Bones, scales of *Upeneus sulphureus* and *Osphronemus gouramy* increase adhesion and decrease IL–1β expression on monocytes against *Streptococcus mutans*

I Dewa Ayu Ratna Dewanti¹, Pujiana Endah Lestari¹, Purwanto Purwanto¹, Erawati Wulandari¹, Risty Widi Endah Yani¹, Sunlip Wibisono², Juris Burlakovs³

¹Faculty of Dentistry of Jember University, Indonesia.
²Faculty of Economics and Business, Jember University, Indonesia.
³Faculty of Health and Life Sciences, Linnaeus University, Sweden

Corresponding author:
I Dewa Ayu Ratna Dewanti (idewadewanti@yahoo.com)

Abstract:
Adhesion and IL–1β on cells immunocompetent are important to prevent the spread of *Streptococcus mutans* infection to the systemic. One of the efforts is to use bones, fish scales *Upeneus sulphureus* and *Osphronemus gouramy*, they suspected contain of immunomodulator components. This study aims to analyze the effect of bone, scales of *U. sulphureus* and *O. gouramy* on adhesion activity and IL–1β expression on monocytes against *S. mutans*. This *in vivo* experimental study using the post test only control group design. Peripheral blood that had been layered with ficoll hypaque, then it added HBSS, Fungizone and Penstripe. The cells were placed in well microtiter plate, then they treated according to groups. Adhesion was stained with Giemza, IL–1β was stained with immunocytochemical. Cells were counted under a light microscope with magnification 400 times per 100 cells. The data obtained were analyzed using ANOVA followed by LSD test. Post test only design and the data obtained were analyzed using ANOVA followed by LSD test. There were no significant differences (p < 0.05) between Bones and scales. They both increase the adhesion of *S. mutans* and decrease the expression of IL–1β in monocytes. Bone and scales *U. sulphureus* and *O. gouramy* increase the recognition of monocytes to *S. mutans* and inhibit the spread of infection to systemic.

Keywords: Cells immunocompetent, gurame fish, in vivo, kuniran fish.

How to cite this article: Dewanti et al. (2020): Bones, scales of *Upeneus sulphureus* and *Osphronemus gouramy* increase adhesion and decrease IL–1β expression on monocytes against *Streptococcus mutans*, Ann Trop & Public Health; 23 (S8): 1253–1258. DOI: [http://doi.org/10.36295/ASRO.2020.23810](http://doi.org/10.36295/ASRO.2020.23810)

Introduction

The increasing awareness of the public likes to eat fish, the more waste (scales and bones) are buried. Until now, the use of fish (bones and scales) is still not widely used, even though the waste contains many components that are beneficial to health. Fish waste that has a composition similar to dentine and bone rich in hydroxyapatite, protein (32%), collagen and it is a source of calcium (1, 2). Fish scales consist of collagen type-I fibrils, and some mineralization with hydroxyapatite (16% to 59% mineral content). The outer layer of fish scales is significantly more mineralized and it often referred to as the "bone layer", while the inner layer ("basal" or "collagen" layer) (3). On the other hand Hydroxyapatite is widely used for bone replacement materials because it has several characteristics, namely bioactive, biocompatible, osteoconductive, non-toxic and not (4). The research has previously proven that bones, scales from Kuniran fish (*Upeneus sulphureus* [G. Cuvier,1829]) and gouramy (*Osphronemus gouramy* [Lacépède, 1801]) contain protein, amino acids, omega-3, omega-6, flavonoids. Allegedly the component is able to act as an immunomodulator. In addition this study have also proven that bones and scales increase the viability of monocytes and salivary leukocyte, so they reduce TNF–expression (5–7). In the market materials to prevent and overcome caries have been found, but there are still many side effects such as allergies. This study hope will later use bones and fish scales as a materials to prevent and overcome dental caries which also acts as an immunomodulator. Dental caries must be prevented, because it can spread to the systemic via blood vessels and cause various systemic diseases, such as endocarditis. This has been proven by the discovery of bacteria (*Streptococcus mutans*) originating from dental caries in patients with endocarditis (8). In addition, the discovery of dental caries bacteria in coronary atherosclerotic plaque specimens of patients who die from heart attacks (9). One way to prevent the spread of *S. mutans* to the systemic is to increase the adhesion of *S. mutans* to immunocompetent cells (one of them is monocytes) and reduce the expression of IL–1β which plays a role in the inflammatory process. So, that will in addition to filling caries it also kills microorganisms that cause caries. In this study used gouramy which was a freshwater fish that was widely cultivated especially in Jember district,
Indonesia\(^{(10)}\). Gouramy is an Indonesian native fish and one of the types of fish commodities that is a priority and is included in the aquaculture production by major commodities 2010 to 2014\(^{(11)}\). While *U. sulphureus* is a fish that is spread in tropical and subtropical waters, including Indonesian waters. This fish including one type of fish that has high potential as an Indonesian fishery resource and one type of fish that is abundant in Indonesia with a low economic value but has a high protein nutritional component\(^{(12,14)}\). The abundance of the two fish, efforts were needed to utilize the waste. Therefore this study want to use bones and scales to overcome dental caries and prevent their spread to systemic. In this study will analyze bones, scales of upeneus and gouramy fish against the adhesion of *S. mutans* to monocyte cells and IL–1 expression.

