THE EFFECT OF CINNAMALDEHYDE MEMBRANE APPLICATION ON THE NUMBER OF MACROPHAGES ON THE INFLAMMATION PROCESS OF LABIAL ULCUS OF WISTAR RATS

Chrisdina Puspita Sari[1)], Nunuk Purwanti[2)], Ika Dewi Ana[2)]

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ABSTRACT

***Introduction**: Macrophage is the inflammatory cell that dominates the chronic inflammation. Macrophage plays an important role in the phagocytic process and secretion of chemical mediators in the early stage of inflammation. Cinnamaldehyde is the major constituent of the cinnamon plant has an anti-inflammatory effect. Cinnamaldehyde can be delivered by membrane-shaped hydrogel polymer made from gelatin to maximize its anti-inflammatory effect.* ***Aim**: The objective of this study was to investigate the effect of cinnamaldehyde membrane on the macrophage numbers on the labial ulcer of Wistar rats.* ***Methods**: Thirty Wistar Rats were divided into five groups, four groups as treatment, and 1 group as a control. An injury of the labial mucosa of Wistar Rat was made by applying acetic acid glacial on the labial mucosal surface. The cinnamaldehyde membrane 1%, 4%, K-diclofenac membrane, and DMSO membrane were applied on the wound of the treatment groups, while the DMSO was applied on the control group one day after injury. Three rats from each group were sacrificed on the third and sixth day. The ulcerative mucosal tissues were collected and processed with Hematoksilin Eosin (HE) staining for histological preparation. The numbers of macrophages were counted in 6 fields using a trinocular microscope. The data were analyzed using a Two-way Analysis of Variance (Two-way ANOVA).* ***Result**: Two-way ANOVA showed significant differences between the treatment and control groups, so were the interaction of the treatment and the day.* ***Conclusion**: The application of cinnamaldehyde membrane affects the numbers of macrophages on the labial ulcer of Wistar Rats.*

***Keywords**: Inflammation, macrophage, ulcer, cinnamaldehyde.*

ABSTRAK

Pendahuluan: Makrofag merupakan sel radang yang dominan pada proses inflamasi kronis. Pada tahap awal inflamasi, sel makrofag berperan dalam proses fagositosis dan sekresi mediator-mediator kimiawi. *Cinnamaldehyde* merupakan kandungan utama tanaman kayu manis yang berpotensi sebagai agen antiinflamasi. *Cinnamaldehyde* dapat dipadukan dengan bahan polimer hidrogel gelatin yang berbentuk membran untuk meningkatkan efektivitas antiinflamasinya. **Tujuan**: Penelitian ini bertujuan untuk mengetahui pengaruh aplikasi membran-*cinnamaldehyde* terhadap jumlah makrofag pada proses inflamasi ulkus bibir tikus Wistar. **Metode**: Tiga puluh ekor tikus Wistar dibagi dalam lima kelompok yaitu empat kelompok perlakuan dan satu kelompok kontrol. Perlukaan pada mukosa bibir tikus dibuat dengan aplikasi asam asetat glasial. Luka pada kelompok perlakuan diberi membran-*cinnamaldehyde* 1%, 4%, membran-K-diklofenak 1%, membran-DMSO dan pada kelompok kontrol diberi DMSO tetes 1 kali sehari. Tiga ekor tikus dari masing-masing kelompok dikorbankan pada hari ke-3 dan ke-6 setelah perlukaan. Jaringan luka diambil, diproses secara histologis dan dilakukan pengecatan menggunakan Hematoksilin Eosin (HE). Penghitungan jumlah sel makrofag dilakukan pada enam lapang pandang menggunakan mikroskop trinokuler. Data jumlah sel makrofag dianalisis menggunakan ANOVA 2 jalur. **Hasil**: Hasil penghitungan jumlah sel makrofag dengan ANOVA 2 jalur menunjukkan perbedaan yang signifikan antara kelompok perlakuan dan kelompok kontrol dan interaksi antara perlakuan dan hari. **Kesimpulan**: Aplikasi membran-*cinnamaldehyde* berpengaruh terhadap jumlah sel makrofag pada proses inflamasi ulkus bibir tikus Wistar.

