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Research Report

The effects of *Curcuma zedoaria* oil on high blood sugar level and gingivitis

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ABSTRACT

Background: *Hyperglycemia is a condition when blood sugar level is higher than normal. Hyperglycemia is also one of diabetes mellitus (DM) symptoms. Hyperglycemia has a correlation with the occurrence of periodontal disease. Curcuma zedoaria oil is known to decrease concentration of serum glucose.* ***Purpose:*** *This study was aimed to determine the effects of Curcuma zedoaria oil on high blood sugar level and gingivitis in rats.* ***Method:*** *This study used twenty-five male Wistar rats, divided into two groups, namely the treatment group and the control group. In the treatment group, fifteen rats were divided into three subgroups (each of which was induced with 10 µl/ml, 30 µl/ml and 50 µl/ml of Curcuma zedoaria oil). The control group was consisted of ten rats, divided into two subgroups, as the positive control group (induced with 10 mg/kg of Glibenclamide) and the negative control group (induced with propylene glycol). Streptozotocin (STZ) (Naclai tesque, Kyoto Japan) with a dose of 40 mg/kg was used to create hyperglycemia condition in those rats. Gingivitis was then made by using silk ligature in those hyperglycemia rats. Silk ligature was twisted at the margin of gingiva anterior mandibular incisors for seven days. After the rats had gingivitis, Curcuma zedoaria oil, glibenclamide and propylene glycol were orally administered for seven days. Their gingivitis condition was observed, and their blood sugar level was measured before and after the induction of STZ and during the treatment. The data obtained were analyzed by using Manova.* ***Result:*** *There were significant differences of blood sugar levels between the treatment group before and after the administration of Curcuma zedoaria oil and the positive control group ($p<0.05$). Healthy gingiva was then found in the treatment group and the positive control group.* ***Conclusion:*** *Curcuma zedoaria oil can decrease blood sugar level and gingivitis.*

Keywords: *Curcuma zedoaria oil; gingivitis; blood sugar level*

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INTRODUCTION

Hyperglycemia is a condition when blood sugar level is higher than normal. Hyperglycemia is also one of diabetes mellitus (DM) symptoms, and it is considered as a metabolic disorder. In metabolic disorder condition characterized by the presence of oxidative stress, the balance between production and inactivation of reactive oxygen species (ROS) is disrupted. ROS has a very important role in various physiological systems, especially for celluler metabolism.[1,2]

Hyperglycemia condition will cause changes in glucose homeostasis metabolism. Pathological mechanism of hyperglycemia involving β cells of the pancreas, for instance, can cause its inability of to produce insulin. Hyperglycemia is also known to be associated with periodontal disease, especially related to the mechanism of glycation end-product (AGE) formation. Organism physiologically produce AGE, but the production of AGE under hyperglycemia condition or augmented oxidative stress can be excessive.[2,3]

AGE is a substance that is able to increase cytokines produced by macrophages, such as tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6), as well as to stimulate the secretion of hepatic protein acute-phase, including C-reactive protein (CRP), fibrinogen, plasminogen

activator/inhibitor and amyloid A serum. AGE is also known to correlate with the occurrence of oral infections and cardiovascular disease, especially in patients with periodontal disease. In addition, AGE can increase the activities of monocyte migration, endothelial permeability, fibroblast permeability and muscular cells, and can also bind to collagen. AGE substance can cause a rapid expansion in the respiratory activity of polymorphonuclear neutrophils (PMN), leading to an increased severity of periodontal tissue destruction and some changes in bone metabolism, especially during healing process, and can also reduce the production of extracellular matrix.[3,4]

