

Anti-inflammatory Effects of *Channa micropeltes* Extract through NF- κ B and TNF- α in Diabetic Rat

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ABSTRACT

Diabetes mellitus (DM) is a disorder of carbohydrate, fat, and protein metabolism caused by impaired insulin secretion, insulin action, or both characterized by hyperglycemia. Hyperglycemic condition is associated with inflammation process leading to complications. Food supplements play an important role in controlling protein metabolism and have anti-inflammatory properties such as *Channa micropeltes* (CM). This study aimed to analyze the effect of CM extract on the levels of molecular markers Nuclear factor kappa beta (NF- κ B) and Tumor Necrosis Factor alfa (TNF- α) in normal and diabetic rats. The experimental design of this investigation was a post-test-only control group design and the samples were the diabetic model group given CM extract at 16 mL/kgBB dose, and negative control group for 14 days, respectively. Serum concentrations of NF- κ B and TNF- α were measured by ELISA. DM-CM group showed decreasing levels of NF- κ B. TNF- α was detected with the lowest level in the N-CM group. The levels of TNF- α declined close to normal levels in the DM-CM group. Hence, the conclusion was the CM at a dose of 16 mL/kg BW for 14 days in Wistar rats can reduce Nf- κ B and TNF- α in groups of normal and diabetic rats.

Keywords: cytokines; diabetes; Nf- κ B; TNF- α ; traditional medicine

INTRODUCTION

Diabetes mellitus (DM) is a combination of diseases whose onset is characterized by a state of hyperglycemia and glucose intolerance as a result of a lack of insulin, decreased insulin activity, or both (Piero et al, 2014). Based on data from the International Diabetes Federation (IDF) in 2019, Indonesia ranks 2nd in Asia with the most DM patients, which is around 10.7 million sufferers from the age range of 20 - 79 years.

Chronic hyperglycemia in DM is associated with long-term damage and multiple organ failure. DM causes changes in the microvasculature, leading to the synthesis of extracellular matrix proteins, and thickening of the basement membrane of the capillaries, which are potential early stages of diabetic microangiopathy. These changes are closely related to an increase in late oxidative stress, low-grade inflammation, glycation end products, and neovascularization of the vasa vasorum which can lead to macrovascular complications (Chawla et al., 2016). The increased levels of advanced glycation end products (AGEs), Receptors for it (RAGE), oxidative stress,

lipoproteins, and hyperlipidemia enhance the expression of nuclear factor- κ B (NF- κ B) (Singh et al., 2014). In hyperglycemic conditions, the release of TGF- β , cytokines, chemokines, and vesicular cell adhesion molecules (VCAMs) are enhanced significantly by NF- κ B activity. Hence, there is up-regulation of some cytokines acting in apoptosis of endothelial cells and inflammatory processes such as CD36, MCP-1TNF- α , IL1 β , and IL6 (Suryavanshi & Kulkarni, 2017).

Diabetes mellitus shows prolonged inflammation due to overexpression of proinflammatory cytokines such as Nf- κ B and TNF- α (Kaur & Choudhury, 2020). NF- κ B can activate innate immunity reactions, cell migration, and proliferation, modulate matrix metalloproteinase expression, secretion and stability of cytokines, and growth factors for wound healing (Ambrozova et al., 2017). Activation of the NF- κ B pathway will result in gene expression consisting of proinflammatory cytokines and chemokines. In a previous study, there was a significant rise of NF- κ B and pro-inflammatory cytokines like TNF- α and IL-1a in pancreatic tissues in diabetic control rats (Ajiboye et al., 2018). TNF- α production was the first pro-inflammatory cytokine recognized for its involvement in insulin resistance pathogenesis

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and type 2 DM (Alzamil, 2020). Suppression of NF- κ B can cause a decrease in pro-inflammatory cytokines such as TNF- α at the site of diabetic wounds (Kaur & Choudhury, 2020). These markers are usually chosen as targets of therapeutic in DM.

