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Research Article
Antioxidant Effect of Channa Micropeltes in Diabetic Wound of Oral Mucosa

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Abstract

Background and Objective: Toman fish (*Channa micropeltes*) contains albumin which possesses antioxidant effect. People in south Kalimantan, Indonesia, use toman fish as an alternative drug to accelerate diabetic wound healing process. In diabetic wound, there is a reduction in superoxide dismutase (SOD) activity and an elevation in malondialdehyde (MDA) level which can lead to the occurrence of delayed wound healing. The objective of this study was to prove the antioxidant effect of *Channa micropeltes* using SOD activity and MDA level in diabetic wound of oral mucosa. **Materials and Methods:** This is an experimental study with post test-only control group design. This study used 24 Wistar rats which divided into three treatment groups. The groups comprised of one group administered with toman fish extract of 16 mL kg^{-1} rat BW per oral, one group as a positive control administered with Haruan fish extract of 13.54 mL kg^{-1} rat BW per oral and one group as a negative control given BR2 feed only. The SOD activity measurement was done through Misra and Fridovich method using spectrophotometer in 480 nm wavelength. The MDA level measurement was done through Thiobarbituric Acid (TBA) test from Buege and Aust method using spectrophotometry in 532 nm wavelength. **Results:** One-way ANOVA test of day 3 showed no significant difference in SOD activity ($p = 0.078$) and MDA level ($p = 0.094$). Meanwhile, statistical result of day 7 showed significant difference in SOD activity ($p = 0.018$) and MDA level ($p = 0.011$). *Post-hoc* LSD test showed significant difference between toman fish extract of 16 mL kg^{-1} rat BW dosage and haruan fish extract of 13.54 mL kg^{-1} rat BW dosage and group given BR2 feed only. **Conclusion:** It can be concluded that *Channa micropeltes* has an antioxidant effect which represented by the increase of SOD activities and the decrease of MDA level in diabetic wound of oral mucosa.

Key words: Antioxidant effect, *Channa micropeltes*, diabetic wound, haruan fish, wound healing, toman fish

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

People with Diabetes Mellitus tend to suffer chronic wound which characterized by prolong healing process. Complication of diabetes mellitus is comprised of the occurrence of delayed wound healing as a result of uncontrolled hyperglycemia. People of South Kalimantan, Indonesia frequently consume toman fish (*Channa micropeltes*) and Haruan fish (*Channa striata*) to accelerate wound healing process[1-3].

Empirically, patent drugs contain haruan extract have been marketed in Indonesia. Haruan fish contains 4.53% albumin which is equal to 13.54 mL kg^{-1} rat b.wt. Based on the previous study, haruan fish is proven to contain albumin as an antioxidant which can accelerate diabetic wound healing process in 8 days[4-7]. Lately, Haruan fish is more difficult to obtain so the presence of alternative resource is required. An alternative resource used as the substitute of Haruan fish is toman fish. Toman fish is known to be in the same family as haruan fish and easy to cultivate because of its fast regenerative property. The administration of toman fish extract 16 mL kg^{-1} rat b.wt., dosage can accelerate wound healing process[8,9].

In diabetic wound healing of oral mucosa, there is an occurrence of prolong inflammatory phase. Acute inflammatory phase of diabetic wound healing process is marked by an elevation of ROS production[10]. Excessive number of ROS will inhibit diabetic wound healing process as an effect of superoxide dismutase (SOD) activity reduction[11-13].

The SOD reduction can increase malondialdehyde (MDA) level which can be overcame by the administration of toman fish extract. Toman fish extract had been proven to contain albumin. Albumin has abundance of sulfhydril (-SH) groups which function as radical scavenger to bind ROS. The binding of ROS will result in an increase of SOD activity which can reduce ROS level in the body. This condition will be marked by the reduction of MDA level in chronic inflammatory phase. In diabetic wound, inflammatory phase then proceeds to proliferative and remodeling phase until wound healing process can be achieved[4,14,15].

