

RESEARCH ARTICLE

Effect of Topical Noni (*Morinda citrifolia* L.) Leaf Extract Paste in Carrageenan-induced Paw Edema on Wistar Rats

Indah Puti Rahmayani Sabirin,¹ Euis Reni Yuslianti²¹Department of Oral Biology, ²Department of Biochemistry and Molecular Biology, Faculty of Medicine, Universitas Jenderal Achmad Yani, Cimahi, Indonesia

Abstract

The inflammatory response is one of natural process in the body to protect itself following tissue injury, but it can cause discomfort. Noni (*Morinda citrifolia* L.) leaf known as a traditional medicament to help reduce the inflammatory effect. The leaves empirically applied as a wrapping on fever or wound. The purpose of this study was to identify the anti-inflammatory effect of topical noni leaf extract paste in 5% and 10% concentration by examination of Wistar rat paw edema induced by λ -carrageenan. Twenty-four Wistar rats divided into four groups, which were negative control, positive control with diclofenac sodium 1% gel, 5% noni leaf, and 10% noni leaf paste groups. Paw edema was induced by intraplantar injection of 1% λ -carrageenan to every rat. Every treatment subsequently applied in the plantar area before injection, and the changed paw volume measured with plethysmometer at minutes 0, 30, 60, and 90. This study was at the Animal Laboratory, Faculty of Medicine, Universitas Jenderal Achmad Yani, Cimahi city in October–December 2017. The result displayed that the minimum volume after 90 minutes was on 5% and 10% noni leaf paste group, which is 1.00 mL. Kruskal-Wallis test result of inflammatory percentage was significantly different among every group in each examination time ($p < 0.05$). Post-hoc test showed that inflammatory reduction on paw edema with noni leaf paste application on both concentrations were significantly different compared to the negative control. However, it was not different from the positive control group. This study showed that application of noni leaf paste in 5% and 10% concentration could help reduce inflammatory response on skin possibly by the active anti-inflammatory ingredients of noni leaf.

Key words: Inflammatory response, noni leaf, paste formula, paw edema

Efek Pasta Ekstrak Daun Mengkudu (*Morinda citrifolia* L.) Topikal terhadap Edema Kaki Tikus Galur Wistar yang Diinduksi Karagenan

Abstrak

Inflamasi adalah proses alami tubuh untuk melindunginya setelah cedera, namun hal tersebut dapat menyebabkan ketidaknyamanan. Daun mengkudu (*Morinda citrifolia* L.) dikenal sebagai obat tradisional untuk menurunkan efek inflamasi yang secara empiris digunakan untuk mengobati demam dan luka. Tujuan penelitian ini adalah mengetahui efek anti-inflamasi pasta ekstrak daun mengkudu melalui pemeriksaan edema kaki tikus yang diinduksi karagenan- λ . Dua puluh empat tikus galur Wistar dibagi menjadi 4 kelompok, yaitu kontrol negatif dengan akuades, kontrol positif (aplikasi gel Na diklofenak 1%), serta perlakuan pasta daun mengkudu 5% dan 10%. Edema dibuat dengan menginjeksi intraplantar tikus dengan 1% karagenan- λ pada tiap kelompok. Tiap-tiap perlakuan diaplikasikan sebelum tikus diinjeksi dan perubahan volume kaki tikus diukur dengan plethysmometer di menit ke-0, 30, 60, dan 90. Penelitian ini dilakukan di Laboratorium Hewan, Fakultas Kedokteran, Universitas Jenderal Achmad Yani, Kota Cimahi pada Oktober–Desember 2017. Hasil pengukuran memperlihatkan penurunan volume edema kaki terkecil setelah 90 menit pada kelompok pasta daun mengkudu 10%, yaitu 1,00 mL. Hasil Uji Kruskal-Wallis terhadap persentase inflamasi berbeda nyata pada tiap kelompok dan tiap waktu pengamatan ($p < 0,05$). Hasil uji beda menunjukkan bahwa penurunan inflamasi kaki tikus pada perlakuan pasta daun mengkudu kedua konsentrasi berbeda nyata dibanding dengan kontrol negatif, tetapi tidak berbeda dengan kontrol positif. Penelitian ini menunjukkan bahwa pasta daun mengkudu 5% dan 10% dapat membantu menurunkan reaksi inflamasi kulit dan efeknya sejalan dengan Na diklofenak karena zat aktif yang bersifat anti-inflamasi dalam daun mengkudu.