One of the therapies that can be applied in controlling the inflammation process is *Channa micropeltes* (CM). People of South Kalimantan consume this fish as a source of protein in meals and to support wound healing post-surgery. This fish is a freshwater fish containing high protein content such as vitamin C, albumin, omega 3 and omega 6 fatty acids, and zinc (Omar et al., 2010). The previous study by Apriasari et al., (2020) revealed that 16 mL/kgBW dose of CM extract could completely close the wounds of diabetic rats on the 14th day. Albumin and arachidonic acid could prevent prolonged inflammation in people with DM and accelerate the wound healing process (Alauddin et al., Andrie & Purwanti 2016; Apriasari et al., 2018). There was no research before that revealed the anti-inflammatory effect of CM on the wound with DM. This study aimed to analyze the effect of CM extract on the levels of molecular markers NF- κ B and TNF- α in the wound healing process of normal and diabetic rats.

MATERIALS AND METHODS

This study has obtained ethical feasibility from the Faculty of Dentistry, University of Lambung Mangkurat with No. 112/KEPKG-FKGULM/EC/IV/2020. This research is a true experimental design with a posttest-only and control design. The study population was Wistar rats (*Rattus norvegicus*) with inclusion criteria of healthy male Wistar rats aged 2-3 months and weighed 200-300 grams. Exclusion criteria were rats that were dead, abnormal (injured), hematuria, and presenting with weight loss that exceeds 10% body weight after adaptation. The sampling technique used was simple random sampling with four treatment groups, there was non-diabetic rat without STZ induction (N), non-diabetic rat with MC dose 16 mL/kg BW (N-CM), the diabetic rat with STZ induction without MC treatment (DM), and diabetic rat with MC dose 16 mL/kg BW (DM-CM).

The *Channa micropeltes* Extract Preparation

The CM was collected from a traditional market located in Martapura, South Kalimantan, Indonesia. The first step is to clean the fish from its scales, blood, head, and abdominal contents. Secondly, the flesh was weighed 18 kg and then

was steamed in a pot for \pm 30 minutes at 70-80°C. For the pressing process, the flesh was then wrapped in a flannel cloth and put into a hand press. The resulting extract was put into a test tube and centrifuged at 6000 rpm for 15 minutes. The centrifugation result was separated from impurities and the liquid phase (oil and water) was taken. The separated extract was stored in a dark glass bottle that was covered with aluminum foil and a clean pack. Then it was stored in a refrigerator with a temperature of \leq 4°C to prevent damage due to oxidation and contamination.

Induction of Diabetes Mellitus

Wistar rats were induced to develop type 1 diabetes mellitus by injecting streptozotocin (STZ) at a dose of 40 mg/kg BW. Blood sugar levels were checked with a glucometer before and after STZ induction. Rats were said to have diabetes mellitus if the blood sugar level \geq 126 mg / dL was checked using a glucometer that was confirmed on the third day after STZ induction.

Experimental Animal Treatment

Diabetic Wistar rats were treated with CM extract at 16 mL/kg BW twice a day (morning and evening) orally using a gastric tube for 14 days. After the 15th day, the rats were sacrificed using ketamine-xylazine in a 1: 1 ratio of 0.1 mL for each rat.

Sample Collection

The samples should be allowed to clot in the collection tubes for at least 30 minutes at room temperature. The serum should be separated from the clot by centrifuging the collection tube for 20 minutes at 2000~3000 rpm.

Determination of Cytokine

Serum concentrations of NF- κ B (catalog No. E0290Ra) and TNF- α (catalog No. E0108Ra), were measured by ELISA by using the sandwich-ELISA kits from Bioassay Technology Laboratory (Shanghai, China). The operating procedure provided by the manufacturer was strictly followed.

Data Analysis and Statistical Evaluation

Data obtained from all groups were processed using SPSS software. The marker levels (NF κ B, and TNF- α) for all groups were presented in the mean rank. The Mann-Whitney U test was used to examine the differences between groups

with a significance level of less than 0.05 ($p < 0.05$).

