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Matrix Metalloproteinase-1 (MMP-1) Expression and Density of Collagen Fibers following Application of Haruan Fish (*Channa striata*) Extract in Inflamed Pulp of Wistar Rat

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ABSTRACT

Pulp perforation in teeth with reversible pulpitis can occur due to iatrogenic factors. This condition can be treated with direct pulp capping using calcium hydroxide. Still, this material can cause necrosis of the superficial layer of the pulp and dentin, which produces tunnel defects in the long term. Therefore, natural ingredients of fish extract containing essential compounds for the process of tissue syntheses such as albumin, amino acids, and several minerals that can play a role in accelerating the wound healing process is being developed. This study was conducted to determine the effect of the application of Haruan fish extracts on MMP-1 expression and the density of collagen fibers in the dental pulp of inflamed Wistar rats. A total of 27 samples were divided into three groups: the negative control group, treatment (extract of Haruan fish), and positive control (calcium hydroxide). The test material was applied to class 1 cavities, made to reach the pulp, and given LPS e-coli then left for 3 hours. Samples were decapitated on 3rd,7th and 14th days. Data were analyzed using the Anova and Chi-Square test. Based on the study, there was a decrease in MMP-1 expression on 3rd,7th and 14th days, but there was no statistically significant difference. The density of collagen fibers was seen as thicker scores on the 7th day, but there was no significant difference in the time of observation. Based on the conducted study, Haruan fish extract has been shown to reduce MMP-1 expression in inflamed pulp and trigger the proliferation of fibroblast cells to increase the density of collagen fibers.

Keywords: Haruan fish extract, MMP-1, the density of collagen fibers

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INTRODUCTION

The dental pulp is a connective tissue that contains nerves, blood vessels, essential substances, interstitial fluid, odontoblast cells, fibroblast cells, and other cellular components.[1,2] Dental pulp complex can adapt to various stimuli to maintain tooth vitality and play a role in secreting dentin.[6] When the pulp tissue is inflamed, it can carry out a defensive reaction.[3,4,5] Several Matrix Metalloproteinases (MMPs) are found in dentin-pulp complexes and healthy tissue, which are involved in the physiological processes during the formation and maintenance of the dentin-pulp complex.[7] One of the MMP included in the collagenase enzyme is MMP-1 expressed by fibroblast cells, macrophage cells, and endothelial cells. In physiological conditions, its expression is low, while the increase in pathological conditions.[8,9] Extracellular matrix in dental pulp contains collagen type I and III. The collagen degradation process is very instrumental in tissue and collagen damage during pulp inflammation.[10]

Pulp perforation in teeth with reversible pulpitis can occur due to iatrogenic factors. This condition can be treated by applying calcium hydroxide in the perforation area.[11] Calcium hydroxide is alkaline, stimulating the formation of reparative dentine on direct contact with the pulp. Still, this material can cause necrosis of the superficial layer of the pulp and dentin.[12,13,14,15]

At present many natural materials are developed, which are biocompatible with hard tissues and synthesis and proliferation.[17,18] Besides, the hydroxyapatite content found in the bones of the Haruan fish (*Channa striata*) play a role in the formation of dental hard tissue.[19]

With a variety of Haruan fish (*Channa striata*) extracts that are useful in inducing the wound healing process, it is of interest to the Author to research more about the effectiveness of Haruan fish (*Channa striata*) on MMP-1 expression and the density of collagen fibers in the inflamed pulp of Wistar rats.

MATERIALS AND METHODS

The production of Haruan fish extract (*Channa striata*) consists of the extraction of flesh and bones of Haruan fish. Haruan fish extract was produced referring to the research conducted by Agustin et al. with steaming and bone extraction methods referring to research conducted by Maulidah et al. by doing bone de-proteinase then mixed with a comparison (1: 1) to form a paste.

Twenty-seven male Wistar rats aged 12-16 weeks with a bodyweight of 250 - 300 g, were used in this study. Wistar rats were divided into three groups randomly; each group consisted of 9 rats. A Class I (Black Class) cavity was made on the occlusal surface of the maxillary right first molar using a low-speed handpiece. The penetration of the pulp was done by using K-file # 15 until a red dot was seen. The cavity was irrigated with a sterile saline solution and dried using cotton pellets. After that, the LPS E-coli was applied and then covered with Cavit. The negative group was closed with Resin Modified Glass Ionomer (RMGI), a permanent restoration material. The treatment group was applied with Haruan fish extract (*Channa striata*), and positive control was used with calcium hydroxide after the sample was left for 3 hours. After the application of the material, the cavity was restored with RMGI. After that,

the examples were decapitated on 3rd,7th and 14th days. The samples were examined immunohistochemically to examine the expression of MMP-1, and Mallory staining was done to evaluate the density of collagen fibers.