Omega-6 is a group of fatty acid derived from arachidonic acid (AA) and chemical mediators such as prostaglandin and lipoxin which playa role in inflammatory phase[16]. In the end of inflammatory phase, wound healing process will proceed to proliferative phase to achieve neovascular formation and re-epithelialization. Furthermore, wound healing process will continue to the maturation phase where synthesis of extracellular matrix and closure of the wound will take place in day 14[3,17].

Studies about *Channa micropeltes* extract for the acceleration of diabetic wound healing is still limited. More study about wound healing should be done. The main objective of this research was to study the effect of *Channa micropeltes* as an antioxidant by investigating SOD activities and MDA levels in diabetic wound of oral mucosa.

MATERIALS AND METHODS

Research preparation: The study was an experimental laboratory research with post-test only control group design. Research procedure was approved by Ethical Clearance Committee of Faculty of Dentistry, Universitas Lambung Mangkurat, Banjarmasin, South Borneo, Indonesia with letter No. 050/KEPKG-FKG ULM/EC/IX/2017. This study was done on December, 2017 at Biochemistry Laboratory of Medical Faculty, Universitas Lambung Mangkurat, Banjarmasin, Kalimantan Selatan, Indonesia. Population of this study was Wistar Rat. Inclusion criteria of the sample were male Wistar rat, weigh 250-300 g, age 2-3 months and in a healthy condition (active and have good appetite). Exclusion criteria of the sample in this study were weight loss more than 10% after adaptation at the laboratory, unhealthy condition (in-active and has no appetite), abnormal rat (presence of wound or disability) and death rat. Experimental animal was divided into 3 groups comprised of negative control group given BR2 feed only, treatment group administered with toman fish extract of 16 mL kg^{-1} b.wt., dosage per oral and a group administered with haruan fish extract of 13.54 mL kg^{-1} b.wt., dosage per oral. Each group consisted of 4 rats which sacrificed in day 3 and 7.

Extract manufacturing: Toman fish or haruan fish used in this study had total weight of 11 kg. Part of the fish used was the flesh of toman fish and haruan fish. Toman fish and haruan fish used was firstly cleaned from the heads, guts and scales. The flesh was then weighed of 9.84 kg and steamed in close pan for ±30 min until 750 mL of yellowish pale liquid was obtained and separated. The flesh of toman fish and haruan fish then put into hydraulic press to proceed. Toman fish and haruan fish extract were put into reaction tube for 7.5 mL separately and centrifuged for 15 min with 6000 rpm speed. The centrifugation resulted in 750 mL liquid, comprised of 50 mL extract which then stored in dark glass bottle covered by aluminum foil and clean pack[18].

Diabetes mellitus induction in Wistar rat: The model of oral mucosa diabetic wound was obtained by injecting streptozotocin (STZ) in male Wistar rat with 35 mg kg^{-1}

dosage. Rat was given BR2 feed twice a day and then examined 7 days later. Glucose level in rats was measured by glucometers which done before and after the administration of STZ. Rat was diagnosed with diabetes when blood glucose level was over 126 mg dL^{-1}. The condition of Wistar rat which suffered with diabetes was limped, in-active and show no appetite[18].

Model of diabetic wound in oral mucosa: Experimental animal used was male Wistar rat which was adapted previously for a week inside the cage at laboratory. Wistar rat right buccal mucosa was taken and measured for wound making. Operator's hands were firstly washed and then covered using sterile gloves. The procedure was continued with sedation using 0.75 mL diethyl ether for 5-10 min until the rat was put to sleep. Incision wound was made at 10 mm length and 1 mm depth on right buccal mucosa of Wistar rat using scalpel and disposable blade number 15, blood was cleaned by sterile aquadest.

