ANTI-INFLAMMATORY EFFECTS OF Channa micropeltes EXTRACT TOWARDS INTERLEUKIN-10 IN DIABETIC RAT

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Abstract
Background: Inhibition of tissue repair is a symptom of prolonged diabetic inflammation that occurs in wounds resulting in delayed healing. Inflammation can be controlled by giving food supplements that have anti-inflammatory properties such as Channa micropeltes (CM). CM has high protein content, including albumin, omega 3 and omega 6 fatty acids, vitamin C, and zinc. Purpose: This study aimed to analyse the effect of the CM extract on the levels of IL-10 in the wound healing process of normal and diabetic rats. Methods: This study was a true experimental design with a post-test-only control group design. The samples were the diabetic model group given CM extract at 16 mL/kg BB dose and negative control group for 14 days. Results: Compared to the control, the concentration of IL-10 in DM group was significantly higher in the DM without CM treatment. Interestingly, we found the level of IL-10 in DM-CM was significantly decreased while the level of TNF-a in DM-CM decreased as well. Conclusion: CM at a dose of 16 mL/kg BW for 14 days in the wound healing process of Wistar rats can increase IL-10 in the normal group but could not increase IL-10 in the diabetic group.

Keywords: Anti-inflammatory, Channa micropeltes, Diabetes, Interleukin-10.

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INTRODUCTION
Diabetes mellitus (DM) is a disorder of carbohydrate, fat, and protein metabolism caused by impaired insulin secretion, insulin action, or both characterized by hyperglycemia. The results of the Indonesia Basic Health Research conducted in 2018 showed that the prevalence of DM in Indonesia based on a doctor’s diagnosis at the age of 15 years was 2%. It showed an increase when compared to the prevalence of DM in a population for 15 years in 2013 was 1.5%. DM and its complications are 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 glycation end products, oxidative stress, low-grade inflammation, and neovascularization of the vasa vasorum which can lead to macrovascular complications. This condition is a continuation of chronic hyperglycaemia begins with cytokine infiltration into vascular tissue and inhibition of tissue function and repair.

Inhibition of tissue repair is a symptom of prolonged diabetic inflammation that occurs in wounds resulting in delayed healing. Diabetics show prolonged inflammation due to overexpression of proinflammatory cytokines such as Nf-kB and TNF-α. Prevention of prolonged inflammatory process needs ingredient can activate anti-inflammatory cytokines such as IL-10 so that diabetic wounds can heal quickly. Suppression of NF-kB can cause a decrease in pro-inflammatory cytokines such as TNF-
at the site of diabetic wounds, increasing the anti-inflammatory cytokine IL-10. After the inflammatory phase occurs, the wound healing process will enter the proliferative phase. This action requires the role of anti-inflammatory cytokines like IL-10. IL-10 will reduce inflammation and produce a conducive environment for the regeneration process of wound healing by acting as a regulator of extracellular matrix (ECM), fibroblasts, and endothelial progenitor cells (EPCs). The role of these three markers in delayed wound healing conditions due to diabetes is certainly different from normal conditions.

One of the diabetic wound therapies can be applied by controlling inflammation process. Inflammation can be controlled by giving food supplements that have anti-inflammatory properties such as Channa micropeltes (CM). The CM or Toman fish is a fish that is widely consumed by the people of South Kalimantan-Indonesia. This fish is a freshwater fish that has high protein content such as albumin, omega 3 and omega 6 fatty acids, vitamin C, and zinc. The research Apriasari (2020) proved that CM extract at a dose of 16 mL/kgBW could completely close wounds and wound contraction in normal Wistar rats on the 7th day and diabetic rats on the 14th day. Albumin and arachidonic acid could prevent prolonged inflammation in people with DM and accelerated the wound healing process. There was no research before that revealed the anti-inflammatory effect of CM on the wound with DM. This study aimed to analyse the effect of CM extract on the level of IL-10 in the wound healing process of normal and diabetic rats.

MATERIAL AND METHOD

This study has obtained ethical feasibility by the Faculty of Dentistry, University of Lambung Mangkurat with No. 075/KEPKG-FKGULM/EC/VII/2021. 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).

CM Extract Preparation

The CM was bought at the traditional market located in Martapura, South Kalimantan, Indonesia. The fish was initially cleaned from its scales, blood, head and abdominal contents, then weighed 18 kg for the flesh that was later steamed in a pot for ± 30 minutes at 70-80°C temperature. The flesh was then wrapped in a flannel cloth and put into a hand press for the pressing process. The resulted extract was put into a test tube and centrifuged for 15 minutes at a speed of 6000 rpm. The centrifugation result was then separated from impurities and the oil and water phases of the extract were taken. The separated extract was stored in a dark glass bottle that was covered with aluminum foil and a clean pack. CM extract was then stored in a refrigerator with a temperature of ≤ 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 ≥ 126 mg / dL was checked using a glucometer that was confirmed on the third day after STZ induction.

Experimental Animal Treatment

The incision wound on the back of the rat was made 1 cm long and 2 mm deep. Before the injury, the rats were anaesthetized for inhalation with 0.75 mL of diethyl ether and kept for 5-10 minutes until the rats fell asleep. The hair of the rats was shaved in the head and abdominal contents, then weighed 18 kg for the flesh that was later steamed in a pot for ± 30 minutes at 70-80°C temperature. The flesh was then wrapped in a flannel cloth and put into a hand press for the pressing process. The resulted extract was put into a test tube and centrifuged for 15 minutes at a speed of 6000 rpm. The centrifugation result was then separated from impurities and the oil and water phases of the extract were taken. The separated extract was stored in a dark glass bottle that was covered with aluminum foil and a clean pack. CM extract was then stored in a refrigerator with a temperature of ≤ 4°C to prevent damage due to oxidation and contamination.

