

Journal of Medicinal and Chemical Sciences

Journal homepage: <u>http://www.jmchemsci.com/</u>



Original Article

An Anti-Inflammatory Dental Pulp of Eugenol Extracted from Clove (*Eugenia Caryophyllata*)

Nendika Dyah Ayu Murika Sari¹* ⁽, Ikmal Hafizi¹ ⁽, Nida Faradisa Fauziyah² ⁽, Ratih Hartinawati¹, Zakia Sholiha¹

¹Faculty of Dentistry, Universitas Muhammadiyah Surakarta, Jalan Kebangkitan Nasional No 101, Penumping, Laweyan, Surakarta, Jawa Tengah, Indonesia

²Faculty of Medicine, Universitas Muhammadiyah Surakarta, Kampus IV UMS, Jalan Ahmad Yani, Gonilan, Kartasura, Sukoharjo, Jawa Tengah, Indonesia

ARTICLE INFO

Article history

Receive: 2023-04-10 Received in revised: 2023-05-22 Accepted: 2023-06-24 Manuscript ID: JMCS-2306-2099 Checked for Plagiarism: **Yes** Language Editor: Dr. Fatima Ramezani Editor who approved publication: Dr. Majid Hajifaraji

DOI:10.26655/JMCHEMSCI.2023.11.10

K E Y W O R D S Eugenol Macrophages Pulpitis

ABSTRACT

Objective: To determine the effective volume of eugenol on day 3 in the inflamed pulp.

Methods: Experimental research with a posttest-only control group design The experimental animals used were 28 male Sprague-Dawley rats. The maxillary right molar tooth was prepared with a size of 1 mm and a depth of 1 mm. Eugenol was dropped on cotton pellets according to the treatment group, namely 0.1 μ L, 0.08 μ L, and 0.02 μ L, and aquadest as a negative control was then put into the cavity and closed with a temporary seal. Rats were decapitated after 3 days, prepared, and stained with hematoxylin and eosin. Results were read with a 100x magnification microscope at 3 fields of view and with imageJ software.

Results: The average number of macrophages in the cotton pellet application group with 0.1 μ L eugenol volume was 22, the 0.08 μ L eugenol volume group was 6.71, the 0.02 μ L eugenol volume group was 44.43, and the negative control group was 34.29. The results of the one-way ANOVA test showed a significance of 0.000 (p<0.05).

Conclusion: Eugenol volumes of 0.08 μ L had the lowest average number of macrophages compared to eugenol volumes of 0.01 μ L and 0.02 μ L, and based on the post hoc test, there was a significant difference (p<0.05).

G R A P H I C A L A B S T R A C T



Introduction

There is still a significant problem with dental and oral health in Indonesia. According to the findings of Riset Kesehatan Dasar conducted in 2018, the percentage of dental and oral health concerns was 57.6% [1].

Pulpitis is a dental and oral health condition that manifests frequently and results in discomfort [2].

According to study conducted in Narayana, 77.2% of respondents had suffered pulpitis [3].

Pulp inflammation is a pathological indication that indicates inflammation and causes discomfort [4].

Treatment should be administered immediately, especially to the youngsters in pain. A therapy with anti-inflammatory substances is one treatment that can release pain, and it is simple and quick to administer, such as eugenol [5].

The plant cloves (Eugenia caryophyllata) is the source of the essential oil known as eugenol [6]. There is empirical evidence that eugenol possesses various biological qualities, including antibacterial, analgesic, anti-inflammatory, antioxidant, anti-mutagenic, and anticarcinogenic actions [7].

Because of its anti-inflammatory properties, eugenol has the potential to take the place of certain nonsteroidal anti-inflammatory medicines, or NSAIDs [8].

Dentists frequently employ eugenol with a cotton pellet placed inside the cavity to treat pulpitis pain. The volume of eugenol used varied in different studies. Eugenol administration in cavities has been demonstrated to reduce pain within 72 hours [9].

No information was provided on the amount of eugenol used in this study. Based on Enggardipta's research [10], using a volume of 0.1 μ L of eugenol overwritten with 500-gram weights reduce inflammatory cells. Ates' research [11] states that 1 drop of eugenol can reduce pain. It has been established that too much eugenol use during pulpectomy treatment with mixed zinc oxide results in pathological root resorption. When used excessively, eugenol may potentially irritate the periapical tissues [12].

