Eosin staining, followed by macrophage counting. Two-way ANOVA and LSD post hoc test ($p>0.05$) were used to analyze data. **Results:** Hyperglycaemia and periodontitis were indicated by fasting blood sugar of 369.6 mg/dL and clinical signs. The macrophage counts reached peak on day 3 then decreased gradually on days 5 and 7. The macrophage counts in the saline group were higher than those in the treatment group. **Conclusion:** Injection of 70% ethanolic extract of *kecombran*g flower as an anti-inflammatory agent effectively reduces macrophage number in hyperglycemic and periodontitis.
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Keywords: *Etlingera elatior*, Hyperglycemia, Periodontitis, Flavonoid, Macrophage

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INTRODUCTION

Hyperglycemia refers to high blood sugar, >200 mg/dL under normal circumstances, >126 mg/dL when fasting, or when HbA1c levels are 6.5% or higher. It is typically attributed to individuals with decreased activity levels, increased obesity, and an aging population (1, 2, 3). Hyperglycaemia is the main characteristic of diabetes, which is diagnosed in 537 million people worldwide and causes 6.7 million deaths a year (4). Individuals with uncontrolled diabetes are more susceptible to periodontitis, but ironically, one increases the risk of diabetes when suffering from periodontitis (5).

Periodontitis is one periodontal disease that inflammation has spread from the gingiva to the cementum and periodontal ligament, damaging the tissue and causing alveolar bone resorption (6, 7). It manifests as gingival inflammation and or recession, loss of clinical attachment, alveolar bone loss, increased probing depth, bleeding on probing (BOP), and tooth mobility (8, 9).

The central feature of both hyperglycaemia and periodontitis is inflammation, which causes an elevation in macrophage count (10, 11). Diabetic individuals with periodontitis exhibit a skewed macrophage population, favouring pro-inflammatory M1 cells over tissue-repairing M2 cells, causing periodontal tissue repair in diabetic patients to be much less efficient (12).

Inflammation in periodontitis and hyperglycaemia is

generally treated with non-steroidal anti-inflammatory drugs (NSAIDs) and hypoglycaemic drugs such as metformin. It is important to note that the use of combination drugs carries the risk of various side effects, such as severe lactic acidosis and acute renal failure (13, 14, 15, 16, 17). To address these concerns, the indigenous Indonesian plant, *kecombrang* (*Etlingera elatior*), offers an accessible, safe, and cost-effective solution due to its flavonoid content (18, 19).

Previous in vivo study has proven the effect of 70% ethanolic extract of *Etlingera elatior* as an antihyperglycemic agent in rats suffering from diabetes due to alloxan induction (20). Due to the lack of previous research regarding the anti-inflammatory potential of *kecombrang* flowers, this study explored the anti-inflammatory effect of ethanol extract of *kecombrang* flowers (*Etlingera elatior*) on the macrophage number in periodontitis-induced hyperglycemic Wistar rats (Rattus norvegicus)

MATERIALS AND METHODS

Ethical clearance was issued by the Research Ethics Committee of the Faculty of Dentistry, Universitas Gadjah Mada (UGM), number 178/KE/FKG-UGM-EC/2022. Research permits at the Plant Systematics Laboratory, Faculty of Biology UGM, Pharmacognosy-Phytochemical Laboratory for Pharmaceutical Biology Unit II, Faculty of Pharmacy UGM, Integrated Research and Testing Laboratory (LPPT) UGM Unit IV for Experimental Animal Breeding (UPHP), and Integrated Research Laboratory Unit, Faculty of Dentistry UGM had been obtained prior to this research.

Raw *kecombrang* flowers were collected from Ngaglik, Sleman, Yogyakarta, and identified as *Etlingera elatior*. The *kecombrang* flowers were determined at the Plant Systematics Laboratory, Faculty of Biology, UGM according to letter No. 0128/S.Tb./VII/2022. The ethanolic extract was obtained through maceration, where the kecombrang flowers were cleaned and dried in an oven at 45°C for 48 hours, processed into a fine powder, dissolved in 70% ethanol, and stirred for 24 hours. The process was repeated twice, with solvent replacement done every 24 hours at room temperature. The results were concentrated using a rotary evaporator at a temperature of 40°C, obtaining 22.60 g of extract from 2000 g of kecombrang flowers. Its flavonoid content was confirmed through phytochemical screening using thin-layer chromatography (TLC), presenting the formation of brownish-yellow stains after being steamed with ammonia under visible blue light at UV 366 nm.