Rats from each group were sacrificed after sedated by inhalation method using diethyl ether on day 3 and 7. Sacrificed rat was then biopsied by taking the tissue of oral mucosa around erythematous area with 3 mm length, 3 mm width and 3 mm in-depth. Biopsied tissue was weighed for 0.2 g and crushed with cold mortar after added with 1 mL physiologic NaCl. Homogenate was moved to microtube and centrifuged with 8000 rpm speed for 20 min. The SOD activity and MDA level examination was done on 500 mL supernatan using spectrophotometer on day 3 and 7. The SOD activity measurement was done through Misra and Fridovich method using spectrophotometer at 480 nm wavelength. The MDA level measurement was done through Thiobarbituric Acid (TBA) of Buege and Aust method using spectrophotometer at 532 nm wavelength. Based on the measurement of SOD activity and MDA level[19]. Normality test was done using Shapiro-Wilk test and homogeneity test using Levene's test.

Statistical analysis: Data is statistically analyzed by One-Way ANOVA with significance level $p<0.05$ and then analyzed using *post-hoc* LSD test.

RESULTS

The statistical result showed that data was normally distributed and homogenous with $p>0.05$ on day 3. The data then proceeded to parametric analysis using one-way ANOVA where p-value = 0.078 ($p>0.05$) for SOD activity and p-value = 0.094 ($p>0.05$) for MDA level on day 3. This showed that there was no significant difference among the treatment groups.

Statistical analysis of SOD activity and MDA level on day 7 showed that data was normally distributed and homogenous with $p>0.05$. The analysis was continued with parametric test using one-way ANOVA. One-way ANOVA test for SOD activity obtained $p = 0.018$ ($p<0.05$) and MDA level obtained $p = 0.011$ ($p<0.05$). This statistical analysis showed that there was a significant difference among the treatment groups. The data then analyzed using *post-hoc* LSD test.

Post-hoc LSD analysis result on SOD activity and MDA level showed significant difference on day 7 between toman fish extract of 16 mL kg^{-1} rat b.wt., dosage group and haruan fish extract of 13.54 mL kg^{-1} rat b.wt., dosage group. There was a significant difference between toman fish extract of 16 mL kg^{-1} rat b.wt., dosage group and BR2 feed-only group.

The elevation of SOD activity on buccal mucosa wound of Wistar rat on group administered with toman fish extract, haruan fish extract and feed only on day 3 and 7. Different SOD activity on rat buccal mucosa wound at each group on day 3 and 7 can be seen in Fig. 1. The highest SOD activity elevation among the three groups respectively are toman fish extract, haruan fish extract and feed-only group in sequence.

A decrease of MDA level on rat buccal mucosa wound which administered with toman fish, haruan fish and feed-only on day 3 and 7. Results in Fig. 2 depicted the

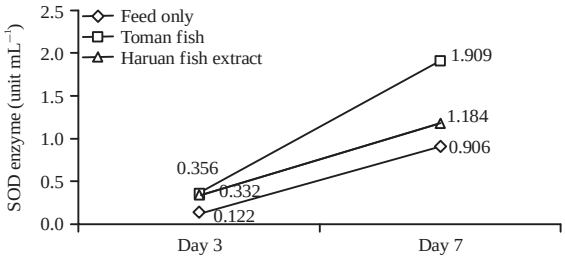

Fig. 1: Mean value of SOD activity (enzyme unit mL^{-1}) on buccal mucosa wound of Wistar-strain rat with diabetes mellitus on day 3 and 7

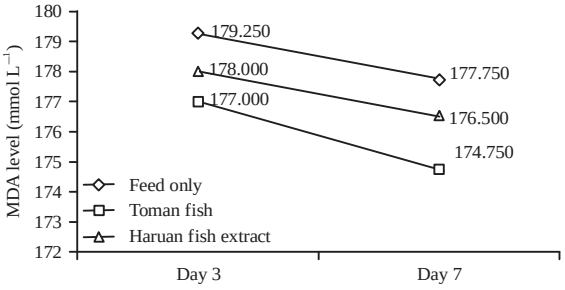

Fig. 2: Mean value diagram of on buccal mucosa wound of Wistar rat with diabetes mellitus on day 3 and 7

different MDA level on buccal mucosa wound of wistar rat with diabetes mellitus in each group on day 3 and 7. The lowest MDA level among the three group respectively were toman fish extract, haruan fish extract and BR2 feed-only group.

