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Expression of HSP-70 In Oral Chronic Inflammation Post-Catfish (*Clarias batrachus*) Oil Application

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Abstract

Chronic inflammation endures for a long time and may cause oxidative stress which can increase Heat Shock Factor (HSF) and Heat Shock Protein (HSP) 70 expression. Cat fish (*Clarias batrachus*) oil contains Polyunsaturated Fatty Acids (PUFA) that act as antioxidants. Aim and

The aim of this study is to evaluate the antioxidant effect of cat fish oil on oral mucosa chronic inflammation through the expression of HSP70.

Rattus norvegicus were subject to incision on the buccal mucosa and randomly divided into three groups, Group 1 as the control group, Group 2 (KP1) administered 5% cat fish oil, and Group 3 (KP2) administered 10% cat fish oil. After 3 days, cat oil was applied topically. On day 4, the rats were sacrificed and prepared for immunohistochemical evaluation with the data being analyzed using One Way ANOVA.

There was a significant difference in HSP70 expression between KP2 and the control group.

10% cat fish (*Clarias batrachus*) oil can downgrade HSP70 expression in chronic inflammation of the oral mucosa.

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Introduction

Inflammation represent the physiological response of the body to injury.¹ It's constitutes a defense mechanism against injury in which leukocyte migrates from the bloodstream, and inflammatory mediators were released into the extravascular tissues.^{2,3} Inflammation localizes and eliminates injurious agents and protects the host against micro-organisms. Inflammation is a self-defense response to injury characterized by a vascular reaction that releases fluids, dissolved substances and cells from the blood vessels to the injured site. Inflammation is a non-specific reaction of the host to infection.²

There are two types of Inflammation: acute and chronic. The former encompasses immediate and early responses to injury and is rapidly resolved.^{1,4} Prolonged inflammation, also referred

to as chronic inflammation, may cause tissue damage that leads to fibrosis.^{1,5} Chronic inflammation generally accepted that the host immune inflammatory response to peri odontogenic microorganism that involved in the development and progression of the disease.⁶ Chronic inflammation occurs when the lesion persists for prolonged periods of time.^{1,7}

The local factor that causing chronic inflammation is hypoxia. Hypoxic cells cause a reduction in ATP production, mitochondrial damage, and free radical accumulation. Decreased ATP production in turn leads the failure of the cellular process, such as reduced glycogen storage capability and lactic acid accumulation that may provoke a decrease in cellular pH.^{5,8}

Chronic inflammation can lead to oxidative stress, characterized by increasing Reactive Oxygen Species (ROS), that causes cells, tissues or organs damage. Oxidative stress increases Heat Shock Factor (HSF) and expression of Heat Shock Protein (HSP) 70 that serves as a protective cell against inflammation.^{9,10,11}

Heat Shock Protein is one of the first protein groups known to be synthesized in

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response to hyperthermia, hypoxia and allows cells to recover from such medical condition.^{10,12} Heat Shock Protein (HSP) regulates the conformation and functions of a large number of cellular proteins in order to protect the body from stress.¹³ Heat Shock Proteins are classified in many groups: 20, 22, 25/27, 60, 70, 90 and 110 (kDa).^{10,11,13} Heat Shock Protein (HSP) 60 and HSP 70 are the most important groups in the reproduction/ regeneration system.^{10,12,14} The expression of HSP 70 will directly stimulate TLR2 signaling in response to tissue damage, causing an inflammatory response.¹⁵

According to research conducted by Chen et al in 2006, inflammation occurs in response to various cellular pressures including infection and heat shock. Heat Shock Protein 70 is an HSP that does not exist under normal conditions, but increases its expression during bouts of illness.¹⁶ Omega-3s in fish oil have been known to have anti-inflammatory, antioxidant, antibacterial and immunomodulatory effects. Optimal fish oils are fish oils those rich in fatty acids that are beneficial to health.^{17,18}

Currently, an expanding body of research on the efficacy of catfish (*Clarias batrachus*) extract in the form of albumin or fish oil as a therapy in various diseases is emerging. Fish oil *C. batrachus* has the highest omega-3 PUFA content compared to that derived from freshwater fish, marine fish and shrimp with a content of 28% of total fatty acid.^{18,19} It is produced from specific fish as a source of omega-3 especially eicosapentaenoic acid (EPA) and docosahexanoic acid (DHA).^{15,20} Fish oil supplementation can control oxidative stress and increase antioxidants. One group of the ingredients is Polyunsaturated Fatty Acids (PUFA) which consisting of EPA and DHA, that have been found to reduce oxidative stress.^{17,20,21}

