THE EFFECT OF EXTRACTS OF *CURCUMA ZEDOARIA* ON THE EXPRESSION OF THE PROINFLAMMATORY CYTOKINE TNF- α

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ABSTRACT

Curcuma zedoaria is known to have antibacterial and antifungal activity, and have the potential to be used as an irrigation solution in endodontic treatment. Endodontic irrigant that is going to be used clinically has to go through a series of research, to ensure effectiveness and safety without adverse side effects. The purpose of this study was to determine the effect of extracts of *C. zedoaria* with concentrations of 5%, 10%, and 20% as endodontic irrigation on the expression of the proinflammatory cytokine TNF-α on Wistar rat. This experimental study used extracts of *C.zedoaria* with concentrations of 5%, 10%, 20%, as tested groups, 5.25% sodium hypochlorite as the positive control, and saline solution as a negative control group. Thirty male adult Wistar rats (*Rattus novergicus*) with an average weight of 150-200 g were included in this study. The dermis of each rat was marked with a circle and 0.1 ml of each irrigant was injected subcutaneously by using a syringe. The evaluation was made 14 days after injection. The expression of cytokine TNF-α was assessed with the IHC method. The percentage of TNF-α expression was evaluated and analyzed by using One-way Anova followed by Tukey Post Hoc test. The results of this study revealed that the highest percentage of TNF-α expression is positive control group (5.25% NaOCl). Furthermore, the higher the concentration of *C. zedoaria* extract, the greater the percentage of TNF-α expression. One-way Anova results showed that there was a significant difference between groups ($p \leq 0.05$). From the results of the Tukey test, it was found there was a significant difference between groups except between the negative control group and the 5% extract group. It can be concluded that *C. zedoaria* extract 10 and 20% can increase TNF-α expression, but its ability is lower than sodium hypochlorite.

Keywords: C. zedoaria, TNF-α, Endodontic irrigant

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1. INTRODUCTION

The main purpose of root canal instrumentation is to reduce the microorganism and to clean the dentinal wall, to eliminate the intracanal and periapical infection. However, previous study showed that there are areas especially in the apical parts that remained untouched by the instruments, and contained tissue remnants and/or bacteria.[1] This suggests that endodontic irrigant with antimicrobial and tissue-dissolving abilities is essential to assist in cleaning those areas.

Several chemical solutions are commonly used as endodontic irrigants such as sodium hypochlorite (NaOCl), ethylenediaminetetraacetic acid (EDTA), and chlorhexidine. Although it has many advantages, the use of chemical irrigation solutions can affect the physical and mechanical properties of dentin.[2] Furthermore, one of the main problems related to the use of irrigation solutions is their toxicity to the periapical tissues.[3]

Recently, the use of herbal ingredients has gained more popularity in dentistry. Herbal plants contain active compounds with pharmacological and therapeutic effects that have been widely synthesized and used in modern medicines.[4] One of which is to be developed as an alternative to irrigation solutions to replace the chemicals. There is evidence that herbal ingredients have antibacterial activity against root canal bacteria so that they have the potential to be used as an alternative irrigation material. The advantages of herbal irrigation materials are safe, readily available, inexpensive, and low microbial resistance.[5,6]

Indonesia has biodiversity and is rich in herbal plants. One of which is genus *Curcuma*, a member of the *Zingiberaceae* family, and consists of about 80 species mainly distributed across Southeast Asia, South Asia, and China.[4] Several in vitro studies have reported that one species of the genus *Curcuma*, namely *Curcuma zedoaria*, has antibacterial activity.[7-9] *C. zedoaria* rhizome extract was also able to clean the smear layer in the apical third of the tooth.[10]

Endodontic irrigants applied to the root canals of the teeth may unintentionally come into contact with the surrounding tissues in the oral cavity, for instance in a wide apical foramen or extreme pressure during irrigation.[4] Fernandes tested the cytotoxic effects of *Curcuma zedoaria* in vitro using fibroblasts derived from oral mucosa, the results showed low cytotoxicity of *C. zedoaria* fluid extract.[11]

Toxicity evaluation of *C. zedoaria* extract needs to be evaluated to ensure their effectiveness and safety without adverse side effects. The purpose of this study was to determine the effect of extracts of *C. zedoaria* with concentrations of 5%, 10%, and 20% on the expression of the proinflammatory cytokine TNF-α on Wistar rat.

2. MATERIAL AND METHOD

This is an experimental study in vivo with a post-test approach with a control group design. The study was conducted at the Biochemistry Laboratory Faculty of Medicine, Universitas

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Sriwijaya for the preparation of *C.zedoaria* extract, and in Eureka Research Laboratory for TNF-α preparation and the IHC test.

The research subjects were thirty male adult rats Wistar strain (*Rattus norvegicus*), 2-3 months old with an average weight of 150-200 g, in a healthy condition marked by a health certificate from the Palembang Livestock Service.