**Materials and methods**

This research was approved by the Ethical Committee Faculty of Dentistry (No. 079/UN25.8/KEPK/DL/2018), University of Jember, Indonesia. The material of this research were as follows: Peripheral blood collection, *S. mutans* (from Microbiology Laboratory, Faculty of Dentistry, University of Jember, Indonesia), Ficoll–hypaque (Sigma), DAB (Diamonobezadinidine/Daco), HRP (horseradish peroxidase/Daco), RPMI (Roswell Park Memorial Institute/Gibco), Immunostaining KIT (Daco), PBS (Phosphate Buffer Saline/Sigma), HBSS (Hank's Balanced Salt Solution/Gibco), FBS (Fetal Bovine Serum/Sigma). The method in this study was explained below. At first the fish bones and scales were separated from the meat, washed and boiled at 80 °C for 60 min, then it dried using an oven for 48 h at 65 °C. The dried bones and scales of the fish were washed and filtered using a 100 mesh filter. Furthermore, powder of bones and fish scales were dissolved in PBS with a ratio of 1:1. The next step, 0.006 L peripheral blood from healthy people was taken and mixed with heparin (anticoagulant). Then, the blood was layered on Ficoll–hypaque, centrifugated (63.33 rad s\(^{-1}\), 30 min, 26 °C). The monocyte layer was taken and added with HBSS in the ratio of 1:1, pipetting. After pipetting, it was centrifugated (57.67 rad s\(^{-1}\), 10 min, 26 °C). The supernatant was discarded, then pellet was suspended with culture medium (RPMI 1640 with 10 % FBS, 0.1 % Penstrep 20 μL and 0.05 % Fungizone 5 μL), then it was incubated for 24 h at room temperature. Monocytes were then layered inside the culture dish and added with RPMI. Afterward, cells were placed on 24–well microtiter plate \(8 \times 10^{3}\) cells/well, then it incubated for 45 min 37 °C, then it was washed \(4 \times\) with HBSS medium. Monocytes were treated according to their groups. Control Group: monocytes untreated, *S. mutans* Group: monocytes + *S. mutans*, Declovenac Group: declovenac + monocytes + *S. mutans*, *U. sulphureus* Scales group: scales of *U. sulphureus* + monocytes + *S. mutans*, *U. sulphureus* Bones Group: Bones of *U. sulphureus* + monocytes + *S. mutans*. Osphronemus gouramy Scales Group: scales of *O. gouramy* + monocytes + *S. mutans*, *O. gouramy* Bones Group: Bones of *O. gouramy* + monocytes + *S. mutans*. All groups were incubated for 2.5 h, the incubation medium was removed. Furthermore, fixation with absolute methanol was 2 min to 3 min. Adhesion analysis was stained with Giemza, the preparations were washed with running water, added Giemza and aquadest buffer (1:4), shaken until they looked faded, washed with running water, then they were dried. IL–1β was stained with immunocytochemical. The preparation was washed with PBS, soaked in Peroxide Blocking Solution at room temperature for 10 min. Preparations were incubated in the Back–Ground Sniper (protein blocking solution) 10 min at room temperature, then the primary antibody was added 20 μL, incubated at 25 °C for 60 min, washed with PBS. A secondary antibody was added to 20 μL, incubated 15 min at 25 °C, washed. TrekAvidin–HRP reagent added, incubated for 10 min, washed. Preparation of chromogen substrate DAB:1 μL Betazoid DAB Chromogen was diluted with 600 μL Betazoid DAB Substarte Buffer, then added to the preparation of 20 μL per preparation, incubated for 10 min, washed with tap water. Furthermore, hematoxylin Mayer stain (counterstain) was added to the preparation, incubated for 1 min to 3 min, washed under tap water, and dried. Cells were counted under a light microscope with magnification 400 times divided 100 cells.

**Statistic analysis:**

Post test only design and the data obtained were analyzed using ANOVA followed by LSD test.

**Results**

ANOVA analysis there were significant differences (\(p < 0.05\)). LSD analysis proved that there were no significant differences (\(p < 0.05\)) between Bones, scales and declovenac group. Adhesion activity and IL–1β in monocytes were treated with more bones and scales not much different from declovenac but are higher than the group that was not given fish scales. They both increase the adhesion of *S. mutans* and decrease the expression of IL–1β in monocytes. Bones and scales has the same ability as declofenac in increasing adhesion of *S. mutans*. The results of this study can be seen in the figure 1, figure 2, figure 3, figure 4.
Figure 1: (A) Microscopic description of S. mutans adhesion on monocyte cells in Control Group (A) S. mutans Group, (B) Declovenac Group, (C) O. gouramy Group Bones, (D) Scales O. gouramy Group, (E) Bones U. sulphureus Group, (F) Scales U. sulphureus Group, (G) Cell lysis (red arrow). Observation using a light microscope with 400 × magnification.