RESULTS

Table 1 showed a decrease in MMP-1 expression in the treatment group, but statistically, there is no significant difference based on the observation time where p (0.528)> 0.05. From the table, it can be seen that the treatment group has decreased MMP-1 expression from 3rd,7th and 14th days, whereas positive and negative control groups have decreased MMP-1 expression from day 3 to 7 but increased on day 14.

Based on the time of observation, collagen fibers density in the treatment group is higher compared to other groups, especially on the 7th day, but there was no statistically significant difference (p=0.233).

DISCUSSION

Matrix metalloproteinase (MMP) is an enzyme that plays a role in the formation of physiological secondary dentin in healthy teeth, matrix degradation during tooth injury. It plays a role in tertiary dentinogenesis and pulp inflammation.[20,21] The balance between MMP-1 and TIMP-1 is the critical control point in network remodeling connections, and imbalance conditions can cause tissue damage.[22]

In the negative group, the MMP-1 expression was seen with a lower value than the other groups. This indicates a mild inflammation caused by mechanical perforation induced by lipopolysaccharide (LPS) of *E. coli* bacteria. This result is consistent with research conducted by Feng Mei et al. that the friction and heat generated due to the use of rotary instruments causes an increase in the that bacteria and the results of its products play a role during the tissue inflammation process through regulation of cytokine production to increase MMP expression or stimulate cells to produce MMP. [21,25]

The decrease in days 7 and 14 in the treatment group was caused by the content of unsaturated fatty acids found in the extract of the Haruan fish (*Channa striata*) functioning as an anti-inflammatory by regulating prostaglandin synthesis which acts as a vasodilator of blood vessels so that regulating infiltration and activation of neutrophils in the inflammatory process and induces wound healing[16]. Besides, palmitic acid in Haruan fish extract can also reduce the production of proinflammatory cytokines that cause MMP-1, MMP-2, TIMP-1 in pulp cells.[21, 26] Other ingredients Zn play a role in cell growth and replication and play a role in the cellular immune response. Cu and Zn play a binding role and optimize the function of the enzyme superoxide dismutation (SOD) to reduce inflammation.[27] Albumin in Haruan fish (*Channa striata*) extract is also able to stimulate Transforming Growth Factor (TGF-β) through macrophages. [28] TGF-β can inhibit the production of MMP-1 through increased proteinase inhibitory activity, namely TIMP (Tissue Inhibitor Metalloproteinase), which can prevent and reduce collagen tissue damage, so that collagen synthesis is increased.[29]

The high expression of MMP-1 on the 3rd day then decreased on the 7th day, indicates inflammation due to mechanical perforation. The application of this bioactive molecular material can trigger cell proliferation and differentiation. However, on the 14th day the expression was slightly increased, this happened because in the application of calcium hydroxide it was likely still experiencing chronic inflammation, high pH in Ca(OH)2 can last for four weeks, causing tissue necrosis in the superficial pulp in contact with the material. Tissue necrosis will be responded to as a lesion and produce an inflammatory signal.[30]

Nelson-Filhoet et al. reported that Ca(OH)2 initially induces the formation of necrotic zones when contact with dental pulp tissue due to high pH (11-12).[31] During the inflammatory process that causes the number of pulp cells to increase simultaneously, fibroblast cells will migrate toward injury, proliferate, and produce large amounts of collagen matrix that will help isolate and repair damaged tissue.[19, 32]

In the Haruan fish extract treatment group, there was no significant difference in the increase in collagen density at each observation time. However, it can be seen in the treatment group with a score of 2 (thick) more than the positive and negative control groups. Collagen formation begins on day 3, more after day 7, and a few weeks later, collagen synthesis is stopped, and enzymatic degradation of the collagen matrix will occur. This condition will contribute to the balance of collagen formation. The balance between collagen deposition and degradation will determine the integrity and strength of the tissue. The mechanism of collagen degradation will occur through proteolytic enzymes (MMP) by degrading collagen and other macromolecular matrices to smaller extracellular peptides. This intracellular degradation is the most critical mechanism in physiological conditions and remodeling of collagen fibroblast tissue.[33]