Kata Kunci: Inflamasi, makrofag, ulkus, *cinnamaldehyde.*

1) Biomedic Department, Faculty of Medicine, Widya Mandala Surabaya Catholic University Surabaya, Surabaya, East Java, Indonesia. # Corresponding author chrisdina@ukwms.ac.id. 2) Biomedic Department, Faculty of Dentistry, Universitas Gadjah Mada, Yogyakarta 3) Biomedic Department, Faculty of Dentistry, Universitas Gadjah Mada, Yogyakarta

INTRODUCTION

Inflammation is a physiological response of tissue to injury or to pathological infection and involves the body's chemical signals[1]. Inflammation works as a body's protective response that aims to clear the initial cause of damage to cells such as microbes, toxins, and the results of cell destruction such as dead cells and other dead tissue[2]. The inflammatory phase is characterized by cardinal signs of redness (rubor), heat (calor), swelling (tumor), pain (dolor), and functional disturbances (functiolaesa)[3].

The circulating cells in the blood vessels involved in inflammation are neutrophils, monocytes, eosinophils, lymphocytes, basophils, and platelets. Tissue cells involved are mast cells confined to the vicinity of blood vessels, connective tissue fibroblasts, and occasionally macrophages and lymphocytes[4]. Monocytes are cells produced by the bone marrow and are included in the granulocyte cell type. Monocytes have many roles at the end of the acute and chronic inflammatory processes. Monocytes differentiate into macrophages and play a role in bacterial phagocytosis. Macrophages will also secrete several chemical mediators, enhancing the acute inflammatory response[5].

Oral ulcers are a form of inflammation in the oral cavity. These ulcers can occur in any area of the oral cavity. Inflammation can be caused by conditions of the oral cavity itself such as poor oral hygiene, protein deficiency, improper prostheses/dentures, burning mouth syndrome from plant toxins, drugs, allergic reactions, radiation therapy, and infection. Stomatitis is a type of ulcer that forms on the mucous membranes of the mouth. The most common type of ulcer is a minor aphthous ulcer (recurrent aphthous stomatitis). Aphthous ulcers are usually round or oval lesions that are yellow in color with a dirty bottom and a reddish outer border[6].

Anti-inflammatory drugs on the market have side effects that are more dangerous than herbal medicines. Cheap and natural inflammatory drugs can be obtained from the herbal medicine industry. Indonesia's biodiversity has a high potential to be used as an area for developing the herbal medicine industry. One of the

herbs that can be developed as an inexpensive anti-inflammatory drug is cinnamon (Cinnamommum sp). Cinnamon is a spice in the form of bark commonly used among Indonesian people. The use of cinnamon in medicine is as an anti-bacterial and painkiller. In dentistry, cinnamon serves to prevent the formation of dental plaque, cure canker sores, and prevent bad breath. The chemical content of cinnamon includes *cinnamaldehyde*, tannin, eugenol, and coumarin, which have health benefits, especially dental and oral health. One of the chemical constituents of cinnamon, namely *cinnamaldehyde*, is known to have anti-inflammatory effects, which significantly resembles Non-Steroidal Anti-Inflammatory Drugs[7]. Research showed that *cinnamaldehyde* has antioxidant, anti-inflammatory, and antiproliferative effects. By utilizing *cinnamaldehyde* with varying concentrations, the research found that at high concentrations, *cinnamaldehyde* has an irritating effect on tissues, while at low concentrations, *cinnamaldehyde* can inhibit the secretion of IL-1β and TNF-α[8].

Cinnamaldehyde is a low molecular weight compound, so it is easily soluble upon application. Saliva can dissolve cinnamaldehyde in the oral cavity, and its release into the body system cannot be controlled. This (menunjuk pada subyek yang mana?) causes the effects of *cinnamaldehyde* will not be seen even in large doses. There needs to be a carrier capable of binding the *cinnamaldehyde* compound so that the release of this compound in the body can be controlled. Currently, a carrier material that uses natural polymer materials has been developed to control the half-life of the active substance used. The natural polymer material that is often used is gelatin. The advantage of using gelatin is that it is easily biodegradable in tissues, making it suitable for use as a carrier[9].