This study aimed to calculate the amount of eugenol that was useful on day 3 in pulp inflammation.

Materials and Methods

Study design

The study was experimental with posttest-only control group design. Twenty-eight male Sprague-Dawley rats from LPPT Unit IV Gadjah Mada University Yogyakarta with inclusion criteria: male, healthy, 2-3 months old, and 300-350 g of body weight.

Research sites

The research was conducted at Unit IV LPPT, Gadjah Mada University, Yogyakarta, and the Faculty of Veterinary Medicine, Gadjah Mada University, Yogyakarta.

Precondition

Rats were randomly divided into four groups, the treatment group with a volume of 0.1 μ L, 0.08 μ L, and 0.02 μ L, and the negative control group with aquadest. Rats were adapted for 7 days by being given a standard feed of 360 g per day and drinking ad libitum.

Pulp inflammation procedure

Rats were anesthetized intramuscularly with ketamine HCL (0.1 mL/100 g of body weight). The maxillary right molar was prepared to form a class I GV black cavity with a diamond round bur number 010 (Edenta, Switzeland) mounted on a micromotor with a speed of 20,000 rpm without cooling water, a size of 1 mm, and a depth of 1 mm. According to the treatment group, eugenol obtained from the dental supplier was dripped on cotton pellets. The cotton pellet that has been dripped with eugenol is inserted into the prepared cavity. The cavity is closed with a temporary filling (cavit).

Procedure for making histological preparations

After three days, the rats were decapitated. Mice were anesthetized intramuscularly with ketamine HCL (0.1 mL/100 gram body weight). The rat jaws were cut at the molar teeth, fixed with 10% formalin for 24 hours, and decalcified for 10 days using 10% EDTA at 40 °C. The tissue was cut with a 0.5-1 cm thickness and washed for 6 minutes under running water. Tissues were dehydrated with alcohol at 70%, 80%, 90%, and 100% (absolute 1), 100% (absolute II), and 100% (absolute III) concentrations for 10 min. Followed by clarification agents, namely xylol I, xylol II, and xylol III, each lasting 5-10 min. Infiltrated with an oven temperature of 56 °C.

Specimens were put in xylol paraffin with a ratio of 1:1, followed by pure paraffin I, II, and III for 60 min.

Paraffin is poured into the paraffin block until it is complete, and air bubbles in the block are removed. Specimens were placed in liquid paraffin, and then cut to a thickness of 4 μ m using a microtome and placed on a glass object [13, 14].

Hematoxylin eosin staining procedure

The dried preparations were deparaffinized in xylol three times (10-15 min each).

Thereafter, it was put in 96% alcohol two times (each for 5 min), washed with running water until the alcohol is gone, and then it was put in hematoxylin paint for 7-10 min.

After that, it was washed with running water until it does not fade.

The preparations were dipped in HCl twice for decolorization, washed again with running water, soaked in water briefly until the colour turns blue, put in eosin paint for 3-5 min, and then washed under running water. Next, it was put into alcohol solution 1, and then into alcohol solution 2, and washed with running water.

After that, the preparation was pressed with paper, wiped with cotton, and put in xylol. The preparation was then pressed again with paper, wiped with cotton, and then mounted and received a laboratory number.

Calculation of the number of macrophages

Readings of preparations were done using a light microscope with a magnification of 100 times. Calculation of the number of macrophage cells with imageJ software in 3 fields of view.

Data analysis

The data were analysed using the Shapiro-Wilk normality test, which showed a significance of 0.059 (p > 0.05), and the homogeneity test with the Levene test, which showed a significance of 0.410 (p > 0.05). The data was then tested by a one-way ANOVA test and a post hoc test.

Result and Discussion

Macrophages from this study were demonstrated by the presence of spherical mononuclear cells with an oval or kidney-shaped nucleus located eccentrically with the cytoplasm and containing granules (Figure 1).

The results showed that the lowest average number of macrophages on day 3 was the eugenol volume of $0.08 \ \mu$ L.