Periodontal disease is likely caused by neutrophilic basic immune response as a result of excessive and uncontrolled production of ROS. This condition then leads to local and peripheral oxidative damage directly and indirectly caused by genes redox-sensitive transcription factors. Nuclear factor kappa B and activator protein-1 can trigger inflammatory mediator of cascade and accelerate cellular aging. Hyperglycemia, thus, has associated with the changes in oral mucosa barrier marked with PMN accumulation, increased matrix metalloproteinase (MMP) and ROS in periodontal tissues. The changes then may trigger inflammation in the periodontium tissue, so the gingival crevice fluid can be detected from the increased level of prostaglandin E 2 (PGE 2) and IL-1β.[2,3,4]

On the other hand, *Curcuma zedoaria* has been studied for oral health maintenance. In dentistry, *Curcuma zedoaria* oil even has been used for its potential therapeutic effects as antimicrobial and anti-inflammatory herbs and for lowering blood sugar levels.[5,6] The chemical composition of herbal extracts is actually depends on several variables, such as growing conditions, soil quality, season and post-harvest handling. The pharmacological effects of the extracts in both liquid and powder are expected to be related to the interaction of chemical substances contained, not only to isolated molecules.

After measured with gas chromatography and mass spectrometry (GC-MS), *Curcuma zedoaria* oil is known to contain several compounds, namely camphene; 1-beta-pinene; mycrene; 1,8-cineole; camphor; beta-elemene; bicyclo [2.2.1] heptane-2-ol, 1,7,7-trimethyl-, exo; borneol L; eremophilene; (+) Calarene; valencene; beta-elemenone; germacrone and 2-ethoxy-6-ethyl-4,4,5-trimethyl-1,3-dioxa-4-sila-2-boracyclohex-5-ene.[7] Some previous studies even indicated that *Curcuma zedoaria* oil can be used as an anti-inflammatory for artificial edema at a dose of 400 mg / kg, [8] increase phagocytic activity of neutrophils [9] and reduce gingival inflammation by decreasing $CD4^+$ expression. [10] Potential phytochemistry of *Curcuma zedoaria* powder can also decrease glucose serum.[11] However, the effects of *Curcuma zedoaria* oil on hyperglycemia accompanied by gingivitis in rats have not been known yet. Therefore, this study was aimed to determine the potential therapeutic effects of *Curcuma zedoaria* oil on the high blood glucose level and gingivitis.

MATERIALS AND METHODS

This study was an experimental study to analyze the biological activities of *Curcuma zedoaria* oil against hyperglycemia and gingivitis in the oral cavity of rats. Determination of *Curcuma zedoaria* was conducted at the Laboratory of Biology Pharmacy, Faculty of Pharmacy, Universitas Gadjah Mada. *Curcuma zedoaria* oil was prepared in Unit II of Integrated Research and Testing Laboratory (LPPT) Universitas Gadjah Mada by using water vapor distillation method. The procedures of this study were approved by the Ethics Committee of Dentistry Faculty of Dentistry, Universitas Gadjah Mada. This study used twenty-five male Wistar rats aged 2 months old obtained from Unit IV of LPPT, Universitas Gadjah Mada. To adapt to the laboratory environment, those Wistar rats were put in individual cages for one week, feed with standard pellets as much as 360 grams per day and administered with mineral water ad libitum.

The procedures of this study were conducted in several stages. To create hyperglycemic condition in those rats, they were fasted for 18 hours before treated. Intraperitoneal injection of *Streptozotocin (STZ)* (Nacalai tesque, Kyoto Japan) was administrated in the abdomen of those rats with a dose of 40 mg/kg in 0.1 M citrate buffer. Those rats were drunk with 5% glucose solution to protect the effects of *STZ*.[12] Those rats were intramuscularly anesthetized into their right thigh by ketamine HCl with a dose of 0.2 ml/200 grams. Gingivitis was made by using silk ligature with size 3.0. Ligation was placed on margin of anterior gingiva mandibular incisors for six days. Silk ligature was checked every day to observe the possibility of the release of ligature. The changes of gingiva were observed, such as reddish color, shiny look, and bleeding easily.