RESULTS

In this study, there were significant differences in each group N: Non-diabetic rat without STZ induction; N-CM: Non-diabetic rat with MC dose 16 mL/kg BW; DM: Diabetic rat with STZ induction without MC treatment; DM-CM: Diabetic rat with MC dose 16 mL/kg BW for NFK β measurement ($P > 0.05$). Figure 1 showed that NF-K β level rat serum of the N-CM group had a lower level than the N group. Whereas in the DM condition, the DM-CM group showed a decreasing level of NF- κ B, namely under 2.5 ng/mL relative number. The data were mean value \pm SD of five rats in each group with a significant value $p < 0.05$.

Figure 2 presents a calculation of TNF- α . The data are mean value \pm SD of five rats in each group with a significant value $p < 0.05$. As shown in Figure 2, TNF- α was detected with the lowest level in the N-CM group. The peak concentration level was reached by the DM group without CM treatment, and levels declined close to normal levels in the DM-CM group.

DISCUSSION

Nuclear Factor Kappa Beta (NF- κ B) is a transcription factor activated by an extracellular response such as an inflammatory response. NF- κ B can induce pro-inflammatory cytokines such as TNF- α . During inflammation, the amount of TNF- α can be increased (Cao et al., 2017; Beserra et al., 2020). Inhibition of NF- κ B level can be one of the treatment methods for inflammatory disorders such as diabetes (Beserra et al., 2020). Diabetes causes prolonged inflammation related to the additional number of NF- κ B and TNF- α (Liu et al., 2017; Portou et al., 2020). In our study, elevated levels of serum NF- κ B and TNF- α have been reported in the diabetic group without CM extract.

The amount of NF- κ B and the pro-inflammatory cytokine TNF- α in normal and diabetic rats can be decreased with the CM-extract. Arachidonic acid is contained in the CM extract and is a derivative of Omega-6-fatty acids which can be used as an anti-inflammatory. The CM extract is rich in fatty acid content, total saturated fatty acids are 29.62% and total unsaturated fatty acids are 27.81% (Apriasari et al., 2020). Arachidonic acid can regulate the enzyme 15-LO (15-Lipoxygenase) so that leukotriene, which is a pro-inflammatory mediator, transitions into lipoxin, which is an anti-inflammatory mediator. Lipoxins can also

weaken proinflammatory M1 macrophages (Chandrasekharan et al., 2015). In addition to omega-6, Toman fish extract contains omega-3 fatty acids. Omega-3 fatty acids are found in the form of docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) (Oscarsson et al., 2017). EPA and DHA can function in reducing proinflammatory cytokines including IL-1 α , IL-1 β and TNF- α . The reduction process occurs because EPA and DHA together with AA synergize in the oxygenase process (cyclooxygenase and lipoxygenase) and eicosanoid synthesis. Eicosanoids produced from EPA and DHA are prostaglandins PGE3 and thromboxane TXA3 which are anti-inflammatory (Simonetto et al., 2019).

A study by Omar et al., which used a modified Folch method revealed that the CM extract contains 19,8% omega-3 (Omar et al., 2010). This fat can inhibit TLR-4 and its downstream cascade including NF- κ B, causing a decrease in pro-inflammatory cytokines such as TNF- α (Marion-Letellier et al., Savoye & Ghosh, 2015; Kaur & Choudhury, 2020). Omega-3 PUFAs can decrease proinflammatory cytokines such as TNF- α through PPAR γ activation, thereby reducing I κ B degradation, which in turn reduces NF- κ B translocation into the nucleus (Marion-Letellier et al., 2015). Polyunsaturated fatty acids (PUFA) such as α -linolenic acid (ALA), Docosahexaenoic acid (DHA), omega-3, and Eicosapentaenoic acid (EPA) can reduce NF- κ B in inflammatory conditions (Marion-Letellier et al., 2015).

