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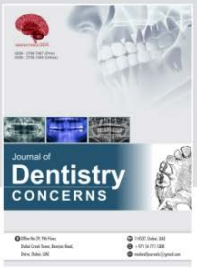

Expression of IL-1α and PMN Leukocytes in Inflamed Pulp of Wistar Rat After Application of Haruan Fish Extract (*Channa striata*)

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Key words: Pulp capping, haruan fish extract, PMN leukocyte cells, IL-1α, Wistar rat

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Abstract: Inflammation of permanent teeth that occurs in reversible pulpitis, pulp capping therapy can be done to keep the pulp healthy. Calcium hydroxide is the gold standard material as a capping agent but it has disadvantages due to its high pH. Haruan fish extract (*Channa striata*) contains active compositions such as albumin, unsaturated fatty acids and important minerals that are needed in the inflammatory process, those can be considered as an alternative medicine to subtitute calcium hydroxide. To determine the effect of the application of haruan fish extracts (*Channa striata*) on the number of PMN leukocyte cells and the expression of IL-1 Alpha; in the inflamed dental pulp of Wistar rat. Samples were 24 Wistar rats. Experimental animals were divided into 3 groups: negative control group, treatment group (application of haruan fish extract), positive control group (application of calcium hydroxide) to be decapitated in a period of 12 and 72 h. Data were analyzed using the Kruskall Wallis and Chi-square test. There was a decreased in the number of PMN leukocyte cells and low expression of IL-1 Alpha from strong staining to no staining on histological examination and statistical tests ($p<0.05$) according to observation time 12 and 72 h in the treatment group. Haruan fish extract is effective in decreasing PMN leukocyte infiltration and reducing IL-1α expression.

INTRODUCTION

Pulp tissue has a defense reaction against mechanical, thermal, chemical or bacterial irritants[1]. Pulp exposed to irritants if not treated, the inflammation of the pulp will get worse which can eventually cause pulp death[2].

Cells associated with inflammation of the pulp connective tissue are polymorphonuclear leukocytes and mononuclear leukocytes[3, 4]. In an inflammatory reaction, one of the mediators that plays a role is IL-1α which is an agonist that causes an inflammatory response[5,6].

Calcium hydroxide has been used as a gold standard for conservative treatment of injured dental pulp[7],

however, a high pH in calcium hydroxide can cause chronic inflammation and cell necrosis in *in-vivo*[8-10]. This is the author's consideration to find alternative natural ingredients that are effective in reducing inflammation in the pulp with minimal side effects.

One of the potential natural materials used is haruan fish. Haruan fish (*Channa striata*) has anti-inflammatory effects and the ability to accelerate wound healing. Based on this, the author would like to know the effectiveness of extract of haruan fish (*Channa striata*) against inflammatory cells of PMN and IL-1α leukocytes in the dental pulp of Wistar rats.

MATERIALS AND METHODS

Making haruan fish extract (*Channa striata*) 100% consists of meat and bone extractionthen mixed in a ratio of 1: 1[2].

Sample was 24 male Wistar rats, body weight between 250-300 g age 12-16 weeks. Samples were then divided into 3 groups consisting of 8 rats then divided into two subgroups for observation of 12 and 72 h. KlasI cavity preparation (Black) on the occlusal surface of the maxillary right molar using a low-speed round bur. The pulp roof perforation in cavity using K-file#15 then the LPS application of *E. coli* bacteria and left for 3 h. In the negative control group none of the ingredients was applied, the treatment group was applied haruan fish extract and the positive control group applied calcium hydroxide ($Ca(OH)_2$). The cavity was then dried and stuffed using RMGI. Samples were then decapitated after 12 and 72 h, then continued with HE staining to see PMN cells and immunohistochemical (IHC) staining to see IL-1α expression.

RESULTS AND DISCUSSION

Table 1 shows that at 12-h observations, the average PMN in the treatment group was 42.25, the positive control was 41.92 and the negative control was 53.50, from the statistical test results obtained p = (0.300)>0.05, which means there was no difference in PMN between groups. While on 72 h observation, the mean PMN in the treatment group was 24.25, the positive control was 38.08 and the negative control was 15.83, the statistical test results obtained p = (0.058)>0.05 which means there was no difference in PMN between groups.

For the assessment of IL-1α expression, in all groups there was a decrease in IL-1α expression from 12-72 h indicated by Table 2 and Fig. 1. The highest IL-1α expression at 12-h observation was produced by the negative control group compared to the treatment group and positive control group. The lowest IL-1α expression (strong staining) at 72 h observation was produced by the treatment group compared to the negative group and positive control group.

The bacterial lipopolysaccharide (LPS) used in this study was the LPS of *Eschericia coli* which is a bacterial virulence factor and an amphytic molecule that contains the main outer layer of the outer membrane of most Gram-negative bacteria[11-13]. Observation on PMN leukocyte examination 12 h after pulp perforation and LPS *E. coli* administration, a high PMN leukocyte infiltration was seen in all groups, this is because inflammation causes PMN leukocytes to enter the blood circulation system and invade the injured area in the first few hours after inflammation begins and will increase within 12 h[14-17].