The average number of macrophages in the cotton pellet application group with 0.1 μ L eugenol volume was 22, the 0.08 μ L eugenol volume group was 6.71, the 0.02 μ L eugenol volume group was 44.43, and the negative control group was 34.29 (Figure 2).

The results of the one-way ANOVA test showed a significance of 0.000 (p < 0.05) (Table 1). These results indicate a relationship between the use of eugenol in the dental pulp of Sprague-Dawley rats and the number of macrophages. To find out the relationship between treatment group and control group, the test was continued with a post hoc test (Table 2).

Based on the study results, the order of number of macrophages in the treatment group from the lowest to the highest was 0.08 μ L, 0.1 μ L, and 0.02 μ L. Inflamed pulp tissue undergoes early vascular changes caused by the effects of bradykinin and fibrinopeptide as direct plasma enzyme mediators that promote vasodilation and increased vascular permeability[15]. Macrophages become active 24 hours after an injury, intending to replace neutrophils. They will continue to proliferate after day 5, at which point they will become the predominant cell type in wounded tissue [16].

Macrophages in body cells are resting and can be activated by various stimuli, such as the presence of cytokines produced by T cells, the presence of inflammatory mediators, and components of the bacterial cell wall [17]. Sari N.D.A.M., et al. / J. Med. Chem. Sci. 2023, 6(11) 2665-2671



Figure 1: The application of cotton pellets wet with 0.02 μL of eugenol to the dental pulpitis of Sprague Dawley rats at 100x magnification under a microscope reading



Figure 2: The average number of macrophages in the dental pulpitis of Sprague-Dawley rats

Table 1: One-way ANOVA test on the effectiveness of using eugenol on the average number of
macrophages in the dental pulpitis of Sprague-Dawley rats

	Sum of Squares	df	Mean Square	F	Sig.		
Between Groups	5552.857	3	1850.952	228.311	0.000		
Within Groups	194.571	24	8.107				
Total	5747.429	27					

Table 2: Post hoc test between groups 0.08 µL with groups 0.1 µL, 0.2 µL, and negative control

Volume Eugenol	Volume Eugenol	Mean Difference	Std. Deviation	Sig.
0,08 µl	kontrol	-27.571*	1.522	0.000
	0.1 µl	-37.714*	1.522	0.000
	0.02 µl	-15.286*	1.522	0.000

Macrophages play a role in the phagocytosis of chronic infection stages and function as antigenpresenting cells (APC) to lymphocytes, which are needed to initiate host responses for adaptive immunity [18].

Pulp inflammation occurs due to the accumulation of inflammatory cells such as phagocytic cells, PMN leukocytes, and monocytes in the extravascular area of the pulp, acting as a pathological sign indicating inflammation and causing pain [4]. When inflammation occurs, the pulp plays a role in fighting infection and preventing tissue damage through immune cells with the help of fibroblasts [18].

Inflammation of the pulp causes monocytes to increase and migrate to the connective tissue, where macrophages will be activated. Macrophages initiate the bacterial component of the inflammatory process by releasing cytokines and chemokines. Activated macrophages will secrete pro-inflammatory cytokines in response to pathogens [15].

Based on the results of this study, the volume of eugenol in the treatment group was 0.08 µL lower than that in the control group. During an infection, eugenol was shown to reduce inflammation, accompanied by decreased TNF and neutrophil infiltration. In the course of acting as an anti-inflammatory, eugenol will decrease the activity of COX-2 as a mediator. This is significant because COX-2 activity plays a part in decreasing the creation of prostaglandin, which subsequently activates the body's defense cells. Eugenol functions as an anti-inflammatory agent by preventing the formation of nitric oxide (NO) and the expression of cyclooxygenase-2 (COX-2). This is accompanied by the activation of lipopolysaccharide (LPS), which has can dampen the effects of inflammation [19].

According to the research findings, the application of 0.1 microliters of eugenol resulted in an average number of macrophages that was greater than the application of 0.08 microliters of eugenol. This may be because of too much eugenol was used, which can be hazardous to the tissue if there's too much of it. At higher concentrations, eugenol acts as a pro-oxidant by elevating ROS production. Eugenol's effects are dependent on the concentration level [20, 21].