Cat fishes belong to the category of freshwater fish, that are widely consumed, especially by Indonesian. One easily cultivated, local species is *Clarias batrachus*, whose oil contains Omega-3 fatty acids that known to play an important role in reducing the inflammation that is associated with many serious health problems such as heart disease, atherosclerosis, myocardial infarction and stroke. This is due to its anti-inflammatory effects and the fact that is also acts as an immunomodulator.^{17,22,23,24}

Research by Kamat, Roy (2015) and Husein, et al (2014) demonstrated that *C. batrachus* fish oil can reduce glucose levels and increase the activity of SOD (Superoxide Dismutase) to reduce tissue damage in alloxan induced animals. Based on this, this research is conducted to know influence of *C. batrachus* oil to expression of HSP 70 on mucosa of oral cavity with condition of chronic inflammation. Research conducted by Kamat and Roy (2015) proved that *C. batrachus* oil can increase SOD at a dose of 0.17 with a concentration of 10% in alloxan-induced rats. These results are used as a reference dose, in this study which it is expected can observe the expression of HSP 70 on the oral mucosa of an oral cavity afflicted by chronic inflammation.

Methodology

Sample

This research was approved by ethical committee 209/KKEPK.FKG/IX/2016. This research consisted of a laboratory-based experiment incorporating posttest-only control group design using a sample of 19, healthy, 2-3 months old male Wistar rats (*Rattus Norvegicus*) weighing 250 grams. The samples were randomly divided into 3 groups: a control group, treatment group 1 given fish oil catfish concentration of 5% and treatment group 2 which was given fish oil catfish concentration of 10%. The 19 male Wistar rats (*Rattus Norvegicus*) were acclimatized for seven days and then incised on the buccal mucosa to create a chronic inflammatory condition by inducing 10% H₂O₂ (Sigma Hexindo, Indonesia) twice a day for 2-3 minutes on three consecutive days. After a chronic inflammatory condition had been induced 5% and 10% catfish oil were administered to the mucosa, after which on the fourth day, the subjects were decapitated at the Research and Industrial Consultation Center, Ketintang, Surabaya, East Java, Indonesia. The tissue was subsequently subjected to immunohistochemical imaging at 400x magnification to enable the number of cells expressing HSP 70 (brown colour) to be calculated (Figure 1).

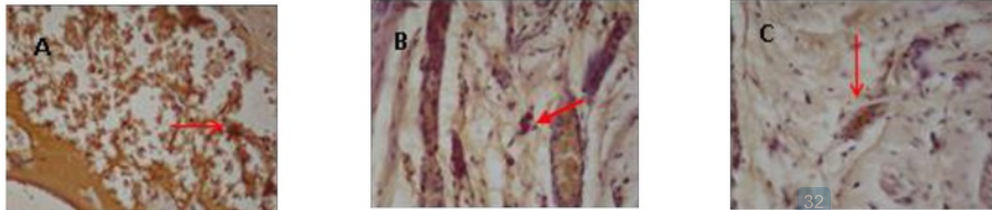


Figure 1. Expression of HSP 70 at 400x magnification. A. HSP 70 expression in the control group, B. Expression of HSP 70 in treatment group with fish oil 5%, C. Expression of HSP 70 in treatment group with fish oil 10%.

Statistical Analysis

Data was analyzed by means of a normality test, specifically a Kolmogorov-Smirnov Test, a homogeneity test using the Levene Statistic Test and a parametric test using One Way ANNOVA, A Tukey test was subsequently conducted to identify the best concentration for lowering HSP 70.

Results

The results showed that HSP 70 expression in the treatment group given fish oil 10% had the lowest amount of HSP 70 expression compared to the 5% fish oil and control group.

In the normality test using the Kolmogorov-Smirnov One-Sample test, the results of all study groups contained significant values greater than 0.05 ($p > 0.05$), which confirmed the research data of all groups to be normally distributed. Furthermore, a Levene-test homogeneity was conducted producing a significant value of 0.214. The variance between groups can, therefore be said to be homogeneous. The comparison between the groups was by means of OneWay ANOVA, employed to determine whether there is a significant difference or not between the control group and the treatment group existed.

Table 1. OneWay ANNOVA expression HSP 70 test results

	Control	KP1	KP2
Group			
Control Group	-	0.223*	0.000*
KP1	0.223*	-	0.000*
KP2	0.000*	0.000*	-

The table 1 shows that there are significant differences between groups exist, viz, the control group and the treatment group 1 ($p = 0,223$), the control group and the treatment group 2 ($p = 0,000$), the treatment group 1 and the treatment group 2 ($p = 0,000$). The next analysis used a Tukey test to determine the best concentration to increase the expression of HSP 70 in a chronically inflamed mucosa oral cavity. From the Table 2, the two treatment groups had the lowest amount of HSP 70 expression.