DISCUSSION

The result of this study showed an increase of SOD antioxidant activity and a decrease of MDA level on day 3 and 7. Toman fish extract administration of 16 mL kg^{-1} rat BW dosage per oral showed satisfactory effect compared to haruan fish extract of 13.54 mL kg^{-1} b.wt., dosage and BR2-only to increase SOD antioxidant activity and decrease MDA level on buccal mucosal wound in rat with diabetes mellitus.

Diabetes mellitus is one of chronic disease caused by metabolic disorder with hyperglycemic condition that induce an increase of advanced glycation end products (AGEs). This is caused by a high blood glucose level on hyperglycemic condition and induces glycation reaction (non-enzymatic reaction between glucose and protein). This reaction can form Schiff base which can produce amadory products. Amadory products can form a very toxic protein called advanced glycation end product (AGEs). This causes a prolong wound healing process in diabetes mellitus patients[20].

Advanced glycation end products (AGEs) are produced from Maillard reaction which is signed by the presence of alkylated amino acid, fluorescence residues and intra or intermolecular cross-linkage. Interaction between AGE and receptor advanced glycation end product (RAGE) will increase the signaling of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase which produces superoxide anion (·O2). This process will contribute to the increase of Reactive Oxygen Species (ROS) production[21,22].

Reactive Oxygen Species (ROS) occurs by the presence of free radical production and antioxidant capacity imbalance[19,21]. The reduction of antioxidant numbers are mostly caused by impairment in the activation of nuclear factor-erythroid-2 related factor 2 (Nrf2). Nrf2 is a transcription factor which regulates genes for the coding of antioxidants and detoxification enzymes. In physiological condition, oxidative stress will trigger the up regulation of endogenous antioxidants and cytoprotective proteins to prevent or limit tissue destruction. This process is mediated by Nrf2 activation which will influence the rate of various antioxidant genes and detoxification enzyme transcription. The interaction of oxidative stress and inflammation shows inseparable relation. Oxidative stress can induce inflammatory stress through NF-kb activation and inflammation can release ROS vice versa[23,24].

An increase in ROS production will result in tissue destruction and dysfunction by attacking, denaturating and modifying molecule structure and function. This process also involves the activation of transcription factor which is sensitive to redox and signal transduction pathway. This condition will increase the occurrence of necrosis, apoptosis, inflammation and fibrosis. Redox systems including antioxidants and detoxification enzymes will protect tissue from destruction caused by ROS. Nrf2 also plays another role in inflammatory process which inhibits the transcription factor of NF-kB to induce the reduction of inflammatory process. Nrf2 dysregulation will induce the reduction of NF-kB transcription factor and increase several genes to encode the expression of inflammatory mediators[25-27].

Inflammation in diabetes mellitus occurs when there is an increase of ROS production. This is caused by the reduction of superoxide dismutase (SOD) activity which is marked by the increase of MDA level as peroksidasi lipid end product. High MDA level is an evidence of low antioxidant status in the body such as the quantity of SOD. However, this state can be overcame by the administration of exogenous antioxidants, one of them is albumin, which can be found in toman fish extract[28-30].

The SOD activity and MDA level statistical analysis on day 3 of diabetic Wistar rat buccal mucosa wound in each treatment groups of BR2, toman fish extract and haruan fish extract shows no significant difference. It is caused by a short administration time of toman fish extract which will result in less number of exogenous antioxidant administered. Wistar rat group on day 3 obtained less number of exogenous antioxidants compared to day 7. This shows that the lower the antioxidant concentration given, the less the antioxidant activity obtained to neutralize excessive amount of ROS in acute inflammatory phase of diabetic wound healing process. The statistical analysis result on day 3 is not significantly different.

Buccal mucosa wound of Wistar rat with diabetes mellitus on day 7 which administered with toman fish extract and haruan fish extract shows significant difference. This can be seen from the result of SOD activity and MDA level statistical analysis result. There is a significant difference caused by the presence of higher albumin content as an antioxidant in treatment group on day 7 compared to day 3.