Those hyperglycemia and gingivitis rats were divided into two groups randomly, namely the treatment group (fifteen rats) and the control group (ten rats). The treatment group was divided into three subgroups, each of which was consisted of five rats and administered orally with 10 µl/ml, 30 µl/ml and 50 µl/ml of *Curcuma zedoaria* oil. Meanwhile, the control group was divided into two subgroups. Five rats as the positive control group induced with 10 mg/kg of glibenclamide and five rats as the negative control group induced orally with propylene glycol. The administration of materials in the treatment and control groups was conducted every day for seven days by using oral gavage. Their gingiva condition was then observed, and their blood sugar level were measured before and after the induction of STZ and after the treatment. The measurement of the blood sugar levels was conducted 24 hours after the injection of STZ. Totally, three rats of the treatment group induced with 10 µl/ml of *Curcuma zedoaria* oil, of the positive control and of the negative control died after 24 hours of STZ injection. Finally, data obtained were analyzed by using Manova statistical test.

RESULTS

The mean of their fasting blood sugar level before the induction of STZ was 89.12 mg/dl, while the mean of their blood sugar level 24 hours after the induction of STZ was 192.24 mg/dl. The blood sugar level of those rats in the treatment and control groups was then observed every day for seven days (Figure 1).

The observation of gingiva after seven days of the ligation showed that all of those rats had gingivitis, characterized by reddish color, shiny look, and bleeding easily. The results of the gingiva observation in the treatment and control groups can be seen in Table 1. Meanwhile, the results of gingivitis and healthy gingiva were showed in Figure 2.

The statistical results of Manova and LSD *post hoc* tests, moreover, showed that the data obtained were normal and homogen (p>0.05). The statistical results of Manova test showed that there was a significant difference of blood sugar level between in the positive control and in the treatment group (p<0.05). It indicated that *Curcuma zedoaria* oil had a significant effect on the decreasing of the fasting blood sugar level. The results of LSD *post hoc* test also showed that *Curcuma zedoaria* oil had a significant effect on the blood sugar level of those hyperglycemia and gingivitis rats as seen in Table 2.

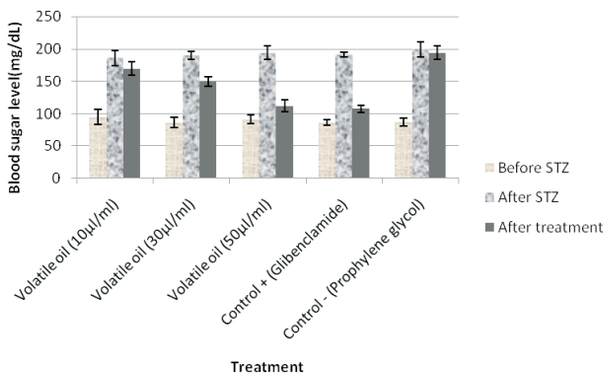

Figure 1. The mean and standard deviation of the blood sugar level (mg/dL) of those rats in the treatment and control groups. The mean of the blood sugar level increased after the STZ injection. The mean of the blood sugar level decreased after the administration of *Curcuma zedoaria* oil and glibenclamide.

Table 1. The results of the gingiva observation in the treatment and control groups. During the observations, the number of rats with healthy gingiva or gingivitis was measured

Group	Number of rats with gingivitis	Number of rats with healthy gingiva
Treatment :		
a. Curcuma zedoaria oil 10 µl/ml	-	4
b. Curcuma zedoaria 30 µl/ml	-	5
c. Curcuma zedoaria 50 µl/ml	-	5
Control :		
a. Positif (*glibenclamide*)	-	4
b. Negatif (*prophylene glycol*)	4	-

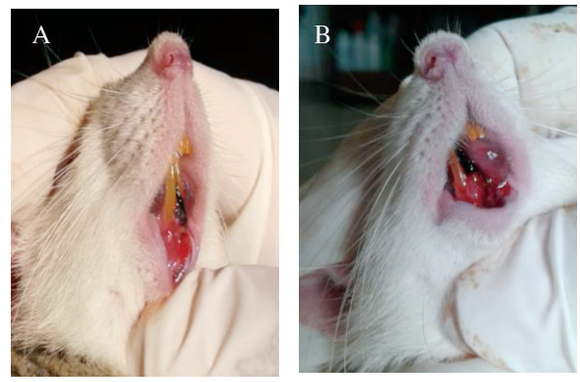

Figure 2. Normal gingiva (A) and gingivitis (B). Gingivitis looked reddish and shiny.