According to Apriasari et al. (2018), the CM extract also contains 18.17% amino acids (Apriasari et al., 2018). Essential Amino Acids (EAAs) have anti-inflammatory properties that can inhibit anti-inflammatory cytokines through the NF- κ B pathway (He et al., 2018). Glycine, Aspartate, Asparagine, Glutamine, and Cysteine can inhibit NF- κ B, whereas Glycine, Phenylalanine, and Arginine can reduce TNF- α (Ren et al., 2019). Glutamine combined with Arginine can also reduce TNF- α (He et al., 2018). Therefore, the outcome of this study indicates that the extract of *Channa micropeltes* possesses inhibition activity in inflammatory markers.

CONCLUSION

From this study, it can be concluded that the *Channa micropeltes* (CM) extract treatment at a dose of 16 mL/kg BW for 14 days can reduce Nf- κ B and TNF- α in groups of normal and diabetic rats. In the future, the CM extract should be extensively carried out in wound healing.

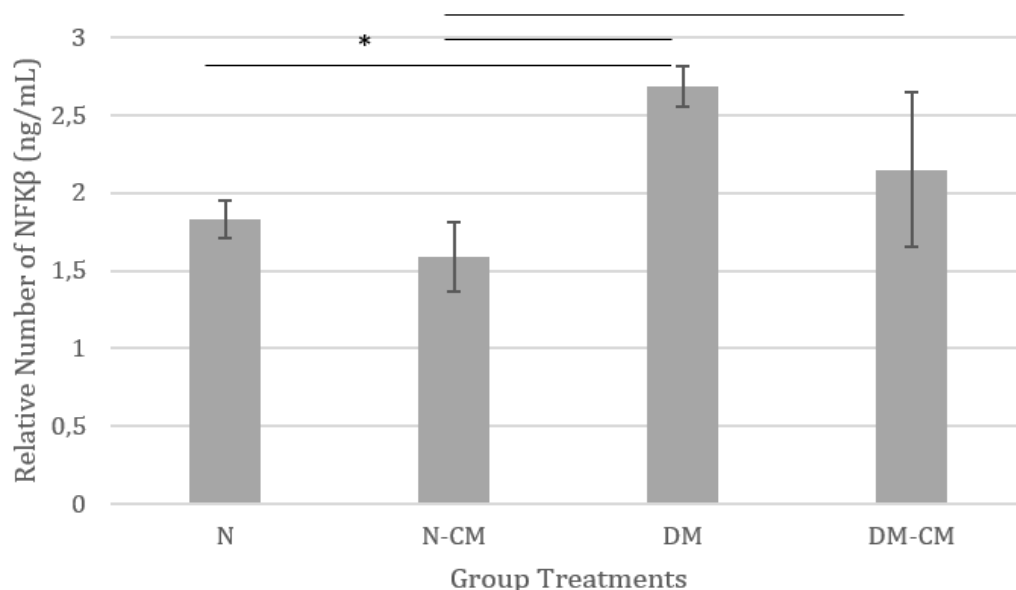


Figure 1. Effect of the CM extracts on the concentration of NF-κβ. N: Non-diabetic rat without STZ induction; N-CM: Non-diabetic rat with MC dose 16 mL/kg BW; DM: Diabetic rat with STZ induction without MC treatment; DM-CM: Diabetic rat with MC dose 16 mL/kg BW ($p > 0.05$)