Table 2. Tukey test results for each treatment group

Tukey Test	Avg
Control Group	64.00
Treatment 1	55.71
Treatment 2	25.71

Discussion

Chronic inflammation is defined as long-term inflammation (i.e. weeks to years) and a stimulant process of active inflammation, tissue injury, and healing. Chronic inflammation is characterized by the infiltration of mononuclear cells such as macrophages, lymphocytes, and plasma cells and tissue destruction and repair including the proliferation of new blood vessels, or angiogenesis and fibrosis. Chronic inflammation is characterized by inflammation that have not healed, tissue injury, repair by scar tissue and the body's immune response.²⁶ Chronic inflammation involves the process of tissue damage and repair over an extended period.^{6,27}

In this research, 19 Wistar rats (*Rattus Novergicus*) were divided into three groups: the control group (KK) and treatment group 1 being given fish oil at a concentration of 5%, while treatment group 2 received fish oil at a concentration of 10%. The buccal mucosa of the oral cavity in the mice were subsequently treated by making cuts, followed by the application of hydrogen peroxide (H₂O₂) 10% for 2-3 minutes twice a day, for three consecutive days in a row until clinical signs of inflammation such as redness, swelling and epithelial disintegration were presented. H₂O₂ was also administered on the grounds that Hydrogen peroxide will break down the epithelial cell membrane resulting in cell death which if not followed by the regeneration process, will culminate in thinning of the cell layer. This will allow oxygen from the blood vessels to penetrate the epithelial layer and enter into the submucosa, so that the process of tissue damage will continue.²⁵ The chronically inflamed mucosa is treated with *C. batrachus* fish oil extract at 5% concentration on KP1 and *C. batrachus* fish oil at a concentration of 10% on KP2. Tissue picking and imunohistokimia staining were then carried out.

Based on the research that has been conducted on 19 male rats (*Rattus novergicus*) obtained data on table 2 amount of expression of HSP 70 that the sample to a control group with a mean of 64.0000, group 1 (*C. batrachus* fish oil 5%) with a mean of 55.7143, group 2 (*C. batrachus* fish oil 10%) with a mean of 25.7143. In this research, the result of HSP 70 expression in the treatment group 2 (*C. batrachus* fish oil 10%) with the mean of 25.7143 then the

decrease compared to the control group and the treatment group 1 (*C. batrachus* fish oil 5%).

This study produced a meaningful result because the significance value obtained was one of $\alpha < 0.05$ which confirms this research as a 95% reliable. Research by Kamat and Roy (2015) showed that *C. batrachus* fish oil can help reduce elevated glucose levels and promote an increase in the activity of SOD (Superoxide dismutase) so as to reduce tissue damage in animals that have been induced with alloxan.^{24,27,28} Superoxide dismutase is an enzyme produced naturally by organisms that consume oxygen and serves as a defense mechanisms, since reactive oxygen is a side effect of metabolism and respiration. Playing an important role, SOD has become one of the alternative means of minimizing tissue damage caused by free radicals.^{10,30,31}

Chronic inflammation may result in oxidative stress, a condition in which an increase in ROS will cause damage to cells, tissues or organs.⁹ In chronic inflammation, a heat shock response occurs that represents the major homeostatic response in addition to the inflammatory response to extracellular insult required as a defense against protein function. Heat Shock Protein (HSP) is a widely available group of proteins and plays an important role as a cytoprotective agent. Heat Shock Protein (HSP) 70 is a protein that does not exist under normal conditions, but increases its expression during period of ill health.

In the study conducted by Lee and Corry (1998), oxidative stress, increased both, HSF and the expression of HSP 70. Heat Shock Factor (HSF) and enhanced expression of HSP 70 is mediated through a SAPK (Stress Activated Protein Kinase) pathway. The SAPK pathway begins when there is inflammation which subsequently activates HSF which binds to the HSE (Heat Shock Element). The bond between HSF and HSE induces the transcription of genes that express the HSP, which in turn will increase the expression of HSP 70.

Clarias batrachus fish oil has the highest omega-3 PUFA content when compared with fresh fish, marine fish and shrimp with a total fatty acid content of 28%. Fatty acids produced from specific fish constitute a source of omega-3 especially eicosapentaenoic acid (EPA) and docosahexanoic acid (DHA). The application of fish oil has the potential to control oxidative stress and increase antioxidants.^{15,19,20}

This oxidative stress will produce ROS, the increased in which, will respond to host to release SOD. The marker of oxidative stress is decreased of HSP 70, which shown after administering *C. batrachus* fish oil in chronic inflammatory conditions.

This study has confirmed a decrease in the expression of HSP 70 in rats with chronic inflammation after being given *C. batrachus* fish oil. In this study the best concentration is one of 10%, which enables the healing process to take place.

Conclusion

This study has confirmed a decrease in the expression of HSP 70 in rats with chronic inflammation after being given *C. batrachus* fish oil. In this study the best concentration is one of 10%, which enables the healing process to take place.

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