Table 2. The results of LSD post hoc test on the effects of *Curcuma zedoaria* oil on the blood sugar level of those hyperglycemia and gingivitis rats

Group	*Curcuma zedoaria* oil 10 µl/ml	*Curcuma zedoaria* oil 30 µl/m	*Curcuma zedoaria* oil 50 µl/m	Control +	Control -
Curcuma zedoaria oil 10 µl/ml	-	20.300 (0.291)	57.700* (0.003)	62.250* (0.004)	-24.250 (0.270)
Curcuma zedoaria oil 30 µl/ml	20.300 (0.291)	-	37.400* (0.004)	41.950* (0.001)	-44.550* (0.013)
Curcuma zedoaria oil 50 µl/m	57.700* (0.003)	37.400* (0.004)	-	-4.550 (0.999)	81.950* (0.000)
Control +	62.250* (0.004)	41.950* (0.001)	-4.550 (0.999)	-	86.500* (0.001)
Control -	-24.250 (0.270)	-44.550* (0.013)	81.950* (0.000)	86.500* (0.001)	-

Table 2 showed the significant effect of *Curcuma zedoaria* oil and glibenclamide on reducing blood sugar level. There were no significant differences of the effects of *Curcuma zedoaria* oil 10 µl/ml and 30 µl/ml, and between *Curcuma zedoaria* oil 10 µl/ml and *propylene glycol*. That there was no significant difference of the effects of *Curcuma zedoaria* oil 10 µl/ml and 30 µl/ml on blood sugar indicated that both doses could decrease blood sugar level almost at the same statistic level. Insignificant difference of the effects of *Curcuma zedoaria* oil 10 µl/ml and *propylene glycol* indicated that both could have almost the same results in decreasing blood sugar level .

DISCUSSION

After having the STZ induction with a dose of 40 mg/kg in 0.1 M citrate buffer intraperitoneally, all of those rats got hyperglycemia. The measurement of their blood sugar level before the induction was 89.12 mg/dL, while after the induction it was 192.24 mg /dl. The blood sugar level of those rats increased around 215%.

STZ compound known as glucosamine-nitrosurea can cause toxic to cells since it can trigger DNA damage. DNA damage causes the activation of poly ADP-ribosylation, allegedly playing an important role in increasing blood sugar level. STZ similar to glucose can be transported to cells by a glucose-transporting protein, glucose transporter type 2 (GLUT2). STZ mechanism to induce an increase in blood sugar level occurs in three phases. However, temporary hypoglycemic effect only occurrs a few minutes up to 30 minutes after STZ injection. In the first phase, blood sugar concentration is increased one hour after STZ administration, and hypoinsulinaemia occurs as a result of inhibition of insulin secretion in the next 2-4 hours. At this stage, the morphological characteristics of beta cells were characterized by intracellular vacuolisasi, dilated rough endoplasmic reticulum, reduced Golgi area, decreased secretory granules and insulin, as well as swollen mitochondria.[13,14]

In the second phase, hypoglycemia condition occurs for approximately 4-8 hours after STZ injection and lasts up to several hours. Nevertheless, this condition is usually looked bad in experimental animals experienced convulsions (seizures) since it can even cause death if not given with glucose soon, particularly when their liver glycogen storage is depleted due to fasting. In the last phase, permanent hyperglycemia morphologically characterized by degranulation and beta cell integrity loss occurs for approximately 12-48 hours. That non-beta cells are still intact shows the characters of selective beta-cells against the action of toxic substance.[13,15]

