

Dentin Matrix Protein-1 (DMP-1) Expression after Application of Haruan Fish Extract (*Channa striata*) on Inflamed Wistar Rat Dental Pulp

Andi Sumidarti*, Christine Anastasia Rovani, Juni Jekti Nugroho, Bulkis Thahir

Department of Conservative Dentistry, Faculty of Dentistry, Hasanuddin University, Makassar, Indonesia
Corresponding Author: a_sumidarti@yahoo.com

ABSTRACT

Haruan fish (*Channa striata*) contains essential compounds such as albumin, amino acids, zinc (Zn), iron (Fe), and hydroxyapatite minerals that can play a role in the process of inflammation, proliferation and cell differentiation. Odontoblast cell activity can be analyzed through DMP-1 expression. The purpose of this study is to determine the effect of administration of extract of Haruan fish (*Channa striata*) on the interpretation of DMP-1 on inflamed rat dental pulp. This study was an experimental laboratory with a posttest only study design with a control group design. The research sample was nine groups of rats with 27 animals divided into 3, 7, and 14 days of observation, and occlusal preparation was performed on the first molar. Calcium hydroxide was given to the positive control group, extract of the Haruan fish in the treatment group, and the group negative control no application. Then the expression of DMP-1 was examined by immunohistochemical examination. Data were analyzed using Anova statistical test, Kruskal-Wallis, and chi-square test. The extract of the Haruan fish (*Channa striata*) can increase the expression of DMP-1 because the calcium and phosphate ion content in the Haruan fish extract can be a carrier of bioactive molecules and can increase the differentiation of odontoblast cells. The expression of DMP1 has increased after applying the extract of the Haruan fish (*Channa striata*) so that it can be considered a dent in biomineral material that can increase the proliferation and differentiation of odontoblast cells.

Keywords: Haruan Fish, Immunohistochemistry, DMP1 Expression.

Correspondence:

Andi Sumidarti
Department of Conservative Dentistry, Faculty of Dentistry, Hasanuddin University, Makassar, Indonesia
Corresponding Author: a_sumidarti@yahoo.com

INTRODUCTION

The dental pulp is a soft connective tissue consisting of cells, extracellular matrix, blood vessels, and nerves. Odontoblasts are the primary cells of the dentin pulp complex¹. Odontoblasts can form a single layer and synthesize extracellular matrix, which then forms dentin. The dentin matrix formation through odontoblast differentiation can be quantified at the protein level or gene expression. Dentin matrix protein-1 (DMP-1) is one of the biomarkers to analyze odontoblast activity.^{1,2}

Dentin matrix protein (DMP-1) is a non-collagen matrix protein present in dentin and bone mineralization matrices because of its highly acidic nature. It can bind Ca²⁺ ions to regulate hydroxyapatite nucleation and inducing differentiation of odontoblast cells into odontoblast like cells.^{1,2}

If the pulp is affected by injury in the form of mechanical trauma, chemicals and bacteria, and their products, the pulp will hold a defense reaction in the form of an inflammatory response and an immune response that can be reversible or irreversible. In reversible pulp, inflammation treatment can be done to maintain the vitality of the dental pulp by forming a reparative dentin or dentinal bridge using an agent or dental material¹.

Calcium hydroxide is one of the dental ingredients that can stimulate dentinal bridge formation. However, long-term research shows that there is a weakness of calcium hydroxide in its binding to dentin, and tunnel defects occur in the structure of the dentinal bridge to facilitate the entry of bacteria that slow down the healing process. A very high pH of 11-13 can cause necrosis of the pulp tissue. These deficiencies are a consideration for finding active natural ingredients with minimal side effects¹.

Natural ingredients received a great deal of attention in the last few decades because they were considered to have smaller side effects. One natural part that is often used as traditional medicine is Haruan fish (*Channa*

striata). Haruan fish extract contains important compounds for tissue synthesis such as albumin, amino acids, minerals, zinc, copper, and iron, which can play an essential role in accelerating the wound healing process, namely in the inflammatory and proliferation phases. The albumin content in the Haruan fish extract can potentially increase the spread of odontoblast cells.^{1,2}

Besides, the calcium content in apatite hydroxide in the bones of the Haruan fish (*Channa striata*) can also stimulate the differentiation of odontoblasts to odontoblast-like cells to increase dentin regeneration to produce reparative dentin, reduce capillary permeability which will lessen the interstitial fluid production and increase concentration in the area that is undergoing odontoblast like cells to increase dentin regeneration to produce reparative dentin, reduce capillary permeability which will lessen the interstitial fluid production and increase concentration in the area that is undergoing odontoblast like cells. Mineralization upon reparative dentin formation¹.