Observation 72 h after the pulp was inflamed PMN leukocyte infiltration decreased in all groups. Because the work of PMN leukocytes as an acute inflammatory cell that plays a role against Foreign bodies and unfagocytosis cell decays is completed more quickly, therefore, the work of PMN leukocytes will be replaced by macrophages whose phagocytic power against bacteria is greater than PMN leukocytes. Haruan fish extracts also calcium hydroxide that was applied was not working

Table 1: Average PMN leukocyte cells based on observation time in each group

	Time			
	12 h		72 h	
Groups	Mean	SD	Mean	SD
Negative control	53.5	23.96	15.83	8.34
Treatment	42.25	18.93	24.25	13.37
Positive control	41.92	20.73	38.08	26.5
p-values	0.300*		0.058*	-

* Kruskall Wallis Test

Table 2: Histological expression of IL-1A based on the observation time in each group

		Groups			
Time	IL-1α	Negative control n (%)	Treatment n (%)	Positive control n (%)	p-values
12 h	No staining	0 (0.0)	1 (25.0)	2 (50.0)	0.355*
	Weak staining	2 (50.0)	1 (25.0)	2 (50.0)	
	Strong staining	2 (50.0)	2 (50.0)	0 (0.0)	
72 h	No staining	0 (0.0)	1 (25.0)	0 (0.0)	0.369*
	Weak staining	2 (50.0)	3 (75.0)	2 (50.0)	
	Strong staining	2 (50.0)	0 (0.0)	2 (50.0)	

*Chi-square test

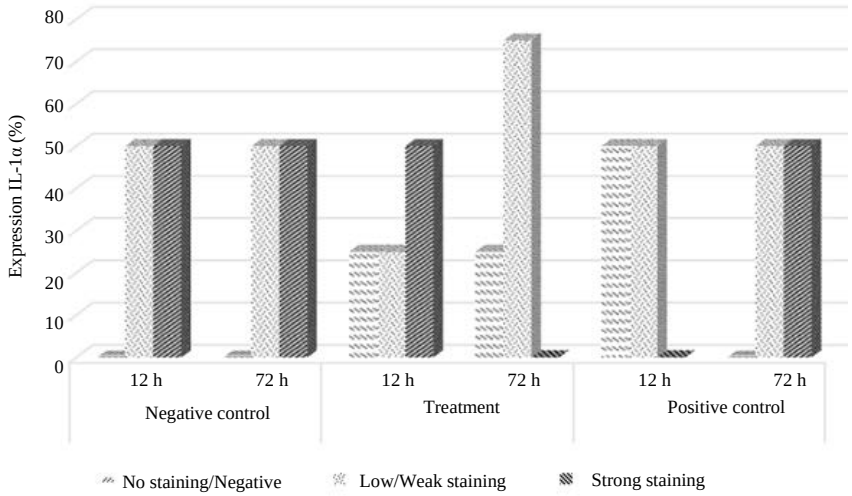

Fig. 1: Diagram of decreased IL-1α expression based on observation time

optimally because of the short duration of application[16-22]. Under normal circumstances, PMN leukocytes are in the blood circulation for only a few hours (6-12 h) before leaving the tissue[23-25].

In accordance with research conducted by Daud and Sadegh the reduction in PMN leukocyte infiltration is also caused by fish containing stearic acid and oleic acid which are very instrumental in inhibiting inflammation by blocking PMN leukocyte activity[14]. In a study conducted by Mat, etc., in addition to oleic acid and stearic acid haruan fish also contain unsaturated fatty acids, EPA, DHA, palmitic acid which can reduce PMN leukocyte infiltration and reduce inflammatory symptoms. While the positive control group using $Ca(OH)_2$ has an alkaline pH that can irritate pulp cells[20, 23, 26].

Calcium hydroxide is antibacterial. However, in a study conducted by Haghgoo, calcium hydroxide can cause abscesses in teeth and caustic actions associated with high pH that can cause necrosis.

Interleukin-1 Alpha (IL-1α) acts as an alarm and can be processed and released at the stage of cell death. IL-1α reaction has been known as an initial mediator of the inflammatory response that arises after injury or tissue necrosis[4, 5, 27, 28]. Interleukin-1α is produced by the activation of macrophages as well as PMN leukocytes, epithelial cells and endothelial cells[29, 30].

Calcium hydroxide, showing high IL-1α expression for observation of 12-72 h. This is in line with research conducted by Haghoo that high pH in calcium hydroxide can cause irritation to pulp tissue can cause abscesses in teeth and caustic actions that can cause necrosis[31-33].

For 12-72 h observations there is no difference in IL-1α scoring in all groups. This occurs because the inflammatory process of endothelial cells, monocytes and macrophages induces IL-1α formation which plays a role in both acute inflammation and chronic inflammation[34, 35].

Expression of IL-1α weakened at the observation time of 12-72 h. This is because the extract of the haruan fish contains albumin compounds which can accelerate the healing process of wounds and unsaturated fatty acids (oleic acid, stearic acid and palmitic acid) which can reduce the production of proinflammatory cytokines, helping to regulate prostaglandin synthesis which is an important role in the inflammatory phase and induces wound healing[36, 37]. Albumin is also the main transport of Zinc (Zn) which plays a role in the immune system, synthesizes protein and maintains the integrity of connective tissue and limits membrane damage due to free radicals during inflammation[33, 37].

Haruan fish extract also contains Cu and Fe. Cu plays an important role in the union of collagen and elastin, is responsible for maintaining the integrity of the myelin membrane, bone formation and the formation of connective tissue. Cu deficiency can cause a decrease in the body's immune response as well as impaired phagocytic function and activity in inflammation. Fe plays a role in oxygen delivery and collagen synthesis. Deficiency of Fe and Zn will result in reduced blood circulation to the tissues[2, 38-43].

CONCLUSION

Based on the description above, extract of haruan fish (*Channa striata*) can help reduce inflammation in the dental pulp of Wistar rats which is characterized by a decrease in the number of PMN leukocyte cells and expression of IL-1α by histological examination seen in observation time is 12 and 72 h.

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