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Research Article

Anti-inflammatory Activity of Ethanol Extract of Lotus (*Nelumbo nucifera* G.) Seed Against White Male Rats Using Paw Edema Method

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Article Info:



Article History:

Received 11 May 2021
Review Completed 17 June 2021
Accepted 22 June 2021
Available online 15 July 2021

Cite this article as:

Fitri K, Khairani TN, Sianturi KT, Leny, Hafiz I, Anti-inflammatory Activity of Ethanol Extract of Lotus (*Nelumbo nucifera* G.) Seed Against White Male Rats Using Paw Edema Method, Journal of Drug Delivery and Therapeutics. 2021; 11(4):1-4

DOI: <http://dx.doi.org/10.22270/jddt.v11i4.4918>

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Abstract

The purpose of this study was to determine the anti-inflammatory activity test of ethanol extract of lotus seeds in rats induced by carrageenan. The research method used was experimental. The object used in this study was male white rats. Observations were made for 6 hours and then the data were analyzed using the one way analysis of variance (ANOVA) test. The anti-inflammatory activity test was carried out on the test animals which were divided into 5 groups, 3 each with the suspension test group with a dose of 200, 300, 400 mg/kg bw, the negative control group was the CMC Na 1% suspension and the positive control was diclofenac Na 2, 25 mg/kg bw. The results showed that the ethanol extract of the seeds of lotus (*Nelumbo nucifera* G) was proven to have an anti-inflammatory effect on carrageenan-induced male white rats at doses of 200, 300 and 400 mg/kg BW with the percentage value of inflammation inhibition at the 6th hour was 65.79, 74.65 and 80.77%, with the most effective dose in reducing anti-inflammatory drugs being 400 mg/kg bw. The ethanol extract of the seeds of lotus (*Nelumbo nucifera*) has anti-inflammatory activity.

Keywords: anti-inflammatory activity, *Nelumbo nucifera*, paw edema

INTRODUCTION

Indonesia is a country with a tropical climate which has quite high biological resource wealth. Plant diversity in Indonesia is estimated to be around 90,000 species with 9,600 identified plant species used as medicinal plants and only a small proportion has been scientifically researched ^{1,2}. Traditional medicine is one of the tangible forms of exploiting these biological resources. One of the plants that are widely known and found in Indonesia is Lotus. Traditionally, the lotus plant is used as a medicinal ingredient. In traditional medicine, all parts of the lotus plant can be used from the roots to the single use flowers or in combination with other traditional medicinal ingredients ³⁻⁵.

Lotus (*Nelumbo nucifera*) or what is known in some lotus literature contains active ingredients such as flavonoids, tannins, saponins, and lignins. Flavonoids are substances that can inhibit the inflammatory process. Flavonoids are able to protect membrane lipids against damaging reductions ⁶⁻⁸. Flavonoids can also inhibit the release of inflammatory mediators such as histamine and prostaglandins which exert anti-inflammatory activity in rats ⁹⁻¹¹. Based on this, an anti-inflammatory activity test was carried out to see the potential of this plant in suppressing inflammation.

This article is a publication of the results of the anti-inflammatory activity test of the ethanolic extract of *Nelumbo nucifera* seeds using the paw edema method on rats. This

study obtained data on inflammation, percent inflammation and percent inhibition of inflammation in the feet of rats. With these data obtained, it will be known the activity and dose of the extract in inhibiting inflammation.

MATERIALS AND METHODS

Plants, Tools and Chemicals

Lotus seeds obtained from Pasar V Marelan, Medan, Indonesia, are then carried out in the first determination at the Faculty of Mathematics and Natural Sciences, University of North Sumatra. After that, 3 kg of seeds are taken, sorted, cleaned and dried at a temperature of 15-30° C.

The tools used in this study were animal cages, rat drinking containers, digital scales, Erlenmeyer, measuring cups, oral gavage, syringes, platsmometers, filter paper, stopwatches, gloves, masks, vacuum rotary evaporators, porcelain cups, blenders. The plant used in the study were lotus seeds (*Nelumbo nucifera*). The chemicals used were ethanol, aquadest, carrageenan, diclofenac sodium tablets, Na CMC, and the tested animal used was male rats.

Plant Extraction

The fine simplicia powder is put into a maceration vessel, then immersed in 96% ethanol solvent. This soaking is carried out for 5 days. Stirring is done so that the solvent is immersed in all the simplicia powder. After 5 days, the soaking results are then filtered using filter paper.

Furthermore, the filtrate is taken and accommodated. The residue of the leaves are macerated again, for 2 days. The filtrate is then collected and concentrated with a rotary evaporator at a temperature of 45°C so that a thick extract is obtained ^{12,13}.

Formulation of Sample Suspension

Extract samples were made in suspension dosage form. 1% Na CMC suspension was used as a negative control as well as the basis for the suspension of the entire sample ¹⁴. Lotus seed extract is formulated in the form of a suspension which can then be given to the test animals at doses of 200, 300 and 400 mg/kg bw.