This study was intended to determine the increase in Dentin Matrix Protein-1 (DMP-1) expression in the dental pulp of Wistar rats that became inflamed after the application of Haruan fish extract.

MATERIALS AND METHODS

This research was an experimental laboratory with a post-test control group design and had obtained ethical eligibility from the research ethics committee of the Faculty of Dentistry, Hasanuddin University. The research sample was the teeth of male Wistar rats (*Rattus norvegicus*) who met the criteria and received perforation pulpal roof action on the maxillary first molar teeth that met the inclusion and exclusion criteria.

This research was started by making an extract of Haruan fish (*Channa striata*) consisting of meat extraction and extraction of Haruan fish bones. Haruan fish meat extract

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was processed by the steaming method. Haruan fish were cleaned from the scales; the contents of the stomach were removed. The meat was cut into small sizes with cross-sections and thickness of ± 1 cm by removing the bones. Haruan fish meat was placed in a container and then put into a pan, steamed at a temperature of 700 (medium heat) for 50 minutes. Haruan fish extract in the form of light yellow liquid was accommodated in a container and then concentrated using a rotary evaporator and then stored in the refrigerator.¹ The extraction of Haruan's bone was done by washing and cleaning fish bones from the remnants. The bones were cut into chips using a bone cutting machine, then rewashed with high-pressure water to remove bone marrow and soft tissue on the bone surface. Fat in the bone was extracted with chloroform: methanol (1:1). The rest of the extraction agent was stopped crushed until ± 60 mesh size was reached. Bone samples were de-proteinase by hydrolysis using sodium hydroxide (NaOH) at a concentration of 1 M at 700C for 7 hours, then deposited for 24 hours and filtered. Bone de-proteinase was dried in an oven at 600 C and screened to a size of 200 mesh ($\pm 75 \mu\text{m}$) or in nm units. Bone de-proteinase was packaged in a closed bottle and sterilized with gamma irradiation¹.

Twenty-seven male Wistar rats, aged 12-16 weeks with a weight of 250-300g, were used in this study. Wistar rats were fed standard (pellets) and drinking water ad libitum. Rats were divided into three groups randomly; each group consisted of 9 mice.

Rats in groups 1, 2, and 3 were anesthetized intramuscularly with Ketamine (65 mg/kg BW) and Xylazine (7 mg/kg BW) dissolved in phosphate-buffered saline (PBS) (Tani-Ishii et al., 1995). A Class I (Classification Black) cavity was made on the occlusal surface of the maxillary first molars using a low-speed handpiece with a round diamond (size $\frac{1}{4}$) BR-49 (Mani Inc., Japan) until it almost reached the pulp roof. The depth of preparation was estimated to be as large as a bur head. The penetration action on the pulp roof was done using K-file # 15 until a red dot was seen. The cavity is then irrigated with a sterile saline solution and dried using cotton pellets. After that, the LPS E Coli was administered and then covered with temporary patches. Distribution of experimental animal groups and application of test materials as follows:

- Group 1: was a Negative Control Group; this group did not apply any test material to the maxillary first molar teeth and was decapitated on 3rd, 7th, 14th days.
- Group 2: a treatment group, application of an extract of forage fish as much as ± 0.5 mg using a ball applicator, and then do the deposition using a permanent resting material RMGI (Resin Modified Glass Ionomer). Decapitation was carried out on 3rd, 7th, 14th days after material application.
- Group 3: a positive control group, the application of calcium hydroxide using an applicator ball, and then carried out by using a permanent restriction material RMGI (Resin Modified Glass Ionomer). Decapitation was carried out on 3rd, 7th, 14th days after material application.

Mandibular bone tissue and teeth were inserted into the paraffin block. After the minimum amount of paraffin blocks had been collected, a brick was cut to make preparations for the immunohistochemical examination. This cutting process used indirect immunoperoxidase

staining using monoclonal antibodies specific to DMP-1 (Santa Cruz Biotechnology®).

Observation using an obtilab microscope at a magnification of 100 and 400 times. Immunohistochemical slides were assessed DMP-1 with the number of positively colored cells divided by the total number of cells multiplied by 100%.

RESULTS

Based on the results, data were obtained and then analyzed using Anova, and Kruskal-Wallis test results were statistically significant if the values were obtained $p < 0.05$.