Thirty-two healthy Wistar rats (*Rattus norvegicus*), male, with an average weight of 200-300 g, were fed and hydrated ad libitum. The rats were induced with hyperglycemia and periodontitis on the same day.

Both groups were anesthetized with a mixture of xylazine 10 mg/kgBB (XylaTM, Netherlands) and ketamine 80 mg/kgBB (KeproTM, Netherlands). The rats were then injected intraperitoneally with a single dose of streptozotocin 40 mg/kg BW (STZ®) dissolved in 1 mL of citrate buffer to induce hyperglycemia, followed by subgingival ligation on the mandibular incisors using 3/0 silk ligature (Serenity®, China) to induce periodontitis (21). Fasting blood glucose (FBG) was measured using GCU's EasyTouch® glucometer 3 days (72 h) after the administration of STZ® to confirm induction of diabetes. The rats were declared to be hyperglycaemic if the fasting blood glucose was 369.6 mg/dL (>200 mg/dL). The ligation was removed 7 days post-induction. Periodontitis and hyperglycemic rats were then evenly separated into two groups: treatment and control.

The treatment group received 70% Etlingera elatior ethanol extract (100 mg/kg BW) intraperitoneally once a day for seven days, while the control group received saline injections with the same amount, method, and frequency. Gingival samples were collected on days 1, 3, 5, and 7 after the first injection, and the rats were euthanized by cervical dislocation. The mandibles were cut and placed in 10% formalin buffer with pH 7.4 for 24 hours in preparation for histological processing. The retrieved specimens were processed, stained with Haematoxylin-eosin, mounted into glass slides, and labelled. The observers counted the macrophages in five visual fields using a light microscope equipped with an Optilab® camera at 400x magnification.

The data obtained were analyzed using two-way ANOVA, followed by the Multi Comparison Least Significant Difference (LSD) post hoc test with a significance level of 95% ($p<0.05$).

RESULT

Data of blood glucose (mg/dL) before and after induced by streptozotocin (40 mg/kg BW) and treatment are shown in Figure 1. All the rats were declared hyperglycaemic after 3 days with fasting blood glucose of 369.6 mg/dL (>200 mg/dL). After 7th days of treatment (*kecombrang*

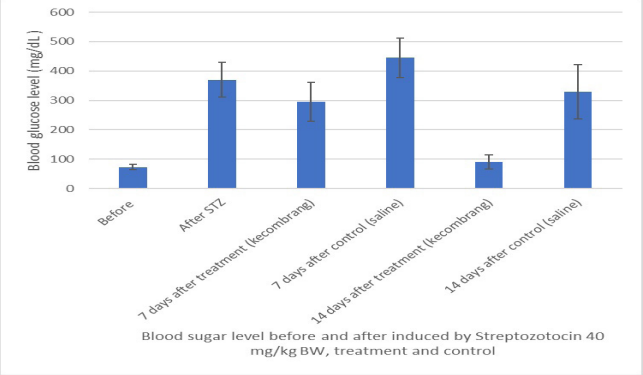

Figure 1: Blood glucose level before and after induced by Streptozotocin 40 mg/kg BW and 7 days after treatment.

flower), the fasting blood glucose decreased with an average of 294 mg/dL while the average the control group was 444,7 mg/dL. In the treatment group, blood sugar levels decreased until on the 14th day blood sugar levels were seen almost to those before STZ induction while the control group showed that their blood sugar was still high (>200 mg/dL).

After the ligature was removed from the rats' mandibular incisors, Wistar rats showed clinical signs of periodontitis, in the form of redness, swelling, tooth mobility, gingival recession, bleeding on probing (BOP), and the formation of periodontal pockets with a depth of 2-3.5 mm.

In this study, we observed the number of macrophages after experimental animals suffered hyperglycaemia and periodontitis then were treated with *kecombrang* flower extract as treatment and saline as negative controls so we did not have data on the number of macrophages before treatment.