The use of eugenol in this study, at a concentration of 0.02 microliters, resulted in the greatest overall number of macrophages compared to both treatment and control groups. This condition is likely produced by the antiinflammatory action that has worn off, which causes an inflammatory response to be triggered once more. When it comes to pulp and fibroblasts, there is also the risk that very low amounts of eugenol could have harmful consequences [22].

Conclusion

It was shown that the usage of cotton pellets soaked in eugenol for three days could significantly lower the number of macrophages that are present in the dental pulpitis of Sprague Dawley rats. When compared to eugenol volumes of 0.01 μ L and 0.02 μ L, eugenol volumes of 0.08 μ L had the lowest average number of macrophages, and the post hoc test results demonstrated that this difference was statistically significant (p<0.05).

Disclosure Statement

No potential conflict of interest was reported by the authors.

Funding

An ethical clearance no. 4456/A.2/KEPK-FKUMS/IX/2022 was obtained from the Faculty of Medicine, University of Muhammadiyah Surakarta.

Authors' Contributions

All authors contributed to data analysis, drafting, and revising of the paper and agreed to be responsible for all the aspects of this work.

Orcid

Nendika Dyah Ayu Murika Sari <u>https://orcid.org/0000-0002-1393-6276</u> Ikmal Hafizi <u>https://orcid.org/0000-0002-9881-0583</u> Nida Faradisa Fauziyah <u>https://orcid.org/0009-0003-9588-6069</u>

References

[1]. Kemenkes R.I., Laporan Riskesdas 2018 Kementrian Kesehatan Republik Indonesia. Lap Nas Riskesdas 2018, 2018, **53**:154 [Google Scholar], [Publisher]

[2]. Shakib P., Rouhi S., Zolfaghary M.R., The role of probiotics in preventing dental caries, *Plant Biotechnology Persa,* 2020, **2**:55 [<u>Crossref</u>], [<u>Google Scholar</u>], [<u>Publisher</u>]

[3]. Kiranmayi G., Anumala L., Kirubakaran R., Estimation of the Prevalence of Pulpitis in the Tertiary Care Hospital in Nellore district-A cross sectional study, *IOSR J Dent Med Sci*, 2019, **18**:63 [Crossref], [Google Scholar], [Publisher]

[4]. Sabir A., Sumidarti A., Interleukin-6 expression on inflamed rat dental pulp tissue after capped with Trigona sp. propolis from south Sulawesi, Indonesia, Indonesia. *Saudi journal of biological sciences*, 2017, **24**:1034 [Crossref], [Google Scholar], [Publisher]

[5]. Gopikrishna V., Grossman's Endodontic Practice, *Wolters Kluwer*, 2021 [Google Scholar], [Publisher]

[6]. Charan Raja M.R., Srinivasan V., Selvaraj S., Mahapatra S.K., Pharmaceutica Analytica Acta Versatile and Synergistic Potential of Eugenol: A Review, Pharm Anal Acta, 2015, **6**:1 [Crossref], [Publisher]

[7]. Manouchehri N., Most Important Medicinal Plants with Anti-Halitosis Property: an Overview, *J Biochem Phytomedicine*, 2023, **2**:1 [Crossref], [Publisher]

[8]. Monsef Rad S.F., Paighambari S.Y., Haji Moradlou A., Mashayekhi F., Qorbani R., Evaluation of electronarcosis and clove oil for short-term anesthesia in common carp, *Cyprinus carpio L*, 1758, *Casp J Environ Sci*, 2016, **14**:299 [Google Scholar], [Publisher]

[9]. Kundabala M., Jagadish S., Ramya S., Efficacy of Ledermix as a root canal medicament in symptomatic teeth: A clinical study, *Journal of Interdisciplinary Dentistry*, 2014, **4**:85 [Google Scholar], [Publisher]

[10]. Enggardipta R.A., Haniastuti T., Handajani J., Efek eugenol terhadap jumlah sel inflamasi pada pulpa gigi molar tikus Sprague Dawley, *Majalah kedokteran gigi Indonesia*, 2016, **2**:66 [Crossref], [Google Scholar], [Publisher] [11]. Ates A.A., Alomari T., Bhardwaj A., Abedelmalek, Gambarini G., Differences in endodontic emergency management by endodontists and general dental practitioners in COVID-19 times, *Brazilian oral research*, 2020, **34**:1 [Google Scholar], [Publisher]