Table 1 showed that gingivitis occured after the ligation using a silk ligature. The results of the study also showed that after the administration of *Curcuma zedoaria* oil, gingiva appeared normal (healthy). These results could be predicted since *Curcuma zedoaria* oil contained curcumin and sesquiterpenoid, which can change gingivitis to be healthy gingiva. Similarly, a study conducted by Kaushik *et al*.[8] also showed that extracts of *Curcuma zedoaria* had anti-inflammatory effects in rats induced with carrageenan. The ability of curcumin contained in *Curcuma zedoaria* oil as an anti-inflammatory can reduce prostaglandins by inhibiting cyclooxygenase mechanism. Sesquiterpenoid contained in *Curcuma zedoaria* oil can also allegedly inhibit the production of PGE and nitric oxide. PGE is one of inflammation mediators. Sesquiterpenoid contained in *Curcuma zedoaria* oil even can inhibit the release of TNF-α from macrophages. Cytokine TNF-α is an inducer of the inflammatory response due to bacterial infection.

In addition, like the results of this study, the results of the previous study also showed that *Curcuma zedoaria* oil has anti-inflammatory against artificial edema in female Wistar rats at a dose of 400 mg/kg. [8] *Curcuma zedoaria* oil administered daily at a dose of 30.6 µl/ml orally for 14 days can also effectively increase the phagocytic activities of neutrophils in rats induced with *Aggregatibacter actinomycetemcomitans*.[9] *Curcuma zedoaria* oil can reduce gingiva inflammation characterized by a decrease in $CD4^+$ expression.[10]

Similarly, in this study *Curcuma zedoaria* oil could decrease blood sugar level in hyperglycemia rats (Figure 1). This result is also supported by a previous study conducted by Rahmatullah *et al*.[11] showing that *Curcuma zedoaria* extract at a dose of 50 mg, 100 mg, 200 mg and 400 mg per kg of body mass can decrease serum glucose concentrations respectively of 36.9%; 39.4%; 41.1% and 55.1% compared to controls. The result of the previous study also suggested that the methanol extract of *Curcuma zedoaria* leaves can decrease blood sugar levels in Swiss albino *mice*. Luteolin and luteolin 7-O-glucoside of flavonoids contained in *Curcuma zedoaria* leaves have abilities to decrese serum glucose level, cholesterol, triglyceride and low lipoprotein density (LDL) level.[16] Rhizome *Curcuma zedoaria* has an ability to decrease blood sugar levels because of α-pinene and curcumin.[17] *Curcuma zedoaria* extract can decrease blood sugar level allegedly through several mechanisms since *Curcuma zedoaria* extract has many abilities to amplify insulin secretion, to increase glucose uptake from the serum, or to decrease the absorption of glucose from intestinum.[11]

In this study, *Curcuma zedoaria* oil was easily absorbed in the digestive tract of those rats, and rapidly reached their bloodstream within 15 minutes. Their blood sugar level, as a result, decreased since *Curcuma zedoaria* oil has an ability to improve the immune system of those rats which blood sugar level was increased due to the STZ induction. This was probably due to the mechanism of *Curcuma zedoaria* oil that could protect beta pancreatic cells so that the damage of beta pancreatic cells induced by STZ was not permanent or worse. Another possibility was that *Curcuma zedoaria* oil has an ability to improve beta pancreatic cells to secrete insulin. This condition could be detected by a decrease in the blood sugar level of those rats after the

administration of *Curcuma zedoaria* oil. In conclusion, the oral administration of *Curcuma zedoaria* oil could decrease blood sugar level and gingivitis in hyperglycemia rats.