Table 1 showed the average comparison of DMP-1 between groups at each observation time. After three days of observation, it was found that there was a difference in the average DMP-1 between the treatment groups, positive control, and negative control. At seven days of observation, it was found that there was a difference in the average DMP-1 between the treatment groups, positive control, and negative control at seven days of view. Whereas on the 14-day observation, the average DMP-1 in the treatment group was 99.33, the positive control was 90.00. The negative control was 36.33. The statistical test results obtained p-value (0.027) < 0.05 , which means that there was a difference in the average DMP-1 between the treatment groups, positive control, and negative control 14-day observation. Following was the expression of DMP-1 in the form of a bar chart.

Based on the diagram above, it could be seen that the treatment group, positive control, and negative control increased expression from 3rd, 7th, 14th days. However, the highest expression was seen in the treatment group day 14, and the lowest in the negative control group.

DISCUSSION

Based on the results of this study, the expression of DMP-1 after the application of Haruan fish extract on the dental pulp of Wistar rats that experienced inflammation, showed an increase in expression of DMP-1 in the treatment group the Haruan fish extract on 3rd, 7th, 14th days. This study was in line with that carried out by Mass LF (2005), which states that DMP-1 appears as an early marker of odontoblast differentiation because its expression increased until the 11th day and was relatively constant after that. This was because the extract of Haruan fish (*Channa striata*) contains inorganic components of mineral bioactive molecules, including calcium (Ca^{2+}) and phosphate (PO_4^{3-}). Calcium contained in Haruan fish bones plays a role in the formation of apatite crystals during bone formation. Hydroxyapatite (HAp) ($\text{Ca}^{10}(\text{PO}_4)^6(\text{OH})_2$) in the bones of the Haruan fish was the main component of mineral that chemically and physically similar to teeth and bones in humans¹.

A significant increase in DMP-1 expression also occurred in the positive control group. This indicated that the bioactive molecular material in calcium hydroxide also triggers the proliferation and differentiation of odontoblast cells for reparative dentin¹.

Calcium hydroxide, which was in direct contact with connective tissue, would cause the formation of necrosis zones, disrupting the psycho-chemical balance and intracellular substance, which would cause rupture of glycoproteins and denatured proteins. According to Holland (1971), the formation of mineralized tissue after contacting calcium hydroxide and connective tissue could

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be seen on days 7 to 10 after application. Holland also reported the presence of superficial granulation tissue between the necrosis zone and the deeper granulation zone. This structure consists of calcium salt and a calcium protein complex. Under the granulation zone, there was a zone of normal cell and pulp proliferation. Near the granular zone, irregular tissue forms a dentine bridge¹.

Dentin the exogenous Protein-1 matrix added to the exposed dental pulp can act as a morphogenic trigger or be a promoter of differentiation of undifferentiated ectomesenchymal cells in the pulp toward the odontoblast lineage. DMP-1 could also act as a transcription component for the activation of odontoblast specific genes¹.

Hydroxyapatite from fish bones was a natural inorganic material that was biocompatible with healthy living tissue. According to Mustafa et al. (2015), hydroxyapatite from natural ingredients such as fish bones have metabolic activity and provide a suitable response compared to synthetic hydroxyapatite. Nano-sized hydroxyapatite had excellent bioactivity because it had a greater surface area. It was able to increase the proliferation and maturation of fibroblasts, which causes tissue regeneration could also stimulate the differentiation of stem cells into odontoblasts into odontoblast-like cells to increase dentin regeneration to produce reparative dentin. This odontoblast initiates ion nucleation to begin the mineralization process. It activates the intramembrane transport mechanism of calcium and phosphate ions to maintain the balance of intracellular ions to increase the mineralization of the reparative dentine matrix².

Hydroxyapatite mineral has osteoconductive properties with pore structure (200-500), pore interconnection, and the same composition as normal bone. These pores are attached and allow the growth of new blood vessels and would be a place to put a new bone matrix. When bone damage occurs, cytokine release and growth factors will occur by platelet cells, macrophages, osteoblasts, and bone matrix such as PDGF, IGF, FGF TGF- and BMPs. The hydroxyapatite mineral had a high affinity to bind these osteoinductive cytokines. The cytokines attached to the hydroxyapatite mineral would stimulate the mesenchymal stem cells found in the hydroxylapatite pores to differentiate into fibroblasts, chondroblasts, and osteoblasts. These three cells would begin the secondary bone healing process and produce a bone matrix suitable for the bone healing phase.^{2,19}

CONCLUSION

Based on the results of this study the expression of DMP-1 after the application of the extract of the forage fish (*Channa striata*) in the dental pulp of the rat which had increased inflammation, so that the extract of the forage fish can be considered as a material that can increase the differentiation of odontoblast cells.