Sample Collection

The samples should be allowed to clot in the collection tubes for a minimum of 30 minutes at room temperature. 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 IL-10 (catalogue No. E0764Ra), was 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 IL-10 level for all groups were presented in the mean rank. The Mann-Whitney U test was used to examine the differences between groups with a level of significance of less than 0.05 (p<0.05).

Results

![Figure 1](Image)

**Figure 1.** Effect of the CM extracts on the concentration of IL-10. 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)

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 NFκB measurement (P > 0.05). Figure 1 showed NFκB level rat serum of N-CM group had lower level than the N group. Compared with the control, the concentration of IL-10 in DM group was significantly higher in the DM without CM treatment. Interestingly, we found that the level of IL-10 in DM-CM was significantly decreased while the level of TNF-α in DM-CM decreased as well (p < 0.05) (Figure 3). These suggested that declined TNF-α may be related to another antiinflammation pathway besides IL-10.

**DISCUSSION**

The CM extract is rich in fatty acids content, total saturated fatty acids are 29.62% and total unsaturated fatty acids are 27.81%. Fatty acids are carboxylic acids formed by hydrogen and carbon atoms. Fatty acids are divided into saturated fatty acids (without double bonds) and unsaturated fatty acids (with double bonds). Unsaturated fatty acids are further divided into monounsaturated fatty acids (MUFA s) which have one double bond in acyls and polyunsaturated fatty acids (PUFA s) which have two or more double bonds. Omega-3 PUFAs can inhibit TLR-4 and its downstream cascade including NF-κB, causing a decrease in pro-inflammatory cytokines such as TNF-α. Omega-3 PUFAs can decrease proinflammatory cytokines such as TNF-α through PPARγ activation, thereby reducing IkB degradation, which in turn reduces NF-κB translocation into the nucleus. Polyunsaturated fatty acids (PUFA) such as α-linolenic acid (ALA), Docosahexaenoic acid (DHA), omega-3, and Eicosapentaenoic acid (EPA) can reduce NF-κB in inflammatory conditions. EPA can also reduce the amount of TNF-α, and has anti-inflammatory properties by increasing CD45+F4/80+CD206+ in wounds on day 10. The CM extract also contains 18.17% amino acids. Amino acids are divided into essential amino acids (EAAs), nonessential amino acids (NEAAs), and conditionally essential amino acids (CEAAs). EAAs are only obtained from nutrition by amino acid transporters, such as Tryptophan, Leucine, and Phenylalanine. NEAAs can be synthesized from certain elements in vivo such as Glutamate, Glutamine, Glycine, and Serine, whereas CEAAs are specific amino acids that are not EAAs or CEAs such as Arginine and Histidine. EAAs have anti-inflammatory properties that can inhibit anti-inflammatory cytokines through the NF-κB pathway. Glycine, Aspartate, Asparagine, Glutamine, Cysteine can inhibit NF-κB, whereas Glycine, Phenylalanine, Arginine can reduce TNF-α. Glutamine combined with Arginine can also reduce TNF-α. TNF-α has an important role in the upregulation and downregulation of T cell activity. T cells control multiple insults simultaneously throughout the body and maintain immune homeostasis. The decrease in TNF-α levels in normal mice was followed by an increase in IL-10 as the pro-inflammatory cytokine. IL-10 is produced by populations of immune cells, such as macrophages, monocytes, dendritic cells, B cells, helper T cells, and regulatory T cells that modulate the function of adaptive immune cells. In addition, IL-10 is also produced by non-immune cells, such as keratinocytes, epithelial cells and tumor cells. Dose of 16 mL/kg BW of CM extract can increase IL-10 in the normal group, because omega-3 levels in CM extract can affect T cell regulation. This is similar to the previous study of Monk et at who reported an increase in CD4+ count. and CD8+ T cells in the spleen of mice fed a high-fat diet of fish fat.
There is no increase of IL-10 level in DM-CM (Figure 1). In diabetes, high glucose levels can trigger resistance in macrophages.5 Macrophages will migrate to the tissue and will initiate the inflammatory process, but when hyperglycaemia occurs, the main marker of the pathophysiological process of diabetes will seek to reduce the anti-inflammatory function of IL-10. This mechanism involves IL-10 inhibition mediated by STAT3 activation.9 This is supported by previous study that IL-10 is less effective in inhibiting the secretion of TNF-α in hyperglycemic whole blood cultures.10 This finding was observed in macrophages exposed to high glucose, which showed IL-10 resistance or hypo responsiveness. This is also supported by the Iyer (2012) study, in diabetes conditions, IL-10 dysregulation can occur which results in the emergence of severity in patients.11 It might give a benefit for this condition because excessive IL-10 level production fails to control the infection.23 The CM extract has not been able to fully restore the state. Further research is needed with additional markers such as T cell levels to be able to learn more about an increase in IL-10 levels in diabetic conditions after administration of CM extract. In conclusion, the CM extract treatment at a dose of 16 mL/kg BW for 14 days in the wound healing process of Wistar rats can reduce Nf-κB and TNF-α in groups of normal and diabetic rats. The CM extract was also able to increase IL-10 in the normal group but could not increase IL-10 in the diabetic group. The authors would like to thank the Institute for Research and Community Service, University of Lambung Mangkurat, for financial support of this project. This research was made possible with funding from University of Lambung Mangkurat through Mandatory Research for Lecturer Grant 2021 (No: 697/UN8/PG/2021).

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