Kata kunci: Daun mengkudu, edema kaki tikus, formula pasta, respons inflamasi

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Correspondence: Indah Puti Rahmayani Sabirin, drg., M.Kes. Department of Oral Biology, Faculty of Medicine, Universitas Jenderal Achmad Yani. Jln. Terusan Jenderal Sudirman No. 1, Cimahi 40533, West Java, Indonesia. Phone: (+6222) 6642781. Mobile: +6285624329427. E-mail: sabirinputi@gmail.com

Introduction

Inflammation is a natural response of the tissue once interact with the source of injuries such as a microorganism, foreign bodies, or physical trauma. Acute and chronic inflammation are two general types of response. Clinically, five cardinal signs of acute inflammation related to inflammatory response pathophysiology, are redness, swelling or edema, fever or warmness, pain, and loss of function. These signs related to the vascular and cellular reaction following the interaction between tissue and injury cause. One of the primary purposes of the inflammatory response is to localize the injury and avoid spreading of microbial agents. The clinical reaction of this response can cause pain, discomfort, or disability. To reduce these unwanted effects, we commonly use anti-inflammatory medication.¹⁻³

Indonesia is a tropical country where using herbal medicine that considered one of its tradition and an essential part of the culture. Noni (*Morinda citrifolia* L.) is a plant grown in any part of Indonesia. Its leaf empirically used for healing wounds and boils by crushing, heating, spreading as well as strapping it to the wound area. Many active constituents in noni leaves responsible as antibacterial, anti-inflammatory, antioxidant, and astringent.⁴⁻⁷

The acute inflammatory process begins with releasing mediators triggered by trauma or invasion of foreign material to the body. Edema is one of the signs of the inflammatory response which would be visible in a few minutes. It will follow by short vasodilation and vasoconstriction of the affected area and decreases when inflammatory reaction ceased. The mediators washed by recovering capillary blood flow.^{1,7}

Previous research showed that topical application of noni leaves in dry and ethanol extract could also promote healing in Wistar rat skin and oral mucosa wound. Through analysis of fibroblast cell count, the diameter of wound reduction, new capillary formation in connective tissue, and inflammatory cells examination. Based on our previous research, the concentration of noni leaf ethanol extract, which was effective on skin and oral mucosa wound healing was in 10% concentration. The paste formula resulted in the most significant wound closure condition as well as inflammatory cells in the wound healing process compared to control was 5% and followed by 10% concentration.⁸⁻¹¹

This research was performed to examine the effect of topical noni leaf ethanol extract in 5% and 10% paste to an inflammatory response in rat plantar area edema induced by λ -carrageenan.¹² Wistar rats used as connective tissue and skin inflammation model in vivo. In this study, ethanol extract of noni leaves formulated into a topical paste. The purpose of the studies eventually is to create topical medication for oral mucosa wound with low toxicity and in the practically better formula to encourage wound healing.

Methods

The research was carried on from October to December 2017 at Animal Laboratory of Faculty of Medicine, Universitas Jenderal Achmad Yani, Cimahi city. Twenty-four Wistar rats (*Rattus norvegicus*) were used as the inflammation model after received ethical clearance from the Health Research Ethics Committee of Dr. Hasan Sadikin General Hospital, Bandung city (No. 924/UN6.C1.3.2/KEPK/PN/2016). The noni leaves were also obtained from Cimahi city and processed as a paste at Pharmacy Laboratory of Faculty of Science, Universitas Jenderal Achmad Yani, Cimahi city.