[12]. Subramaniam P., Gilhotra K., Endoflas Zinc Oxide Eugenol and Metapex as Root Canal Filling Materials in Primary Molars — a comparative clinical study, *Journal of Clinical Pediatric Dentistry*, 2011, **35**:365 [Crossref], [Google Scholar], [Publisher]

[13]. Wijayanti G.E., Setyawan P., Kurniawati
I.D. A Simple Paraffin Embedded Protocol for Fish
Egg, Embryo, and Larvae, *Scripta Biologica*, 2017, **4**:85 [<u>Crossref</u>], [<u>Google Scholar</u>], [<u>Publisher</u>]

[14]. Keklikoglu N., Akinci S., Comparison of three different techniques for histological tooth preparation, *Folia histochemica et cytobiologica*, 2013, **51**:286 [Crossref], [Google Scholar], [Publisher]

[15]. Ago Harlim., Buku Ajar Ilmu KesehatanKulit dan Kelamin Imunologi Inflamasi, *FK UKI*,2018 [Google Scholar], [Publisher]

[16]. Dewi P.S., Setiawan D.S., Lendir Bekicot Meningkatkan Jumlah Sel Makrofag Pada Penyembuhan Luka Pasca Pencabutan Gigi Marmut, *Interdental Jurnal Kedokteran Gigi (IJKG)*, 2020, **16**:1 [Crossref], [Google Scholar], [Publisher]

[17]. Hayati Z., Maulina N., KA RAP. Imunologi Dan Infeksi. First Edit. (Dina Alia, ed.). Syiah Kuala University Press; 2021 [Google Scholar], [Publisher]

[18]. Nurhapsari A., Kusuma A.R.P., Indraswary R, Widyasari F., Rahma D.A., Efek Coenzyme Q10 Terhadap Jumlah Sel Inflamasi Pada Model Tikus Pulpitis Akut, *Syifa'MEDIKA: Jurnal Kedokteran dan Kesehatan*, 2021, **12**:65 [Crossref], [Google Scholar], [Publisher]

[19]. Dwintanandi C., Nahzi M.Y.I., Raharja S.D., Pengaruh Ekstrak Kulit Manggis (Garcinia Mangostana Linn.) Terhadap Jumlah Makrofag Pada Inflamasi Pulpa Studi In Vivo Pada Gigi Molar Rahang Atas Tikus (Rattus Norvegicus) Wistar Jantan, *Dentino: Jurnal Kedokteran Gigi*, 2016, **1**:151 [Crossref], [Google Scholar], [Publisher]

[20]. Nisar M.F., Khadim M., Rafiq M., Chen J., Yang Y., Wan C.C., Pharmacological Properties and Health Benefits of Eugenol: A Comprehensive Review, Oxidative Medicine and Cellular Longevity, 2021, 2021 [Crossref], [Google Scholar], [Publisher]

Barboza J.N., da Silva Maia Bezerra Filho [21]. C., Silva R.O., Medeiros J.V.R., de Sousa D.P., An overview on the anti-inflammatory potential and antioxidant profile of eugenol, Oxidative Medicine and Cellular Longevity, 2018, 2018 [Crossref], [Google Scholar], [Publisher]

Escobar-García M., Rodríguez-Contreras [22]. K., Ruiz-Rodríguez S., Pierdant-Pérez M., Cerda-Cristerna B., Pozos-Guillén A., Eugenol toxicity in human dental pulp fibroblasts of primary teeth, Journal of Clinical Pediatric Dentistry, 2016, 40:312 [Crossref], [Google Scholar], [Publisher]

HOW TO CITE THIS ARTICLE

Nendika Dyah Ayu Murika Sari, Ikmal Hafizi, Nida Faradisa Fauziyah, Ratih Hartinawati, Zakia Sholiha. An Anti-Inflammatory Dental Pulp of Eugenol Extracted from Clove (Eugenia Caryophyllata). J. Med. Chem. Sci., 2023, 6(11) 2665-2671. DOI: https://doi.org/10.26655/JMCHEMSCI.2023.11.10

URL: https://www.jmchemsci.com/article 173997.html