The gelatin hydrogel system is part of the Drug Delivery System (DDS) method because the release of the active substance in it can be controlled. The concept of the Drug Delivery System used is to extend the half-life of a substance/compound by

controlling the release through the degradation of the carrier material, namely gelatin. *Cinnamaldehyde* inserted in the hydrogel can control the release of the active substance and the degradation of the hydrogel to increase the anti-inflammatory effect of *cinnamaldehyde*[10].

SUBJECT AND METHODS

The research subjects were 30 male Wistar rats aged 12 weeks weighing ± 180-200 grams. Each rat will be given the application of glacial acetic acid on the buccal mucosa for 20 seconds once a day. These rats were divided into five groups. The first group consisted of 3 control rats without treatment, the second group treated with hydrogel membrane without active substances, the third group treated with 1% *cinnamaldehyde* hydrogel application, the fourth group given 4% *cinnamaldehyde* application treatment, and the fifth group treated with diclofenac-potassium hydrogel application as a positive control. Each group was observed on days 3 and 6 post-treatment.

Gelatin hydrogel material is made in a gelatin system to obtain a composite derived from natural collagen. The gelatin used is Gelatin from Bovine Skin powder (Nitta). The hydrogel membrane was cut to 5x5 mm and then immersed in 1% and 4% *cinnamaldehyde* and 1% diclofenac potassium.

To create ulcers on the lip mucosa of Wistar rats is done by sticking filter paper measuring 2 x 2 mm containing 100% acetic acid for 20 seconds[11]. Ulcer formation is characterized by a change in the color of the mucosa to white. Lip ulcers were formed two days after acid exposure then each Wistar rat was given a hydrogel membrane application according to the treatment group. Three rats in each group were sacrificed randomly on days 3 and 6, then continued with making histological preparations on the area of the labial mucosa covered with ulcers with HE dye (Hematoxylin Eosin). Observation of the number of macrophage cells was carried out using a trinocular light microscope at 1000x magnification.

RESULTS

The results of the macrophage cell count in the form of the average number of cells and standard deviation in each day and treatment

group were tabulated in **Table 1** and a bar chart (**Figure 1**) as below.

Table 1. The Average and Standard Deviation of Macrophage Number in Each Group (DSMO)**.**

Group	Day	Mean±Standard Deviation
Membran-*cinnamaldehyde* 1%	3	5.85±1.41
	6	7.45±1.78
Membran-*cinnamaldehyde* 4%	3	5.00±1.22
	6	6.30±2.05
Membran-Kalium diklofenak 1%	3	5.96±1.94
	6	7.19±1.69
Membran-DMSO	3	6.30±2.28
	6	5.02±2.33
DMSO tetes	3	7.07±1.08
	6	6.23±1.09

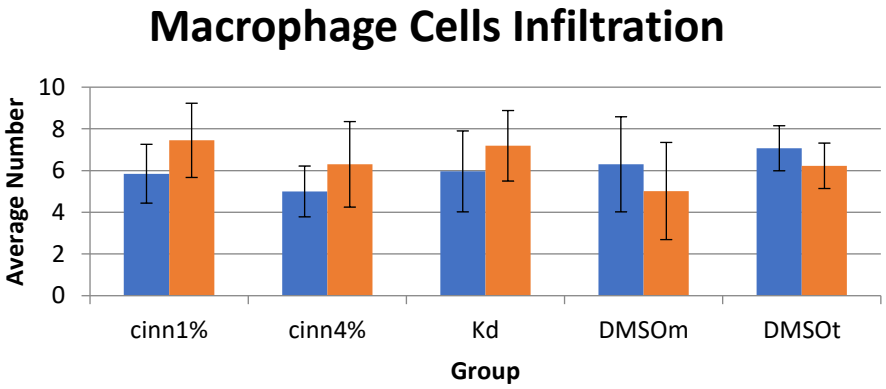

Figure 1. Average Number Of Macrophage Cells in Various Treatment Groups and Days (Cinn 1%= membrane-cinnamaldehyde 1%, Cinn 4%=membrane-cinnamaldehyde 4%, Kd= potassium diclofenac- membrane, DMSOm=membrane-DMSO group, DMSOt= DMSO drip)