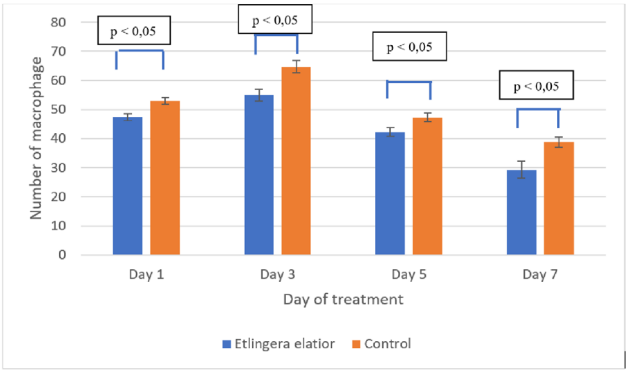

Figure 2: Number of macrophages in the treatment group and control group on days 1, 3, 5, and 7 post-injections.

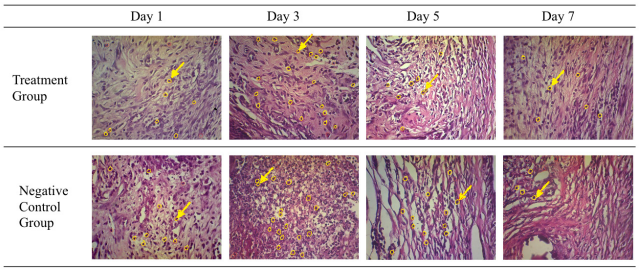

Figure 3: Histological evaluation of macrophage in the treatment group and control group on days 1, 3, 5, and 7 post-injections (magnification 400x)

The results showed macrophage count reached its peak on day 3, followed by a gradual decrease on day 5 and day 7. This trend was found in both treatment and control groups.The data were interpreted as normal ($p > 0.05$) and homogenous ($p > 0.05$) using Shapiro-Wilk and Levene test. The average macrophage count in the treatment group was significantly less than that in the control group ($p < 0.05$) (Fig. 2). Histological observation can be seen in Figure 3.

DISCUSSION

This study explored the anti-inflammatory effect of ethanol extract of *kecombrang* flowers (*Etlingera elatior*) on macrophage count in periodontitis-induced hyperglycaemic Wistar rats. Upon streptozotocin (STZ) injection, the Wistar rats showed clinical signs of diabetes in the form of 3Ps, namely increased frequency and volume of urine (polyuria), increased food consumption (polyphagia), and increased water consumption (polydipsia), malaise, and fasting blood glucose <200 mg/dL. Injection of STZ at medium doses in the range of 40-55 mg/kg BW was carried out to induce partial insulin secretion disorders (21). STZ entered cells via low-affinity GLUT2 transporters, predominantly affecting pancreatic β cells due to their heightened glucose uptake. The methyl nitrosourea group in STZ causes DNA methylation, leading to energy depletion and cell necrosis.

After 7 days of cervical ligation of the mandibular incisors, the Wistar rats showed clinical signs of periodontitis in the form of redness, swelling, accumulation of plaque, teeth mobility, gingival recession, bleeding on probing (BOP), and the formation of periodontal pockets. Cervical ligation of rat teeth using 3/0 silk ligature could induce periodontitis (22). The initial lesion phase occurs 2-4 days after plaque accumulation (23, 16). On the 7th day after plaque accumulation, an early lesion phase occurred, followed by an established and advanced lesion phase, in which the gingiva lost its attachment and showed clinical signs of periodontitis according to the findings in this study.

The chronic inflammatory phase of periodontitis is characterized by chronic inflammatory cell migration. One of them is macrophages, which are differentiated monocytes. Monocytes in blood vessels migrate to connective tissues following signals from apoptotic neutrophils, which are induced by stimulants such as lipopolysaccharide, IL-8, TNF-α, and macrophage chemoattraction protein-1 (MCP-1) (24). Fibroblasts in connective tissue produce Granulocyte Macrophage Colony Stimulating Factor (GM-CSF), which induces the differentiation of monocytes into macrophages (25).