The high percentage of collagen density score 2 (thick) in the treatment group showed that the albumin content of the Haruan fish extract (*Channa striata*) was able to stimulate the proliferation of fibroblast cells, thus confirmed the result of previous in vitro study that HFE could increase the spread of odontoblast-like cells.[34] Increased fibroblasts will increase collagen synthesis, accumulation, and remodeling process. The more fibroblasts in the injured area, the integration of collagen also begins, thus accelerating the wound healing process.[27,28] Albumin and Zn are also crucial for wound healing because this protein can bind Zn and then transport it in blood plasma. Zn deficiency reduces the wound healing process. Moreover, Cu plays a significant role in several enzyme functions, especially amine oxidation and pyridoxal phosphate.[35]

CONCLUSION

This research provides information about the content of Haruan fish (*Channa striata*) extract, such as albumin, Zn, Cu, Fe, and fatty acids, which have anti-inflammatory effects of suppressing the formation of proinflammatory cytokines. So that MMP-1 expression decreases and triggers cell proliferation in the creation of new collagen that will form an increased density of collagen, which indicates the tissue healing process.

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Table 1. Differences in MMP-1 Expression between Groups Based on the Time of Observation.

Group	Observation			p-value
	3rd day	7th day	14th day	
Negative Control	9.10 ± 5.43	7.33 ± 4.21	18.17 ± 2.02	0.038*
Treatment	63.33 ± 26.81	48.33 ± 24.19	38.23 ± 26.67	0.528
Positive Control	83.80 ± 2.59	66.10 ± 13.38	69.43 ± 10.86	0.152

Note: Anova test, p <0.05 *significant

Table 2. Distribution and Analysis Table of Differences in the Density of Collagen Fibers.

Group			Density			Total	p-value
			0	1	2		
Negative Control	3rd day	n (%)	3 (100)	0 (0)	0 (0)	3 (100)	0.029*
	7th day	n (%)	0 (0)	2 (66.7)	1 (33.3)	3 (100)	
	14th day	n (%)	0 (0)	3 (100)	0(0)	3 (100)	
Treatment	3rd day	n (%)	0 (0)	2 (66.7)	1 (33.3)	3 (100)	0.223
	7th day	n (%)	0 (0)	0 (0)	3 (100)	3 (100)	
	14th day	n (%)	0 (0)	3 (100)	0 (0)	3 (100)	
Positive Control	3rd day	n (%)	1 (33.3)	2 (66.7)	0 (0)	3 (100)	0.325
	7th day	n (%)	0 (0)	3 (100)	0 (0)	3 (100)	
	14th day	n (%)	0 (0)	3 (100)	0 (0)	3 (100)	

Chi-Square test with p <0.05 * significant

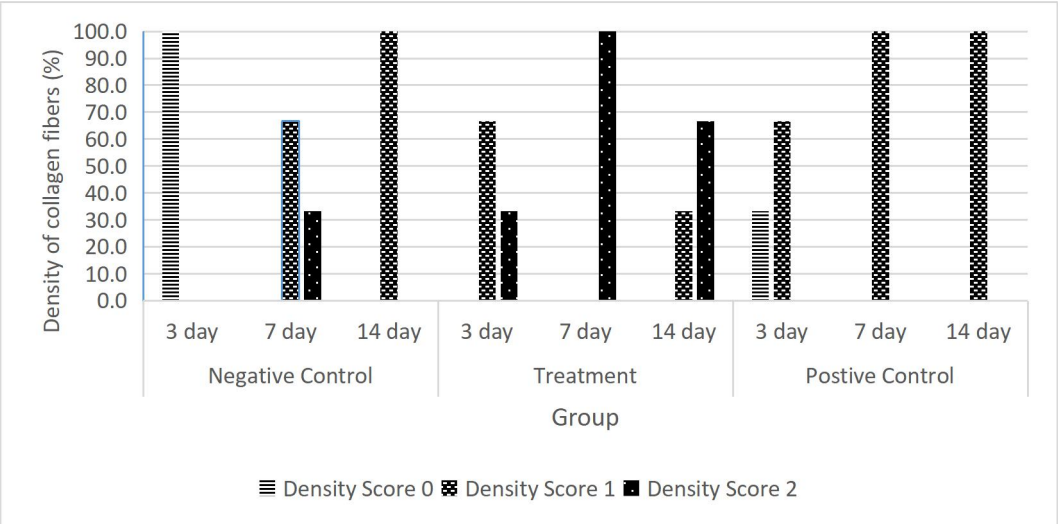

Figure 1. Density of Collagen Fibers Based on the Time of Observation of the Group.