Table 1 and **Figure 1** show that on day 3, the macrophage cells in the drip DMSO group (control group) were the highest compared to the 1% membrane-*cinnamaldehyde*, 4% membrane-*cinnamaldehyde*, diclofenac potassium-membrane, and membrane-DMSO groups. On day 6,

the macrophage cells in the 1% membrane-*cinnamaldehyde* group were the highest compared to 4% membrane-*cinnamaldehyde*, 1% membrane-K-diclofenac, membrane-DMSO, and drip DMSO. The results of histological observations of oral mucosal ulcers of Wistar rats in various treatments and days can be seen in **Figure 2**.

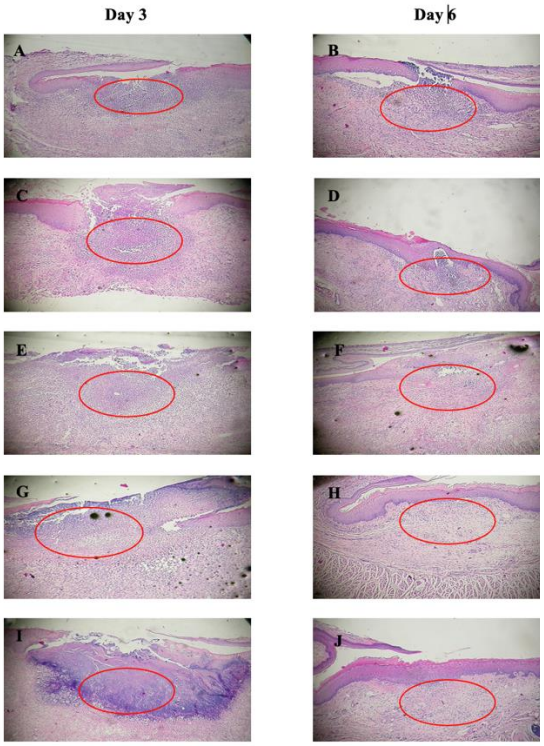

Figure 2. Infiltration of inflammatory cells in labial mucosal ulcers of Wistar rats with HE staining is indicated by a red circle (40x magnification). A-B) 1% *cinnamaldehyde*-membrane. C-D) membrane-*cinnamaldehyde* 4%. E-F) membrane-K-diclofenac 1%. G-H) membrane-DMSO. I-J) DMSO drops

The results of the 2-way ANOVA test showed that the number of macrophage cells was significantly different in various treatment variables, among *cinnamaldehyde* 4%, *cinnamaldehyde* 1%, K-diclofenac, membrane-DMSO, and DMSO drops groups($p<0, 05$). The number of macrophage cells did not differ significantly on the different variables on the day of observation, on day three, and day six ($p>0.05$). The interaction of treatment and day of observation on the number of macrophage cells was significantly different ($p<0.05$). After that, the post hoc LSD test was carried out to see significant differences between groups in the number of macrophage cells.

On day 3, there was a significant difference ($p<0.05$) between the 1% membrane-*cinnamaldehyde* group and the drip DMSO group, between the 4% membrane-*cinnamaldehyde* group and the membrane-DMSO group, between 4% membrane-*cinnamaldehyde* group with drops DMSO group.

Meanwhile, on day six, there was a significant difference ($p<0.05$) between the 1% membrane-*cinnamaldehyde* group and the 4% membrane-*cinnamaldehyde* group, between the 1% membrane-*cinnamaldehyde* group and the membrane-DMSO group, between the membrane-*cinnamaldehyde* group. 1% with drip DMSO group, between membrane-*cinnamaldehyde* group 4% and membrane-DMSO group, between the membrane-K-diclofenac group and membrane-DMSO group, and between membrane-DMSO group and drip DMSO group.