The results of the histological observation (Figures 2 and 3) showed an increase in the macrophage count in the control and treatment groups and reached a peak on day 3. The highest peak number of macrophages occurred on day 3 after treatment or 10 days after induction of STZ or binder to induce periodontitis. This is probably because the experimental animal model has suffered chronic inflammation since the induction of hyperglycaemia and periodontitis. The macrophage count in the control group was higher than that in the treatment group on all days of observation. A decrease in the macrophage count

was seen starting on day 5 or 12 days after induction hyperglycaemia and periodontitis.

On days 1 to 7 after ligation, an acute to chronic inflammatory phase occurred, characterized by red gingiva, swelling, gingival recession, and the occurrence of periodontal pockets. Macrophages appeared at the site of inflammation 2–3 days after injury and phagocytized debris, pathogens, and remaining apoptotic neutrophils (26, 27). In this study, it appeared that there were many macrophages on day 1 after treatment or 8 days after induction hyperglycaemia and periodontitis, which reached a peak on day 3. In this study we did not count the number of macrophages after STZ and periodontitis induction so we did not have data before treatment and control. The decrease in the average macrophage counts on days 5 and 7 in the negative control group that was given saline injection showed the tissue's normal physiological response to inflammation. Macrophages undergo apoptosis after carrying out their duties in the form of phagocytosis of bacteria and their products and apoptotic neutrophils. Macrophage apoptosis occurs 8-16 hours after phagocytosis is completed (28).

The decrease in the macrophage count starting on day 5 after treatment indicated a lesion that occurred at the established stage. The macrophage count in the treatment group was lower than that in the control group, possibly due to the active component of the *kecombrang* flower. In the treatment group, the decline in the macrophage count indicated the success of the 70% *Etlingera elatior* ethanolic extract injection. *Kecombrang* flowers (*Etlingera elatior*) contain 763 mg QE/100 g flavonoids, which show anti-inflammatory properties by inhibiting prostanoid biosynthesis (COX, LOS, and iNOS) (29, 30). Flavonoids act as dual inhibitors of the enzymes cyclooxygenase-2 (COX-2) and 5-lipoxygenase (5-LOX) (31). Arachidonic acid (AA) produces metabolites in the form of prostaglandins and leukotrienes through metabolic processes mediated by COX and 5-LOX enzymes. The COX enzyme is a catalyst for converting AA into prostaglandin G2 (PGG2), then into prostaglandin H2 (PGH2), which then transforms into a number of types of prostaglandins and thromboxane.

COX-1 plays a role in the integrity of the haemostatic system, gastric mucosal defense, kidney function, and platelet aggregation, while COX-2 plays a role in inflammation. The prostanoid inhibition process acts to inhibit COX-2 without interfering with COX-1 function, so gastrointestinal and renal toxicity do not occur. In addition to COX-2, 5-LOX is a catalyst for the oxidation of AA to leukotrienes. LTB4, as a leukotriene mediator, regulates innate immune responses and chronic inflammation. Flavonoids affect several arachidonic acid pathways, resulting in an effective treatment because they inhibit the effects of COX-2 and 5-LOX without interfering with COX-1 action. Flavonoids could activate antioxidant pathways that render an anti-inflammatory effect. Flavonoids may inhibit the secretion of enzymes, such as lysozymes and β-glucuronidase, and inhibit the secretion of arachidonic acid, which reduces inflammatory reactions (32). COX-2 inhibitors could reduce the macrophage count in gingival tissue by inhibiting the production of prostaglandins (33).

The pathway of *kecombrang* flower extract in the peritoneum could induce macrophages in the oral cavity, which can be through a complex mechanism involving the immune system. The active content of *kecombrang* flower extract is likely to interact with receptors expressed on the surface of macrophages including mannose receptors, scavenger receptors, dectin-1 receptors, tuftsin, CD44 cluster of differentiation, folate-β receptors, and phosphatidylserine receptors 2. This mechanism such as polymer nanoparticle-based drug delivery through the peritoneal cavity, is one method that can target macrophages, although further research is still needed (34).