Albumin is a secondary antioxidant which can bind metal ion in ROS formation process. It can act as an oxygen binder and also as hydroperoxide analyst to radical compound and free radical obtained from oxidative process. Albumin is an extracellular antioxidant comprised of abundance sulphydryl (-SH) groups which function as free radical binder. (-SH) group

in albumin can react with ROS. Albumin helps SOD to prevent ROS and lipid peroxide formation. Albumin can also react with aldehyde resulted from peroxide lipid and inhibit lipid peroxidation process. Albumin which reacts with polyunsaturated fatty acid (PUFAs) can act as a defense mechanism from radical destruction[31-33].

Polyunsaturated fatty acids is fatty acids which contain more than one double bond. Polyunsaturated fatty acids can produce a product called MDA. Malondialdehyde is one of aldehyde compound which is formed by peroxide lipid process. This shows that excessive MDA level can be constrained by albumin as one of exogenous antioxidant which can help the activity of endogenous antioxidant such as SOD[32,33].

Albumin as exogenous antioxidant can increase SOD through the signaling of nuclear factor erythroid2-related (Nrf2) which is an important cytoprotective transcription factor[34]. Nrf2 will be activated by the presence of oxidants, electrophiles or endoplasmic reticulum stress. Nrf2 is comprised of 6 domains: Neh1 contains bZIP structure which is needed for binding DNA and forming dimer, Neh2 site can interact with Keap1, Neh 3,4,5 which intervenes Nrf2 transactivation by binding with CREB binding protein (CBP) and Neh6 domain which functions in the negative regulation. In inactive condition, Nrf2 is located in cytoplasm and inactively bind with Kelch-like ECH associated protein 1 (Keap) repressor molecule to form Nrf2-Keap1 complex[27,35].

Keap1 is comprised of several cysteine residues which act as a sensor on intracellular redox status. Nrf2 will be rapidly degraded by proteasome ubiquitine pathway. Signal from ROS and electrophile will cause Nrf2 dissociation from Keap1 and then Nrf2 will be translocated to nucleus. In nucleus, NRf2 binds with regulatory sequence called antioxidant response element or electrophile response elements (ARE/EpRE) located in promotor region of gene which encodes antioxidants and detoxification enzymes[36,37].

This process is mediated by heterodimerization of Nrf2 with other transcription factor, such as small Maf which presence in the nucleus. This process then increase the transcription of various related antioxidants, detoxification enzymes and proteins such as catalase, superoxide dismutase, NADPH quinone oxidoreductase, heme oxygenase-1, glutathione S-transferase, glutathione peroxidase, thioredoxin, which detoxificate various damaging xenobiotic[38].

Nrf2 activation induces the increase of endogenous antioxidant enzyme, SOD, by binding the antioxidant response element (ARE)[39]. The increase of SOD activity can neutralize excessive number of ROS in the form of superoxide anion through superoxide dismutase enzyme catalytic reaction. Superoxide dismutase catalytic enzyme reaction will change superoxide anion to form hydrogen peroxide (H_2O_2) through oxidation and reduction reaction of metal cofactor which presence in SOD[40]. Hydrogen peroxide is then catalyzed into water (H_2O) and air (O_2). Hydrogen peroxide in lower rate can modulate various pathways for wound healing so SOD will increase. The elevation of SOD activity can suppress excessive quantity of ROS which also followed by the decrease of MDA level. This can accelerate wound healing process in diabetes mellitus patients[11,41].

CONCLUSION

Based on this research, there was a significant difference in the administration of toman fish extract of 16 mL kg^{-1} b.wt., dosage, haruan fish extract of 13.54 mL kg^{-1} b.wt., dosage and rat given BR2 feed only in increasing SOD activity and decreasing MDA level on buccal mucosal wound of Wistar-strain rat with diabetes mellitus on day 3 and 7. It can be concluded that *Channa micropeltes* has an antioxidant effect by increasing SOD activities and decreasing MDA levels in diabetic wound of oral mucosa. This research can be used as an alternative medicine to accelerate diabetic wound healing, both wounds that occur on the oral mucosa or skin. It can be used as a basis for the next research to find the new drug.

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