In the treatment and day interactions, there was a significant difference ($p<0.05$) between the 1% membrane-*cinnamaldehyde* group on day three and the 1% membrane-*cinnamaldehyde* group on day 6, between the membrane-*cinnamaldehyde* 4% group on day three and the membrane-*cinnamaldehyde* 4% group on day 6, and between the membrane-DMSO group on day three and the membrane-DMSO group on day six.

DISCUSSION

When the injury occurs, macrophages as non-specific defense cells react by phagocytosis, producing bactericidal substances, namely reactive oxygen species (ROS), TNF- and IL-1β cytokines that can recruit other inflammatory cells from the blood to tissues[12]. The number of macrophage cells peaked on day three and began to decrease on day seven because there was a process of emigration to the surrounding lymphatic tissue and apoptosis[13]. Reducing the excessive number of macrophage cells is an important process in healing inflammation because it can reduce the secretion of cytokines TNF-α and IL-1β. High concentrations of TNF can cause pathological effects, such as tissue necrosis, stimulate prostaglandins production, and lead to disease severity[14]. Thus, inhibiting the cytokines TNF- and IL-1β is the key to controlling inflammation.

This study showed significant differences in the number of macrophage cells of the labial mucosal tissue of Wistar rats in various treatments. The number of labial ulcer macrophages of Wistar rats treated with 1% and 4% *cinnamaldehyde* membrane was significantly different from the number of macrophage cells in the

control group (DMSO drops). In the 4% *cinnamaldehyde* group, the number of macrophages was the lowest compared to the other groups, while the highest number of macrophages was in DMSO/ control group). Previous studies demonstrated that *cinammaldehyde* were able to inhibit cytokines produced by macrophages, TNF-α and IL-1β[15]. The amount of TNF influences the number of macrophage cells- and IL-1β cytokines secreted by macrophage cells. If secretion of these cytokines is inhibited, macrophage cells are recruited into the tissue are also less and less.

On day 3, the number of macrophage cells in labial ulcers of Wistar rats treated with 1% membrane-K-diclofenac application and 1% and 4% membrane-*cinnamaldehyde* on day 3 was not significantly different. The day 3 result means the anti-inflammatory effectiveness of 1% and 4% *cinnamaldehyde*-membrane is equivalent to 1% diclofenac-potassium membrane. Another previous study also suggested that *Cinnamomum burmanii*, most of which the main content is *cinnamaldehyde,* has the highest anti-inflammatory activity among all types of herbal plants which is equivalent to Non-Steroidal Anti-Inflammatory Drugs[16].

On day six, the number of macrophage cells was significantly different between treatments. The number of macrophage cells in the labial mucosal ulcer of Wistar rats treated with 1% *cinnamaldehyde*-membrane was significantly different from the number of macrophage cells in the DMSO drops group (control). The highest number of macrophage cells was precisely in the 1% *cinnamaldehyde*-membrane treatment compared to the other four treatments. The number of macrophage cells 4% *cinnamaldehyde*-membrane group was significantly higher than the membrane-DMSO group. This result was presumably because prolonged contact of *cinnamaldehyde* on the mucosa (48 hours) can be irritating and cause a burning sensation and inflammation.

In this study, exposure to 1% and 4% *cinnamaldehyde* for five days was suspected to cause the increased number of macrophages in the labial

ulcers of Wistar rats observed on day 6. Another study states that the lowest concentration of *cinnamaldehyde* that cells can tolerate is about 3%. Irritation of low intensity but persistent over a long time, can lead to chronic inflammation. The result from these studies further strengthens the notion that *cinnamaldehyde* concentrations of 1% and 4%, which are applied for a long time, can increase the chronic inflammatory process characterized by excessive accumulation of macrophages. The use of *cinnamaldehyde* that does not irritate mucous membranes is less than 0.125% and within 24 hours[17].

CONCLUSION

Membrane-*cinnamaldehyde* 1% and 4% can significantly reduce the number of macrophage cells in labial ulcers of Wistar rats on day 3. The number of macrophage cells in the membrane-*cinnamaldehyde* 4% was less than the number of macrophage cells in the membrane-*cinnamaldehyde* 1%

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