The flavonoid content in *kecombrang* flowers (*E. elatior*) is known to have antihyperglycemic activity through inhibiting the enzymes α-glucosidase and α-amylase and also has functions as an anti-inflammatory. Inhibition of this enzyme is thought to limit carbohydrate absorption and reduce postprandial sugar absorption so that it can regulate the hyperglycemia status of diabetes sufferers, thereby reducing blood glucose levels (35). It is hoped that intraperitoneal administration of *kecombrang* flower extract can be developed as a systemic treatment for hyperglycaemia. This is because the natural antioxidant content of *kecombrang* flowers has potential as an ingredient for preventing or treating DM (36). Other study showed E. elatior as a natural source that has anti-inflammatory and antioxidant compounds so it may could manage diabetes and prevent diabetic nephropathy (37).

Reducing systemic inflammatory conditions is thought to have an effect on reducing periodontitis through the mechanism of peritoneal resident macrophages (PRM) which play an important role in immune surveillance in the peritoneal cavity. These macrophages initially originate from embryonic progenitor cells and can be replenished by bone marrow-derived monocytes during inflammation and aging (38), although the direct pathway from the peritoneum to the oral cavity is not well-established. Further research is needed to fully understand the complex mechanisms.

CONCLUSION

This study concluded that injecting 70% ethanol extract of *Etlingera elatior* as an anti-inflammatory agent successfully reduced the macrophage number in periodontitis-induced hyperglycemic *Rattus norvegicus*.