Figure 2: Diagram of adhesion on monocytes to S. mutans on treatment with bones, scales of O. gouramy and U. sulphureus.
Figure 3: Microscopic description of IL–1β expression on monocyte cells in Control Group (A) S. mutans Group, (B) Declovenac Group, (C) O. gouramy Group Bones, (D) Scales O. gouramy Group, (E) Bones U. sulphureus Group, (F) Scales U. sulphureus Group, (G) Cells expressed IL–1β (black arrow), cell lysis (red arrow). Observation using a light microscope with 400 × magnification.

Figure 4: Diagram of IL–1β expression on monocytes to S. mutans on treatment with bones, scales of O. gouramy and U. sulphureus.

Discussion
Bones, fish scales U. sulphureus and O. Gouramy increase of adhesion and decrease of IL–1β against S. mutans were suspected they contain various amino acids, Calcium, Phosphorus, flavonoids, omega–3, omega–6. Ratnaet.al proved that bones and scales of U. sulphureus and O. gouramy contained amino acids, including alanine, leucine and isoleucine, valine, arginine, proline, glutamic acid, histidine, glycine, serine. Amino acids play a role in forming antibodies, providing energy, strengthening the immune system, reducing inflammation and allergies, healing wounds. Many studies have suggested that the presence of omega–3 and omega–6 inhibits eicosanoid Arachidonic Acid receptor activity, especially on the COX pathway. Inhibition of COX activity will reduce the production of proinflammatory cytokines such as IL–1 beta. It is known that IL–1β stimulate the formation of inflammatory mediators via the nitric oxide synthase (NOS) pathway, which in turn will cause activation of nuclear factor kappa–B (NF–kB) and NF–κB is found in the cytoplasm as an inactive complex bond with NF–κB inhibitor protein (IxB). Various stimuli from inside and outside the cell can cause activation of IxB kinase (IKK) in the cytoplasm which will cause phosphorylation and degradation of the complex bonds of NF–Kb and IxB kinase (IKK). As a result the heterodimer of NF–κB (p50/p65) will experience translocation from the cytoplasm into the nucleus. Furthermore, in the cell nucleus, subunits p50/p65 will bind to a number of gene promoters and activate the transcription of target genes involved in the inflammatory response. As a result, the expression of this enzyme will increase when it gets an inflammatory stimulus, so that it will cause an increase in IL–1β as an
Dewanti *et al.* (2020): The effect of bone as immunomodulator May 2020 Vol. 23 Issue 8

... inflammatory mediator (20). If this happens continuously it can cause tissue damage. Signs of local inflammation was characterized by the accumulation of leukocytes, such as monocytes (21).

In the group of monocytes that were only induced by *S. mutans*, it appeared that many monocytes were lysed compared to other groups. The number of lysis cells is thought to be due to resistance by monocyte cells which exceeds the ability of cells to *S. mutans*, whereas groups of bones and scales have the ability to protect cells characterized by fewer lysis cells than the *S. mutans* group, it suspected antioxidants such as flavonoids and amino acids from scales and bones could protect cells from the attack of *S. mutans* bacteria. Monocytes have receptors that can recognize *S. mutans*. Also, monocytes cells release IL–1β, and TNF–α which are known to be factors that trigger adhesions, especially IL–1β known as immunoregulators (22).

Branch–chain amino acids (BCAAs) have been associated with immunomodulation since around the mid–1970 and mid–1980 and have been used in nutritional therapy for critically ill patients. Evidence shows that BCAAs can directly contribute to immune cell function, helping to restore the impaired immune system. Branched chain amino acids, especially leucine, are involved in the biological mechanisms of insulin action, protein synthesis, mitochondrial biogenesis, inflammation, and lipid metabolism (23). Aromatic amino acids (tyrosine, phenylalanine, tryptophan, histidine) and positively charged amino acids (alanine, lysine, histidine) are immunogenic (24).

**Conclusion**

Bone and scales U. sulphureus and O. gouramy increase the recognition of monocytes to *S. mutans* and inhibit the spread of infection to systemic.

**Acknowledgement**

A big thank you to Research University of Jember, Indonesia which gives an opportunity to obtain research grants (No.058/SP2H/LT/DRPM.2018) and RISTEKDIKTI–Kementerian Riset, Teknologi dan Pendidikan Tinggi, Indonesia [Ministry of Research, Technology and Higher Education, Indonesia] which has provided funding for this study.

**References**

Dewanti et al. (2020): The effect of bone as immunomodulator May 2020 Vol. 23 Issue 8

14. Pramusti, and Suparjo R. Uji efek antihipertensi kecap ikan kuniran (Upeneus Sulphureus) dengan dosis yang berbeda pada tikus (Rattus norvegicus) yang dibuat hipertensi [Test the antihypertensive effect of turmeric fish sauce (Upeneus sulphureus) with different doses in rats (Rattus norvegicus) made hypertension]. [Thesis]. Universitas Brawijaya. 2018. [in Bahasa Indonesia].


1258