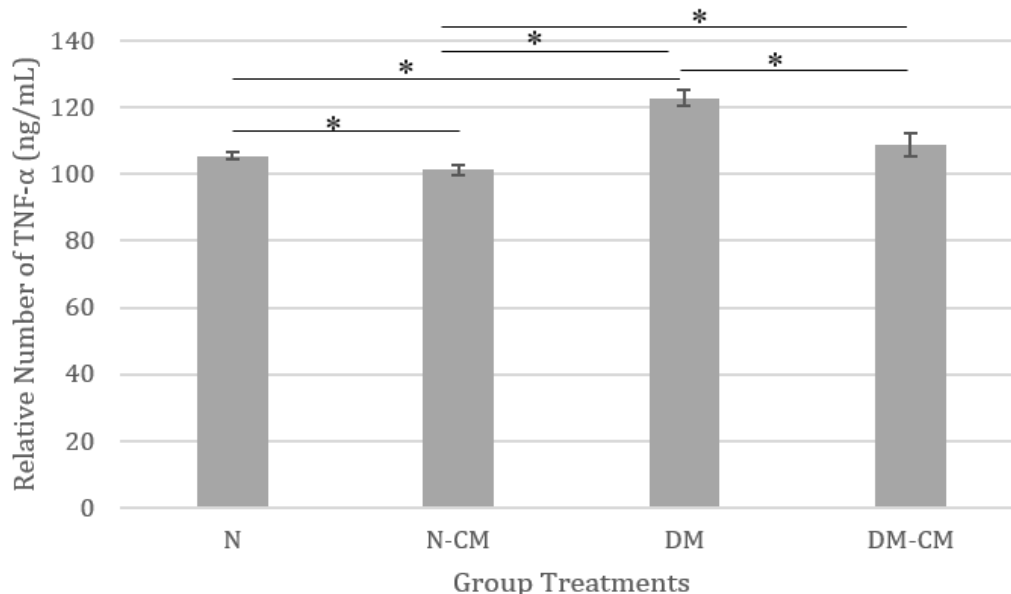


Figure 2. Effect of the CM extracts on the concentration of TNF-α. N: Non-diabetic rat without STZ induction; N-CM: Non-diabetic rat with MC dose 16 mL/kg BW; DM: Diabetic rat with STZ induction without MC treatment; DM-CM: Diabetic rat with MC dose 16 mL/kg BW ($p < 0.05$)

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REFERENCES

Ajiboye, B.O., Ojo, O.A., Akuboh, O.S., Abiola, O.M., Idowu, O. & Amuzat, A.O. (2018). Anti-hyperglycemic and anti-inflammatory activities of polyphenolic-rich extract of *syzygium cumini* linn leaves in alloxan-induced diabetic rats. *Journal of Evidence-Based Integrative Medicine*, 23.