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REFERENCES

1. Ceriello A, Motz E. Is oxidative stress the pathogenic mechanism underlying insulin resistance, diabetes, and cardiovascular disease? The common soil hypothesis revisited. Arterioscler Thromb Vasc Biol 2004; 24(Suppl5): 816–23.
2. Iain LC C, Matthews JB. The role of reactive oxygen and antioxidant species in periodontal tissue destruction. Periodontol 2007; 43:160–232.
3. Pietropaoli D, Tatone C, D'Alessandro, Monaco A. Possible involment of advanced glycation end products in periodontal diseases. B Int J Immunopathol and Pharmacol 2010; 23(Suppl 3): 683–91.
4. Bohlender JM, Franke S, Stein G, Wolf G. Advanced glycation end products and the kidney. Am J Physiol Renal Physiol 2005; 289: F645–F59.
5. Chen IN, Chang CC, Wang CY, Shyu YT, Chang TL. Antioxidant and antimicrobial activity of Zingiberaceae plants in Taiwan. Plant Foods Hum Nutr 2008; 63: 15–20.
6. Marinho BVS, Araújo ACS. Use mouthwash in gingivitis and dental biofilm. Intern J Dentistry 2007; 6: 124–31.
7. Hartono M, Nurlaila, Batubara I. Potensi Temu Putih (Curcuma zedoaria) sebagai antibakteri dan kandungan senyawa kimia. Prosiding Pengembangan Pulau-Pulau Kecil, 2011. p. 203-12.
8. Kaushik ML, Jalalpure SS. Antiiflammatory efficacy of Curcuma zedoaria Rosc root extract. Asian J Pharm Clin Res 2011; 4(3): 90-2.
9. Handajani J. Temu putih (Curcuma zedoaria Rosc., Zingiberaceae) volatile oil increased phagocytic activity of neutrophils exposed to A. actinomycetemcomitans. Proceeding The International Symposium on Oral and Dental Sciences, 17-18 Januari 2013. p. 51-7.
10. Handajani J. Minyak atsiri temu putih (Curcuma zedoaria Rosc., Zingiberaceae) menurunkan ekspresi $CD4^+$ pada gingiva terpapar A. actinomycetemcomitans. Majalah Kedokteran Gigi 2013; 20(1): 9-12.
11. Rahmatullah M, Azam NK, Pramanik N, Sania Rahman N, Jahan R. Antihyperglycemic activity evaluation of Rhizomes of Curcuma zedoaria (Christm.) Roscoe and fruits of Sonneratia caseolaris (L.) Engl. Int J Pharm Tech Res 2012; 4(1): 125-29.
12. Sunmonu TO, Afolayan AJ. Evaluation of Antidiabetic activity and associated toxicity of artemisia afra aqueous extract in wistar rats. Evidence-Based Compl Alt Med 2013; 1-8.
13. Lenzen S. The mechanism of alloxan- and streptozotocin-induced diabetes. Diabetologia 2008; 51: 216-6.
14. Konrad RJ, Mikolaenka I, Tolar JF, Liu K, Kudlow JE. The potential mechanism of the diabetogenic action of streptozotocin: inhibition of pancreatic β-cell O-GlcNAc-selective N-acetyl-β-D-glucosaminidase. Biochem J 2001; 356: 31-41.
15. Akbarzadeh A, Norouzian D, Mehrabi MR, Jamshidi Sh, Farhangi A, Verdi AA, Mofidian SMA, Rad L. Induction of diabetes by streptozotocin in rats. Ind J Clin Biochem 2007; 22(2): 60-4.
16. Shoha J, Jahan H, Mamun AA, Hossain MT, Ahmed S, Hosain MM, Rahman S, Jahan R, Rahmatullah M. Antihyperglycemic and antinociceptive effects of Curcuma zedoaria (Christm.) Roscoe leaf extract in Swiss albino mice. Adv Nat Appl Sci 2010; 5: 6-8.
17. El-Moselhy MA, Taye A, Sharkawi SS, El-Sisi S, Ahmed A. The antihyperglycemic effect of curcumin in high fat diet fed rats. Role of TNF-α and free fatty acids. Food Chem Toxicol 2011; 49: 1129-40.