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REFERENCES

1. Hargreaves, K. M., Goodis, H. E., & Tay, F. R. (Eds.). (2012). *Seltzer and Bender's dental pulp* (p. 512). Chicago: Quintessence.
2. Karanxha, L., Park, S. J., Son, W. J., Nör, J. E., & Min, K. S. (2013). Combined effects of simvastatin and enamel matrix derivative on odontoblastic differentiation of human dental pulp cells. *Journal of endodontics*, 39(1), 76-82. <https://doi.org/10.1016/j.joen.2012.10.013>
3. Kim, J. Y., Kim, D. S., Auh, Q. S., Yi, J. K., Moon, S. U., & Kim, E. C. (2017). Role of protein phosphatase 1 in angiogenesis and odontoblastic differentiation of human dental pulp cells. *Journal of endodontics*, 43(3), 417-424. <https://doi.org/10.1016/j.joen.2016.10.013>
4. Martini, D., Trirè, A., Breschi, L., Mazzoni, A., Orsini, G., Teti, G., ... & Ruggeri Jr, A. (2013). Dentin matrix protein 1 and dentin sialophosphoprotein in human sound and carious teeth: an immunohistochemical and colorimetric assay. *European Journal of Histochemistry: EJH*, 57(4). <https://dx.doi.org/10.4081%2Fejh.2013.e32>
5. Lourenco Neto, N., Marques, N. C., Fernandes, A. P., Rodini, C. O., Sakai, V. T., Abdo, R. C. C., ... & Oliveira, T. M. (2016). Immunolocalization of dentin matrix protein-1 in human primary teeth treated with different pulp capping materials. *Journal of Biomedical Materials Research Part B: Applied Biomaterials*, 104(1), 165-169. <https://doi.org/10.1002/jbm.b.33379>
6. Njeh, A., Uzunoğlu, E., Ardila-Osorio, H., Simon, S., Berdal, A., Kellermann, O., & Goldberg, M. (2016). Reactionary and reparative dentin formation after pulp capping: Hydrogel vs. Dycal. *Evidence-Based Endodontics*, 1(1), 1-9. <https://doi.org/10.1186/s41121-016-0003-9>
7. Roberts, H. W., Toth, J. M., Berzins, D. W., & Charlton, D. G. (2008). Mineral trioxide aggregate material uses in endodontic treatment: a review of the literature. *Dental materials*, 24(2), 149-164. <https://doi.org/10.1016/j.dental.2007.04.007>
8. Hartini PS, Dewi N, Hayatie L. (2015). Haruan fish extract (*Channa Striata*) reduces macrophage amounts in the inflammatory phase healing process of dentofacial wounds. 14(1): 6-10.
9. Agustin, R., Dewi, N., & Raharja, S. D. (2016). Efektivitas ekstrak ikan haruan (*Channa Striata*) dan ibuprofen terhadap jumlah sel neutrofil pada proses penyembuhan Luka Studi in vivo pada mukosa bukal tikus (*Rattus Norvegicus*) Wistar. *Dentino*, 1(1), 68-74. <http://dx.doi.org/10.20527/dentino.v1i1.424>
10. Kumala, Y. R., Rachmawati, D., & Hersanto, K. (2017). Stimulasi dentin reparatif direct pulp capping menggunakan ekstrak ikan teri (*Stolephorus* sp). *E-Prodenta Journal of Dentistry*, 1(2), 45-53. <https://doi.org/10.21776/ub.eprodenta.2017.001.0.2.1>
11. Agustin, R., Dewi, N., & Raharja, S. D. (2016). Efektivitas ekstrak ikan haruan (*Channa Striata*) dan ibuprofen terhadap jumlah sel neutrofil pada proses penyembuhan Luka Studi in vivo pada mukosa bukal tikus (*Rattus Norvegicus*) Wistar. *Dentino*, 1(1), 68-74. <http://dx.doi.org/10.20527/dentino.v1i1.424>
12. Maulidah, M., Irnamanda, D. H., & Panjaitan, F. U. A. (2018). Biocompatibility test of haruan fish (*Channa striata*) bone hydroxyapatite to fibroblast cell as periodontal pocket therapy (In Vitro Study on BHK-