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REFERENCES

1. Mouri MT, Badireddy M. Hyperglycemia. In: StatPearls [Internet]. Treasure Island (FL) : StatPearls Publishing; 2022 [cited 2022 October 5]. Available from: https://www.ncbi.nlm.nih.gov/books/NBK430900/. PMID: 28613650
2. Petersmann A, Mьller-Wieland D, Mьller UA, Landgraf R, Nauck M, Freckmann G, Heinemann L, Schleicher E. Definition, Classification and Diagnosis of Diabetes Mellitus. Exp Clin Endocrinol Diabetes. 2019 Dec;127(S 01):S1-S7. doi: 10.1055/a-1018-9078. Epub 2019 Dec 20. PMID: 31860923.
3. Viswanath B, Choi CS, Lee K, Kim S. Recent Trends in The Development of Diagnostic Tools For Diabetes Mellitus Using Patient Saliva. TrAC, Trends Anal Chem 2017; 89: 60-67. https://doi.org/10.1016/j.trac.2017.01.011.
4. International Diabetes Federation. IDF Diabetes Atlas, 10th ed. Brussels: International Diabetes Federation; 2021. Available at: https://www.diabetesatlas.org
5. Kocher T, Kunig J, Borgnakke WS, Pink C, Meisel P. Periodontal complications of hyperglycemia/diabetes mellitus: Epidemiologic complexity and clinical challenge. Periodontol 2000. 2018 Oct;78(1):59-97. doi: 10.1111/prd.12235. PMID: 30198134.
6. Graziani F, Karapetsa D, Alonso B, Herrera D. Nonsurgical and surgical treatment of periodontitis: how many options for one disease? Periodontol 2000. 2017 Oct;75(1):152-188. doi: 10.1111/prd.12201. PMID: 28758300.
7. Preshaw PM, Alba AL, Herrera D, Jepsen S, Konstantinidis A, Makrilakis K, Taylor R. Periodontitis and diabetes: a two-way relationship. Diabetologia. 2012 Jan;55(1):21-31. doi: 10.1007/s00125-011-2342-y. Epub 2011 Nov 6. PMID: 22057194; PMCID: PMC3228943.
8. Kumar S. Evidence-Based Update on Diagnosis and Management of Gingivitis and Periodontitis. Dent Clin North Am. 2019 Jan;63(1):69-81. doi: 10.1016/j.cden.2018.08.005. Epub 2018 Oct 29. PMID: 30447793.
9. Kwon T, Lamster IB, Levin L. Current Concepts in the Management of Periodontitis. Int Dent J. 2021 Dec;71(6):462-476. doi: 10.1111/idj.12630. Epub 2021 Feb 19. PMID: 34839889; PMCID: PMC9275292.
10. Galicia-Garcia U, Benito-Vicente A, Jebari S, Larrea-Sebal A, Siddiqi H, Uribe KB, Ostolaza H, Martнn C. Pathophysiology of Type 2 Diabetes Mellitus. Int J Mol Sci. 2020 Aug 30;21(17):6275. doi: 10.3390/ijms21176275. PMID: 32872570; PMCID: PMC7503727.
11. Matthews DC. The relationship between diabetes and periodontal disease. J Can Dent Assoc. 2002 Mar;68(3):161-4. PMID: 11911811.
12. Almubarak A, Tanagala KKK, Papapanou PN, Lalla E, Momen-Heravi F. Disruption of Monocyte and Macrophage Homeostasis in Periodontitis. Front Immunol. 2020 Feb 26;11:330. doi: 10.3389/fimmu.2020.00330. PMID: 32210958; PMCID: PMC7067288.
13. Artasensi A, Pedretti A, Vistoli G, Fumagalli L. Type 2 Diabetes Mellitus: A Review of Multi-Target Drugs. Molecules. 2020 Apr 23;25(8):1987. doi: 10.3390/molecules25081987. PMID: 32340373; PMCID: PMC7221535.
14. Chatterjee S, Khunti K, Davies MJ. Type 2 diabetes. Lancet. 2017 Jun 3;389(10085):2239-2251. doi: 10.1016/S0140-6736(17)30058-2. Epub 2017 Feb 10. Erratum in: Lancet. 2017 Jun 3;389(10085):2192. PMID: 28190580.
15. Gugliandolo E , Fusco R, D'Amico R, et al. Anti-inflammatory effect of ATB-352, a H2S -releasing ketoprofen derivative, on lipopolysaccharide-induced periodontitis in rats. Pharmacol Res. 2018;132: 220-231. DOI: 10.1016/j.phrs.2017.12.022
16. Newman MG, Elangovan S, Dragan IF, Karan AK. Newman and Carranza's Essentials of Clinical Periodontology - An Integrated Study Companion. 1st ed. Elsevier; 2020.
17. Chan NN, Fauvel NJ, Feher MD. Non-steroidal anti-inflammatory drugs and metformin: a cause for concern? Lancet. 1998 Jul 18;352(9123):201. doi: 10.1016/s0140-6736(05)77806-5. PMID: 9683213.
18. Ginwala R, Bhavsar R, Chigbu DI, Jain P, Khan ZK. Potential Role of Flavonoids in Treating Chronic Inflammatory Diseases with a Special Focus on the Anti-Inflammatory Activity of Apigenin. Antioxidants (Basel). 2019 Feb 5;8(2):35. doi: 10.3390/antiox8020035. PMID: 30764536; PMCID: PMC6407021.
19. Juwita T, H P Pakpahan W, M Puspitasari I, Mekar Saptarini N, Levita J. Anti-inflammatory Activity of Etlingera elatior (Jack) R.M. Smith Flower on Gastric Ulceration-induced Wistar Rats. Pak J Biol Sci. 2020; 23(9):1193-1200. doi: 10.3923/pjbs.2020.1193.1200. PMID: 32981250.
20. Fitrianita A, Yardi Y, Musir A. Uji efek antihiperglikemia ekstrak etanol 70% daun kecombrang (Etlingera elatior) pada tikus sprague dawley dengan penginduksi aloksan. Jurnal Ilmiah Farmasi. 2018;14(1):9-16. https://doi.org/10.20885/jif.vol14.iss1.art02
21. Husna F, Suyasna FD, Arozal W, Purwaningsih EH. Model Hewan Coba pada Penelitian Diabetes.