- Alauddin, A., Andrie, M. & Purwanti, N.U. (2016). Uji Efek Ekstrak Ikan Gabus (*Channa striaa*) pada Luka Sayat dengan Tikus Putih Jantan Galur Wistar yang diberikan secara oral. *Journal of Knowledge Management*, 2(2).
- Alzamil, H. (2020). Elevated Serum TNF- α Is Related to Obesity in Type 2 Diabetes Mellitus and Is Associated with Glycemic Control and Insulin Resistance. *Journal of Obesity*, 2020.
- Ambrozova, N., Ulrichova, J. & Galandakova, A. (2017). Models for the study of skin wound healing. The role of Nrf2 and NF- κ B. *Biomedical Papers*, 161(1), 1–13.
- Apriasari, M.L., Ainah, Y., Febrianty, E. & Carabelly, A.N. (2018). Antioxidant Effect of *Channa Micropeltes* in Diabetic Wound of Oral Mucosa. *International Journal of Pharmacology*, 15(1).
- Apriasari, M.L., Syahadati, M.A. & Carabelly, A.N. (2020). Clinical Analysis of *Channa micropeltes* for Treating Wound of Diabetes Mellitus. *Berkala Kedokteran*, 16(1).
- Beserra, F.P., Gushiken, L.F.S., Vieira, A.J., Bérigamo, D.A., Bérigamo, P.L., Souza, M.O. de, Hussni, C.A., Takahira, R.K., Nóbrega, R.H., Martinez, E.R.M., Jackson, C.J., Maia, G.L. de A., Rozza, A.L. & Pellizzon, C.H. (2020). From inflammation to cutaneous repair: Topical application of lupeol improves skin wound healing in rats by modulating the cytokine levels, NF- κ B, Ki-67, growth factor expression, and distribution of collagen fibers. *International Journal of Molecular Sciences*, 21(14).
- Cao, Y., Liu, X., Li, Y., Lu, Y., Zhong, H., Jiang, W., Chen, A.F., Billiar, T.R., Yuan, H. & Cai, J. (2017). Cathepsin L activity correlates with proteinuria in chronic kidney disease in humans. *International Urology and Nephrology*, 49(8).
- Chandrasekharan JA, Sharma-Walia N. (2015). Lipoxins: nature's way to resolve inflammation. *J Inflamm Res*, 30;8:181-92. doi: 10.2147/JIR.S90380. PMID: 26457057; PMCID: PMC4598198.
- Chawla, A., Chawla, R. & Jaggi, S. (2016), Microvascular and macrovascular complications in diabetes mellitus: Distinct or continuum?. *Indian Journal of Endocrinology and Metabolism*, 20(4), 546–553.
- He, F., Wu, C., Li, P., Li, N., Zhang, D., Zhu, Q., Ren, W. & Peng, Y. (2018). Functions and Signaling Pathways of Amino Acids in Intestinal Inflammation. *BioMed Research International*, 2018.
- Kaur, P. & Choudhury, D. (2020). Modulation of Inflammatory Dynamics by Insulin to Promote Wound Recovery of Diabetic Ulcers. *Wound Healing*.
- Liu, T., Zhang, L., Joo, D. & Sun, S.C. (2017), *NF- κ B signaling in inflammation, Signal Transduction and Targeted Therapy*, 2.
- Marion-Letellier, R., Savoye, G. & Ghosh, S. (2015). Polyunsaturated fatty acids and inflammation, *IUBMB Life*, 67(9).
- Omar, M.N., Yusoff, N.S.A.M., Zainuddin, N.A. & Yunus, K. (2010). ω -Fatty acids from Malaysian giant snakehead (*Channa micropeltes*) fish oil. *Oriental Journal of Chemistry*, 26(1).
- Oscarsson J., Hurt-Camejo E. (2017). Omega-3 fatty acids eicosapentaenoic acid and docosahexaenoic acid and their mechanisms of action on apolipoprotein B-containing lipoproteins in humans: a review. *Lipids Health Dis*, 10;16(1):149. doi: 10.1186/s12944-017-0541-3.
- Piero, M.N., G.M. Nzaro, J.M. Njagi. (2014). Diabetes mellitus – a devastating metabolic disorder, *Asian Journal of Biomedical and Pharmaceutical Sciences*, 04 (40) ,1-7.
- Portou, M.J., Yu, R., Baker, D., Xu, S., Abraham, D. & Tsui, J. (2020), Hyperglycaemia and Ischaemia Impair Wound Healing via Toll-like Receptor 4 Pathway Activation in vitro and in an Experimental Murine Model, *European Journal of Vascular and Endovascular Surgery*, 59(1), 117–127.
- Ren, W., Liao, Y., Ding, X., Jiang, Y., Yan, J., Xia, Y., Tan, B., Lin, Z., Duan, J., Jia, X., Yang, G., Deng, J., Zhu, C., Hardwidge, P.R., Li, J., Zhu, G. & Yin, Y. (2019), Slc6a13 deficiency promotes Th17 responses during intestinal bacterial infection. *Mucosal Immunology*, 12(2).
- Simonetto, Marialaura, Marco Infante, Ralph L. Sacco, Tatjana Rundek, and David Della-Morte, (2019). A Novel Anti-Inflammatory Role of Omega-3 PUFAs in Prevention and Treatment of Atherosclerosis and Vascular Cognitive Impairment and Dementia, *Nutrients* 11, no. 10: 2279. <https://doi.org/10.3390/nu11102279>
- Singh, V.P., Bali, A., Singh, N. & Jaggi, A.S. (2014). Advanced glycation end products and diabetic complications, *Korean Journal of Physiology and Pharmacology*, 18(1).
- Suryavanshi, S. V. & Kulkarni, Y.A. (2017). NF- κ B: A potential target in the management of vascular complications of diabetes, *Frontiers in Pharmacology*, 8(NOV).