As a positive control, 20 tablets of Diclofenac sodium were taken, crushed and then weighed. The active ingredient of diclofenac sodium in 20 tablets of diclofenac sodium is 25 mg. The total active ingredient in 20 tablets is 500 mg. Weigh the tablet equivalent of 2.25 mg/kg, then put it in a mortar and add 1% w/v Na-CMC suspension gradually while grinding until homogeneous, the volume is sufficient to 10 ml.

Measurement of Anti-Inflammatory Activity

The test animals used were male white rats weighing 180-250 g which were adapted for 1 week for the acclimation process. During this process, rats are maintained so that their food and drinking needs can be fulfilled. The rats were

fasted for 8 hours before treatment but water was still given ¹⁴.

Before testing, the rats were weighed and marked, then each rat was measured the initial volume (Vo) of the feet. After that each rat was induced by 0.1 ml of carrageenan intraplantar, after 30 minutes each rat was given a suspension of ethanol extract of lotus seeds based on the dose of each rat. were given 1% Na CMC, group 2 were given diclofenac sodium solution orally and groups 3,4 and 5 were given suspension of lotus seed extract at a dose of 200, 300 and 400 mg/kg bw. at 1, 2, 3, 4, 5, and 6 hours. The anti-inflammatory activity given by a drug/test preparation is expressed as the percentage of inflammation. The data obtained from the five groups were analyzed by one way analysis of variance (ANOVA) ¹⁵.

RESULT AND DISCUSSION

The measurement of λ -carrageenan-induced rat foot edema volume was carried out at intervals of 1 hour for 6 hours. The change in the volume of the rats' feet can indicate the amount of inflammation that occurs in the soles of the rats' feet caused by λ -carrageenan. The results of the measurement of edema values can be seen in Table 1. The percent inflammation results obtained were statistically tested to see the differences between each treatment group. The results of the calculation of the percentage of inflammation in each group can be seen in Table 2, and the percentage of inflammation inhibition in Figure 1.

Table 1: Edema values measurement

No	Groups	Volume (ml) \pm SD						
		0 hour	1 hour	2 hours	3 hours	4 hours	5 hours	6 hours
1	Negative control	4.94 \pm 0.20 [#]	5.42 \pm 0.18 [#]	5.76 \pm 0.18 [#]	6.19 \pm 0.28 [#]	6.57 \pm 0.15	6.95 \pm 0.22	6.81 \pm 0.34
2	Positive control	4.63 \pm 0.25	4.89 \pm 0.34	5.20 \pm 0.27	5.52 \pm 0.32	5.34 \pm 0.34 [*]	5.08 \pm 0.27 [*]	4.76 \pm 0.18 [*]
3	EEN 200 mg/kg	4.26 \pm 0.25 [#]	4.61 \pm 0.29 [#]	4.91 \pm 0.38 [#]	5.21 \pm 0.32 [#]	5.22 \pm 0.32 ^{##}	5.00 \pm 0.65 ^{##}	4.81 \pm 0.68 ^{##}
4	EEN 300 mg/kg	4.91 \pm 0.59 [#]	5.25 \pm 0.58 [#]	5.61 \pm 0.66 [#]	5.96 \pm 0.67 [#]	5.89 \pm 0.67 [#]	5.65 \pm 0.51 ^{##}	5.38 \pm 0.68 ^{##}
5	EEN 400 mg/kg	5.17 \pm 0.42 [#]	5.50 \pm 0.46 [#]	5.86 \pm 0.53 [#]	6.22 \pm 0.55 [#]	6.06 \pm 0.55 [#]	5.81 \pm 0.70 ^{##}	5.54 \pm 0.39 ^{##}

Explanation: EEN (Ethanol Extract of *Nelumbo nucifera*), # not significantly different from the positive control ($p>0.05$), * significantly different from negative control ($p<0.05$)

Table 2: The percentage of inflammation

No	Groups	Percentage of Inflammation (%) \pm SD					
		1 hour	2 hours	3 hours	4 hours	5 hours	6 hours
1	Negative control	9.82 \pm 1.81 [#]	16.71 \pm 2.02 [#]	25.29 \pm 1.04 [#]	33.13 \pm 1.47	40.72 \pm 1.29	37.76 \pm 1.70
2	Positive control	5.56 \pm 1.49	12.41 \pm 0.79	19.37 \pm 0.26	15.47 \pm 1.82 [*]	9.87 \pm 1.02 [*]	3.02 \pm 1.71 [*]
3	EEN 200 mg/kg	8.12 \pm 1.03 [#]	15.09 \pm 2.34 [#]	22.20 \pm 2.91 [#]	22.60 \pm 0.26 [*]	17.49 \pm 0.96 [*]	12.90 \pm 1.69 [*]
4	EEN 300 mg/kg	7.08 \pm 1.06 [#]	14.45 \pm 3.63 [#]	21.68 \pm 3.07 [#]	20.19 \pm 2.13 ^{##}	15.29 \pm 1.89 [*]	9.53 \pm 1.19 [*]
5	EEN 400 mg/kg	6.44 \pm 0.78 [#]	13.37 \pm 2.36 [#]	20.40 \pm 1.08 [#]	17.22 \pm 1.62 ^{##}	12.47 \pm 1.41 ^{##}	7.28 \pm 1.08 ^{##}