White turmeric rhizome was obtained from the Research Institute for Spices and Medicinal Plants (Balittro) Manoko, West Java. The rhizomes were cut into small pieces and then dried in the oven at 45℃ for 2 days. After drying, the pieces were pulverized with a blender until they became a uniform fine powder. The simplicia was then extracted by maceration using 96% ethanol as a solvent for three days. The extract was then filtered with filter paper to separate the filtrate and debris. Maceration was carried out three times. Each filtrate produced from each maceration process is then evaporated at 40℃ for three days to evaporate the solvent to obtain a 100% extract. The extract was then diluted using 5% tween to concentrations of 5%, 10%, and 20%.

The experimental procedures were conducted after approval by Medical and Health Research Ethics Committee, Faculty of Medicine, Public Health and Nursing, Gadjah Mada University. Experimental animals were divided into three experimental groups and three control groups (n=5). Group 1 as normal control; group 2, negative control with normal saline; group 3, positive control with 5,25% NaOCl solution; group 4, 5, and 6 was treated with 5%, 10%, and 20% *Curcuma zedoaria* extract, respectively.

The dorsal skin of the rats was shaved and cleaned with 10% iodine solution, following general anesthesia using Biopentyl (combination of ketamine, xylazine, and chlorpromazine) 0.1 ml/10 g body weight. A circle was marked on the dermis of each rat. Using a syringe, 0.1 mL of each solution was injected subcutaneously into the circle. For the normal control group, the needle of an empty syringe was introduced in the circle, but no solution was injected. Evaluations were made 14 days days after injection, and the rats were euthanized.[12] Excision was conducted in the circled area with a little bit of normal skin tissue around 0.5 cm from the edge of the wound, and then added into a 10% formalin solution.

Immunohistochemical staining was performed followed by the demonstration of proteins in paraffin-embedded tissue sections. The primary antibody used was TNF-α anti-rat primary antibody, and followed by a secondary antibody anti IgG. The TNF-α expression was assessed using the immunohistochemistry (IHC) method, with a 400× magnification microscope. The inflammatory cells and surrounding tissues were observed and photographed. The increase of brown-colored area indicated an increase in TNF-α expression.[13]

The mean of TNF-α expression in each group was statistically analyzed using SPPS with one-way ANOVA statistical analysis, followed by Tukey's post hoc test.

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3. RESULTS

The results depicted in Table 1 showed the mean value of TNF-α expression in each group. It can be seen that the highest percentage of TNF-α expression is positive control group (5.25% NaOCl). In addition, the percentage of TNF expression increased with increasing extract concentration.

Table 1. The mean of TNF-α expression

Group (n=5)	Percentage of T TNF-α expression
Normal control	3,014
Negative control (normal saline)	13,094
Positive control (5.25% NaOCl)	24,812
5% *C. zedoaria* extract	14,322
10% *C. zedoaria* extract	18,142
20% *C. zedoaria extract*	21,082

There was a statistically significant difference between groups as determined by one-way ANOVA ($p \leq 0.05$). A follow-up test to determine which group was significantly different was carried out by using the Tukey's Post Hoc analysis (Table 2).

Table 2. The percentage of TNF-α expression for each group

Group	Normal control	Negative control (saline)	Positive control (5.25% NaOCl)	5% *C. zedoaria* extract	10% *C. zedoaria* extract	20% *C. zedoaria* extract
Normal control		0.000*	0.000*	0.000*	0.000*	0.000*
Negative control (normal saline)			0.000*	0.644	0.000*	0.000*
Positive control (5.25% NaOCl)				0.000*	0.000*	0.001*
5% *C. zedoaria* extract					0.001*	0.000*
10% *C. zedoaria* extract						0.001*
20% *C. zedoaria* extract						

P<0.05 denotes significant statistically difference by Tukey test.

The results of the Tukey test found there was a significant difference between groups, except between the negative control group and the 5% extract group.

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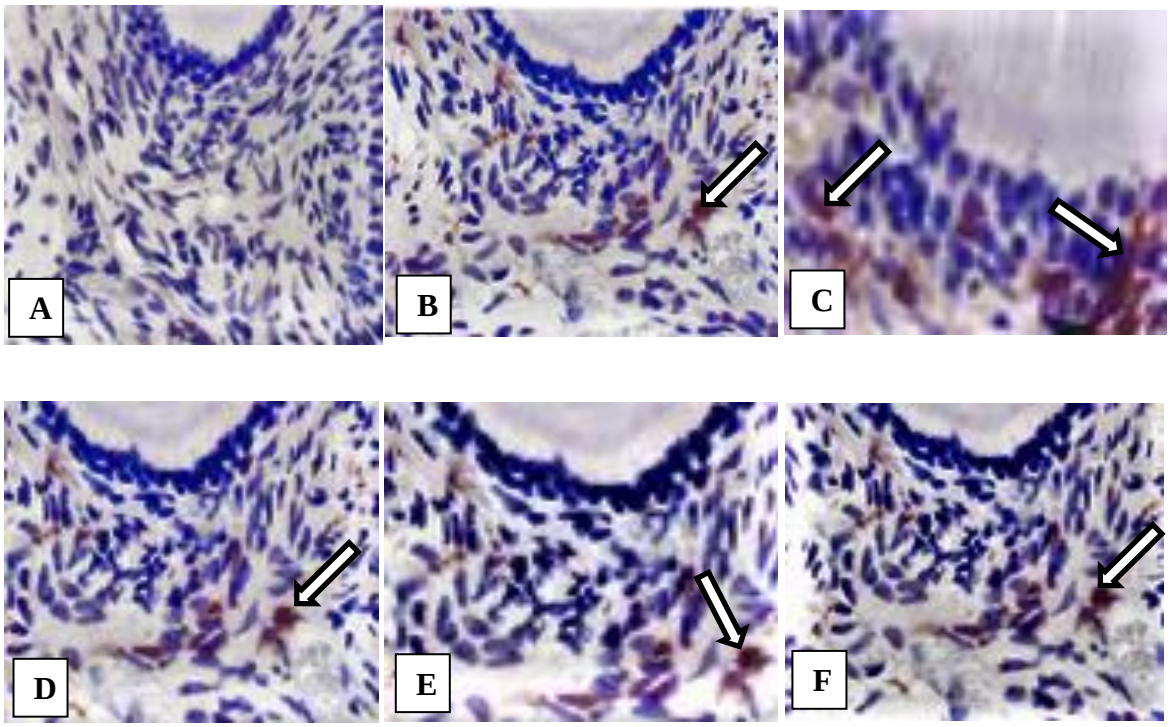