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- 21 Fibroblast Cell with Hydroxyapatite of Haruan Fish Bone (*Channa striata*) as Bone Graft Material). *Dentino*, 3(2), 150-155. <http://dx.doi.org/10.20527/dentino.v3i2.5370>
13. Massa, L. F., Ramachandran, A., George, A., & Arana-Chavez, V. E. (2005). Developmental appearance of dentin matrix protein 1 during the early dentinogenesis in rat molars as identified by high-resolution immunocytochemistry. *Histochemistry and cell biology*, 124(3-4), 197-205. <https://doi.org/10.1007/s00418-005-0009-9>
 14. Janebodin, K., Horst, O. V., & Osathanon, T. (2010). Dental pulp responses to pulp capping materials and bioactive molecules. *Cu Dent J*, 33, 229-48.
 15. Mohammadi, Z., & Dummer, P. M. H. (2011). Properties and applications of calcium hydroxide in endodontics and dental traumatology. *International endodontic journal*, 44(8), 697-730. <https://doi.org/10.1111/j.13652591.2011.01886.x>
 16. Mizuno, M., Miyamoto, T., Wada, K., Watatani, S., & Zhang, G. X. (2003). Type I collagen regulated dentin matrix protein-1 (Dmp-1) and osteocalcin (OCN) gene expression of rat dental pulp cells. *Journal of cellular biochemistry*, 88(6), 1112-1119. <https://doi.org/10.1002/jcb.10466>
 17. Mustafa, A. Chemical Composition and Amino Acid Profile of Channidae Collected from Central Kalimantan, Indonesia. *International Journal of Science and Technology (IJSTE)*, Vol.2No.4, December 2013,25-29ISSN: 2252-5297
 18. Octiara, E. (2015). Dentin reparatif dan growth factor yang berperan dalam dentinogenesis reparatif: reparative dentin and the role of growth factor in reparative dentinogenesis. *Dentika Dental Journal*, 18(3), 294-299.
 19. Setiawan, M. R., Dewi, N., & Oktavianti, I. K. (2015). Ekstrak ikan haruan (*Channa striata*) meningkatkan jumlah neokapiler pada penyembuhan luka (Extract of haruan (*Channa striata*) increases neocapillaries count in wound healing process). *Journal of Dentomaxillofacial Science*. 14(1):1-5.

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Table 1. DMP-1 Expressions between Groups at Each Observation Time

Observation		Mean± SD	p-value
3 Day	Treatment	57.67±6.81	0.000*
	Positive control	80.00±0.00	
	Negative control	21.33±3.51	
7 Day	Treatment	70.67±21.83	0.010*
	Positive control	87.67±2.52	
	Negative control	35.00±10.00	
14 Day	Treatment	99.33±1.15	0.027**
	Positive control	90.00±5.00	
	Negative control	36.33±8.50	
Note: * Anova test		**Kruskall-Wallis test	p (<0.005)

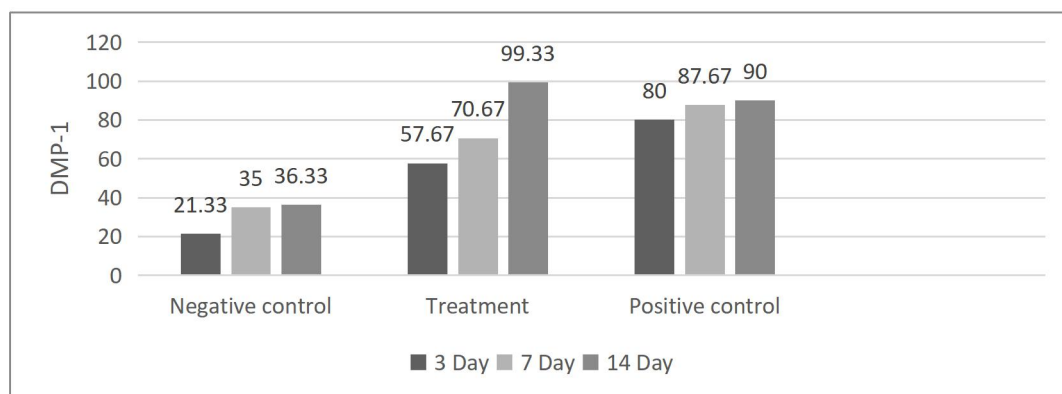


Figure 1. Diagram percentage of DMP-1 expression based on the observation time in each group