Twenty-four Wistar rats were used in this study to represent edema as a clinical inflammatory reaction. Before the treatment, all rat paws measured with plethysmometer for initial tissue volume (V_0). Afterward, each topical treatment (distilled water, noni leaf paste, and diclofenac sodium) applied according to the groups. Paw edema induced by intraplantar injection of 0.1 mL λ -carrageenan 1% in left rear paw. Measurement of paw volume (V_t) was with plethysmometer after every treatment. Carrageenan induction carried out every 30, 60, and 90 minutes.¹²⁻¹⁴ The percentage of inflammation measured by the formula, in which, V_0 was tissue volume right after induction, and V_t was paw volume after induction.

$$\frac{V_t - V_0}{V_0} \times 100\% = \text{percentage of inflammation}$$

Maceration method used to acquire noni leaves ethanolic extract, which then processed into paste formula. Five kilograms of leaves dried to obtain 1 kg of semisolid leaves. They subsequently processed into powder, which was ready to be made ethanolic extract using 96% of ethanol. The powder was put into a macerator

and left for 24 hours, so all the powder become liquid extract. Next, rotary evaporator used to evaporate the liquid to thicken the extract. The result is a thick dark green ingredient of pure ethanolic extract of noni leaves. Noni leaves paste made with the pure ethanolic extract. The extract mixed using a spatula with a paste based formula. The base consists of 20% gelatine, 15% ZnO₂ paste, 2.5 mg of glycerine, 0.5% Sodium benzoate, and water.¹⁵ To make the paste in 5% and 10% concentration, we combined 100 grams base as explained above with 5 grams and 10 grams, subsequently, of pure ethanolic extract of noni leaf. The paste obtained had light-greenish hue with semisolid consistency, which was thicker and hydrophobic compared to gel formula.

Inflammatory cells infiltration count of all groups analyzed using the Kruskal-Wallis test for nonparametric test. The data were not normally distributed according to the normality test result using the Shapiro-Wilk test ($p > 0.05$).¹⁶ Statistical analysis was using a confidence interval of 95% ($p < 0.05$)—the data analysis performed with SPSS

software for Windows.

Results

Examination in 30, 60, and 90 minutes showed that inflammatory response marked by paw edema induction on 5% paste and 10% paste treatment group was reduced quicker compared by the control group. Observation in minute 30 and 60 after carrageenan induction indicated that inflammatory response reduction was better among both noni leaf paste treatment group. However, after 90 minutes of observation, the positive control group with 1% diclofenac sodium also demonstrated a decrement of inflammatory percentage. The lowest inflammatory percentage reduction appeared to be in 10% noni paste group in minute 90. Examination of rat paw edema with plethysmometer showed that on the group with 10% noni leaf paste treatment, the difference between normal paw volume and after 90 minutes of induction was the lowest (Table 1).

Kruskal-Wallis test performed on the data

Table 1 Mean Paw Edema Volume (mL) in Every Observed Minute After Induction with λ -carrageenan

Groups	Normal Volume	30'	60'	90'
Negative control	0.71	1.36	1.36	1.36
Positive control	0.70	1.15	1.26	1.03
5% noni leaf paste treatment	0.76	1.05	1.05	1.01
10% noni leaf paste treatment	0.73	1.10	1.10	1.00

Table 2 Inflammatory Percentage in Minute 30, 60, and 90

Inflammatory Percentage After λ -carrageenan Induction	Mean Rank	p Value*
Minute 30	Negative control	0.000
	Positive control	
	5% paste treatment	
	10% paste treatment	
Minute 60	Negative control	0.002
	Positive control	
	5% paste treatment	
	10% paste treatment	
Minute 90	Negative control	0.001
	Positive control	
	5% paste treatment	
	10% paste treatment	

*Kruskal-Wallis test, $p < 0.05$ =significant



Figure 1 Noni Paste Application on Rat Paw Along with Carrageenan Induction



Figure 2 Measurement of Paw Volume with Plethysmometer

showed that the reduction of inflammatory percentage on every group had a significant difference ($p < 0.05$). On the other hand, Mann-Whitney post hoc test was significantly different only between both negative and positive control groups with both treatment groups on every observation time, but it was not significant among 5% and 10% paste treatment group.