Pharmaceutical Sciences and Research. 2019;6(3): 131-1. DOI: 10.7454/psr.v6i3.4531
22. Lin SK, Wu YF, Chang WJ, Feng SW, Huang HM. The Treatment Efficiency and Microbiota Analysis of Sapindus mukorossi Seed Oil on the Ligature-Induced Periodontitis Rat Model. Int J Mol Sci. 2022 Aug 2;23(15):8560. doi: 10.3390/ijms23158560. PMID: 35955695; PMCID: PMC9369273.
23. Kurgan S, Kantarci A. Molecular basis for immunohistochemical and inflammatory changes during progression of gingivitis to periodontitis. Periodontol 2000. 2018; 76(1):51-67. doi: 10.1111/prd.12146. Epub 2017 Nov 29. PMID: 29194785.
24. Guyton AC, Hall, JE, Textbook of Medical Physiology. Philadelphia: Elsevier; 2006.
25. Shi Y, Liu CH, Roberts A, et al. Granulocyte-macrophage colony- stimulating factor (GM-CSF) and T-cell responses: what we do and don't know. Cell Research. 2006;16(2): 126-33. https://doi.org/10.1038/sj.cr.7310017
26. Larjava H. Oral wound Healing: Cell Biology and Clinical Management, United Kingdom: Wiley-Blackwell; 2012.
27. Lorencini M, Silva JA, de la Hoz CL, Carvalho HF, Stach-Machado DR. Changes in MMPs and inflammatory cells in experimental gingivitis. Histol Histopathol. 2009; 24(2):157-66. doi: 10.14670/HH-24.157. PMID: 19085832.
28. Kirschnek S, Ying S, Fischer SF, HΔcker H, Villunger A, Hochrein H, HΔcker G. Phagocytosis-induced apoptosis in macrophages is mediated by up-regulation and activation of the Bcl-2 homology domain 3-only protein Bim. J Immunol. 2005; 174(2):671-9. doi: 10.4049/jimmunol.174.2.671. PMID: 15634885.
29. Chan EWC, Lim YY, Wong, SK. Photochemistry and Pharmacological Properties of Etlingera elatior: A Review. Pharmacogn J. 2021;3(22): 6-10. DOI: 10.5530/pj.2011.22.2
30. Ernilasari, Walil K, Fitmawati, et al. Antibacterial activity of leaves, flowers, and fruits extract of Etlingera elatior from Nagan Raya District, Indonesia against Escherichia coli and Staphylococcus aureus. BIODIVERSITAS. 2021;22(10): 4457-4464. https://doi.org/10.13057/biodiv/d221039
31. Md Idris MH, Mohd Amin SN, Mohd Amin SN, et al. Flavonoids as dual inhibitors of cyclooxygenase-2 (COX-2) and 5-lipoxygenase (5-LOX): molecular docking and in vitro studies. Beni-Suef Univ J Basic Appl Sci. 2022;11:117. https://doi.org/10.1186/s43088-022-00296-y
32. Al-Khayri JM, Sahana GR, Nagella P, Joseph BV, Alessa FM, Al-Mssallem MQ. Flavonoids as Potential Anti-Inflammatory Molecules: A Review. Molecules. 2022 May 2;27(9):2901. doi: 10.3390/molecules27092901.
33. Preethanath RS, Ibraheem WI, Anil A. Pathogenesis of gingivitis. Oral Diseases. 2020 Mar 6:1-9. DOI: 10.5772/intechopen.91614
34. Sahle FF. Polymeric Nanoparticles-Based Drug and Gene Delivery to Macrophages. In: Gupta S, Pathak YV. (eds) Macrophage Targeted Delivery Systems. Springer, Cham; 2022. https://doi.org/10.1007/978-3-030-84164-5_7
35. Juwita T, Melyani Puspitasari I, Levita J. Torch Ginger (Etlingera elatior): A Review on its Botanical Aspects, Phytoconstituents and Pharmacological Activities. Pak J Biol Sci. 2018 Jan;21(4):151-165. doi: 10.3923/pjbs.2018.151.165.
36. Noordin L, Wan Ahmad WAN, Muhamad Nor NA, Abu Bakar NH, Ugusman A. Etlingera elatior Flower Aqueous Extract Protects against Oxidative Stress-Induced Nephropathy in a Rat Model of Type 2 Diabetes. Evid Based Complement Alternat Med. 2022;2022:2814196. doi: 10.1155/2022/2814196.
37. Widyarini T, Indarto D, Soetrisno S, Purwanto B. Modulation effects of Etlingera elatior ethanol extract as anti-inflammatory on chronic kidney disease in mice with hypertension and diabetes. J Popul Ther Clin Pharmacol. 2022; 29(4):e140-e149. doi: 10.47750/jptcp.2022.988.
38. Jayakumar P, Laganson A, Deng M. GATA6+ Peritoneal Resident Macrophage: The Immune Custodian in the Peritoneal Cavity. Front Pharmacol. 2022 Mar 23;13:866993. doi: 10.3389/fphar.2022.866993.