Figure 1. Expression of TNF-α by immunohistochemistry (IHC) method. (A) Normal control group, (B) Negative control group, (C) Positive control goup, (D) 5% *C. zedoaria* extract, (E) 10% *C. zedoaria* extract, (F) 20% *C. zedoaria* extract, with 400× magnification. The TNF-α expression was marked as brownish color as shown by arrows.

4. DISCUSSION

Most dental materials will have direct contact with hard and soft tissues in the oral cavity. It is, therefore, critical to have a thorough understanding of the biocompatibility and toxicity of materials used in dental treatments. One of them is the endodontic irrigant used in root canal treatment to eliminate pathogenic microorganisms in the root canal.

The most commonly used irrigation solution is sodium hypochlorite, with a concentration of 0.5-5.25%. One of the most important disadvantages of sodium hypochlorite is its toxicity to soft tissues, confirmed by various studies. Uğur et al. evaluated the cytotoxicity of sodium hypochlorite, chitosan, and propolis as endodontic irrigant on human fibroblast cell lines. The result showed that the toxicity of sodium hypochlorite was the highest among other solutions ($p < 0.05$). It is in line with the current study, where the TNF-α expression was highest in the sodium hypochlorite used as the positive control group, marked by brownish color seen in Figure 1.[14]

Recently, herbal plants have been increasingly researched and developed as alternatives to chemical drugs. The genus *Curcuma* is known for its active ingredients and has been used as traditional medicine for a long time. Among *Curcuma* species found in

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Indonesia, *C. longa* and *C.zedoaria* are some of the most popular. Rhizome was the most widely used part of the plant for traditional medicine formulas, it contains active compounds including curcumin, curcuminoid, dimethoxycurcumin, and bisdemethoxycurcumin.[4] The rhizome extracts and volatile oil obtained from rhizome of *C.zedoaria* contain constituents that showed antimicrobial and antifungal activity. Israr reported the antibacterial activity of *C.zedoaria* against *S. epidermitis*, *E. coli*, *P. vulgaris* and *Salmonella typhi para B*, but no activity against *S. aureus.*[15,16] These properties make *C.zedoaria* have the potential to be used as an irrigant in root canal treatment.

In the current study, the rhizome of c.zedoaria was used for making the test solutions with the concentration of 5%, 10%, and 20%. The results showed that the increase in the percentage of TNF-α expression was directly proportional to the concentration of the extract. The results showed that the increase in the percentage of TNF-α expression was directly proportional to the concentration of the extract. Significant differences appeared in all test groups. Although the highest TNF-α expression was in the 20% extract group, the percentage was lower than the sodium hypochlorite group with a significant difference.

Mandroli et al. conducted cytotoxicity evaluation using MTT assay on human periodontal ligament fibroblasts. The result showed that fibroblast cells were viable which means no cytotoxicity was detected for curcumin at any of the concentrations used (100%, 50%, and 25%) by MTT assay.[16]

TNF-α belongs to the proinflammatory cytokines, that are produced mainly by macrophages and T-cells, and also by cells such as endothelial cells and fibroblasts. The stimulation of TNF alpha production includes infection and inflammation.[17]

The positive control group (rats treated with saline) in this study showed a higher percentage of TNF expression than the normal control group, and it was not significantly different from the 5% Curcuma extract. The TNF-α is a component of immunity that is normally present in healthy tissues. This is indicated by TNF expression in the normal control group in this study, even though the percentage was low. TNF-α will remain expressed in small amounts of skin tissue, and will only increase if the skin has a wound or disease. Hence, it can be an indicator of how high the inflammatory activity in the tissue is.

5. CONCLUSION

C. zedoaria extract 10 and 20% can increase TNF-α expression, but its ability is lower than sodium hypochlorite

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