Discussion

Inflammation is a response of micro vascularized body tissue toward the source of injury. This response, including its vascular and cellular responses, serves as a protector from advanced damage from injuries in the body. The inflammatory response classified as acute and

chronic inflammation, which differs from their vascular reactions, cells involved, duration, and occurrence of fibrosis. The acute inflammatory process generally involves mediators. They had a part in vasodilation and increased vascular permeability such as nitric oxide, prostaglandin, histamine, bradykinin, C3a, C5a, and oxygen metabolites. Edema is one of the clinical signs of the acute inflammatory response used as a marker for studies about factors that influenced the inflammatory process. Edema caused by λ -carrageenan induction observed as soon as the

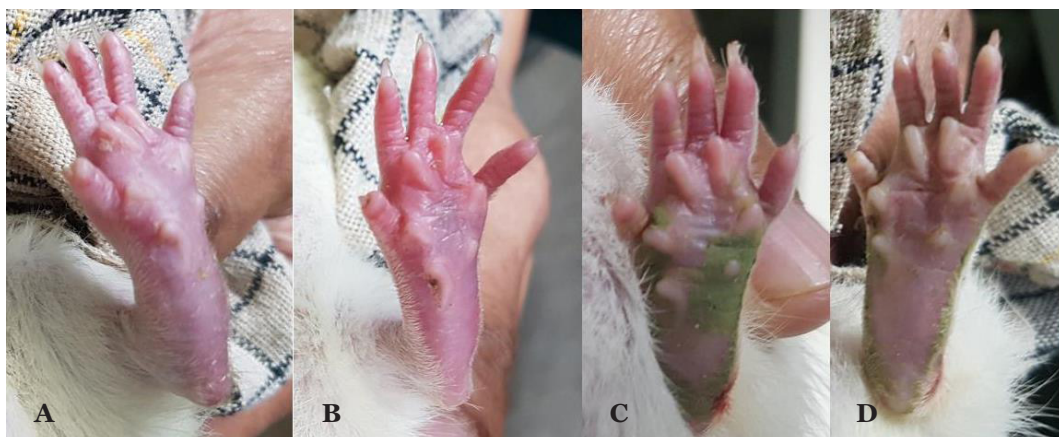


Figure 3 Observation of Rat Paw on Minute 90

(A) Negative control; (B) Positive control; (C) Treatment with 5% noni leaf paste; (D) Treatment with 10% paste

tissue injected.^{1,7,12}

Topical preparation is one form of medication generally used in reducing pain or acted as a barrier for the affected area in the skin or mucosa. Anti-inflammatory topical medication, such as diclofenac sodium that used in this study, generally given to alleviate discomfort caused by the inflammatory reaction around the skin or joint. This preparation, as well as the oral administered ones, acted by inhibiting the work of cyclooxygenase enzyme so that the prostaglandin would not be synthesized from arachidonic acid, and also by restraining leucocyte adhesion. The effects caused by thromboxane and prostaglandins supposed to be synthesized, like pain and tissue damage, will also be repressed. Unfortunately, these anti-inflammatory drugs can also cause side effects such as hypersensitivity, GIT disturbance, and vascular disorder which can be life-threatening.^{1,3,17}