Explanation: EEN (Ethanol Extract of *Nelumbo nucifera*), # not significantly different from the positive control ($p>0.05$), * significantly different from negative control ($p<0.05$)

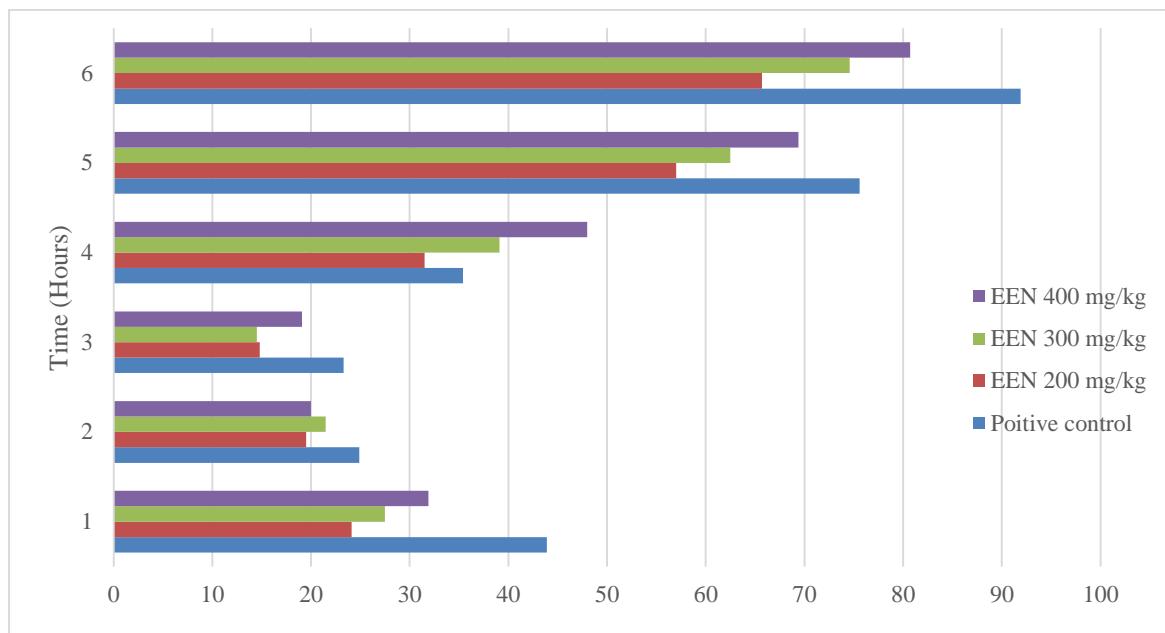


Figure 1: The percentage of edema inhibition

The anti-inflammatory effect is thought to be due to the activity of secondary metabolites contained in the ethanolic extract of seroja seeds, namely flavonoids, saponins, and tannins. One of the secondary metabolites that are thought to have anti-inflammatory activity is flavonoids. The mechanism of action of flavonoids can be through several pathways by inhibiting the activity of cyclooxygenase (COX) and lipoxygenase. The mechanism of action of flavonoids in the process of inhibiting inflammation in two ways, namely inhibiting arachidonic acid and the secretion of lysosomal and endothelial enzymes so that proliferation and exudation of the inflammatory process. The inhibition of the release of arachidonic acid from inflammatory cells will lead to less availability of arachidonic substrate for the cyclooxygenase pathway and the lipoxygenase pathway. The mechanism of action of flavonoids can inhibit cyclooxygenase, so it is likely that the anti-inflammatory effect caused by cyclooxygenase inhibition is the first step of the pathway to eicosanoids such as prostaglandins and thromboxanes. Flavonoids show more than one hundred kinds of bioactivity. The bioactivity shown includes antipyretic, analgesic, and anti-inflammatory effects ¹⁶⁻¹⁸.

Tannins have antioxidant activity that acts as an anti-inflammatory in various ways, namely by inhibiting the production of oxidants (O₂) by neutrophils, monocytes and macrophages. Inhibiting the production of O₂ oxidants will reduce the formation of H₂O₂ which results in the production of hypochlorous acid (HOCl) and OH being inhibited. Directly inhibits the process of reactive oxidants such as hydroxy radicals (OH) and hypochlorous acid. The anti-inflammatory mechanism of saponins is by inhibiting the formation of exudate and inhibiting the increase in vascular permeability ¹⁹⁻²¹.

CONCLUSION

This study proved that the ethanolic extract of *Nelumbo nucifera* seeds had anti-inflammatory activity against carrageenan-induced rats at a dose of 200-400 mg/kgbw.

ACKNOWLEDGMENTS

Authors acknowledge the Research and Public Service Center or LPPM Institut Kesehatan Helvetia, Medan, Indonesia.

CONFLICT OF INTEREST

All authors have no to declare.

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