The use of herbal medicine had been very popular in countries which had a large variety of plants. Studies of herbal medicine are necessary to create an extract that is proven to be beneficial, more practical to use, and possibly has lower side effects. Noni plant (*Morinda citrifolia* L.) is a tropical plant which is very common in Indonesia. The plant can grow in any location since it was relatively durable against high temperature and weather and does not depend on special treatment in a plantation. It commonly grows as a garden tree in towns, although it also found in forests. In Indonesia, many parts of the plant such as fruit and leaves, believed to have the benefit to cure some illness and empirically used as traditional medicine.^{4,7,18}

The authors had accomplished some previous studies using ethanol extract of noni leaf. The outcome showed that the topical extract gel formula could help skin and oral mucosa wound to heal faster compared to povidone-iodine solution. The result is from examining wound closure time, CD34 count, acute inflammatory infiltration, and fibroblast cell count on Wistar rats. Topical paste formula of noni leaves ethanolic extract also may accelerate oral mucosa wound healing compared to gel formula. The result is through an examination of wound width, histological appearance of connective tissue, and tissue MDA level on the remodeling phase. The 5% and 10% concentration paste used in this study because our previous study showed that in the 14th day of wound healing, the clinical wound closure was the most significant, compared to 2.5% and 20%

concentration. Other previous studies by Palu,¹⁹ Nayak et al.,⁵ and Rasal et al.⁶ showed that topical oral administration of ethanol extract of noni was effective on optimal skin wound healing. It also can help mitigate skin erythema induced by UV-B exposure according to the study by West.²⁰ Antibacterial properties of noni leaves extract against *Staphylococcus aureus*, which related to wound healing as well, had also studied by Kumar et al.²¹ in 2010. In vitro study of noni leaf extract on macrophage by Saraphanchotiwithaya and Sripalakit²² showed that it had an inhibitory effect to TNF- α compared to dexamethasone and indomethacin.^{9-11,19-22}

Noni leaf possesses active constituents, such as flavonoid, xeronine, alkaloids, saponin, tannin, scopoletin, triterpenoids, anthraquinone, iridoid glycosides, and ascorbic acid. Many of it worked on improving the wound healing process, through antioxidant, antibacterial, and anti-inflammatory activity. Studies showed active anti-inflammatory constituent in noni leaf which effects were alkaloids, tannin, xeronine flavonoid iridoid glycosides, and scopoletin. The mechanism of the constituents was to inhibit inflammatory enzyme production such as COX, and matrix metalloproteinases, as well as cytokines.¹⁸⁻²⁴ Inflammatory percentage examined indicated that topical noni leaf paste used, either in 5% or 10% concentration, had a better anti-inflammatory effect compared to the negative control. It had a similar effect with diclofenac sodium topical gel as a positive control, mainly in the 90 minutes of observation. The result means one of the chemical composition on the noni paste extract reducing the acute inflammatory reaction. The reaction would occur in prolonged time if no medication applied to the edema.

There was a significant difference between the treatment group and negative control group, but there was not any difference between the two treatment groups with noni leaf paste. It suggested that a 5% deviation in the concentration of extract did not influence the anti-inflammatory effect of the noni leaf paste. The result of this study was following our previous studies. 5% noni leaf ethanolic extract paste concentration improved clinical wound closure on the oral mucosa. It improved wound healing by histopathologic examination of fibroblast and inflammatory cells findings.^{9,11,25} According to the findings, we considered improving this study by observing the side effects and a more prolonged period of examination.

Conclusions

This study about the effect of noni leaf in 5% and 10% extract in paste formula on inflammatory response marked by paw edema indicated that it could reduce inflammatory response similar to the effect of diclofenac sodium 1% topical gel as a standard prescription for inflammatory reaction externally. The result which was statistically significant and yet better than negative control, could be a base for further research on inflammatory response, for example by measuring inflammatory rate with other methods in vivo, try other dosage possible according to previous studies, examination of side effects, as well as considering the study on other form of injury such as burns or vesicles.

Conflict of Interest

There is no any conflict of interest in this study nor in the information stated in this article.

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