2ND Makassar International Conference on Pharmaceutical Sciences (MICPS) 2023
Jurnal Fitofarmaka Indonesia, 2023; 10(3) 95-100
http://jurnal.farmasi.umi.ac.id/index.php/fitofarmakaindo/index

Potential of Extract of *Tamarindus indica* L Leaves an Anti-Inflammatory on Carrageenan Induced Wistar Rats

Aulia Wati[1]*, Sukmawati Sukmawati[2], Bayu Putra[1]
[1]Laboratory of Biopharmaceutical Pharmacology, Faculty of Pharmacy, Universitas Muslim Indonesia, Indonesia
[2]Laboratory of Pharmaceutical Chemistry, Faculty of Pharmacy, Universitas Muslim Indonesia, Indonesia

Article info
History
Submission: 21-10-2023
Review: 19-11-2023
Accepted: 05-12-2023

***Email:**
aulia.wati@umi.ac.id

DOI: 10.33096/jffi.v10i3.1107

Keywords:
antiiflamation; Tamarindus indica L*; carrageenan*

Abstract
Tamarind leaves are used in traditional medicine for inflammation, stomach pain, rheumatism, and sore throats. The aims of this study was to determined the potential of extract tamarind leaf as an anti-inflammatory in Wistar rats. This study was used experimental rats divided into 5 groups, namely group I was given Na. CMC, group II was given diclofenac sodium dose of 5.136 mg/kgBW, groups III, IV, and V were given ethanol extract of tamarind leaves at a doses of 250, 500 and 1000 mg/kgBW. The test animals induced with 1% lambda carrageenan by intraplantar after one hour given test preparation orally. Measurements of edema volume and thickness of the rats were carried out before and after induction for 7 hours at 1 hour intervals using a pletysnometer and calipers. The research data was calculated statistically using the one way anova test and Post Hoc LSD. The results of the study showed that the extract group at a dose of 1000 mg/kgBW had an anti-inflammatory effect not significantly different compared to the diclofenac sodium group at a dose of 5.136 mg/kgBW. Therefore, obtained results showed that the extract of tamarind leaf has an anti-inflammatory effect with an effective dose of 1000 mg/kgBW.

I. Introduction

Tamarindus indica L is a type of tropical plant that grows in Indonesia. Tamarind leaf extract (*Tamarindus indica* L) contains active compounds in the form of flavonoids, tannins, glycosides and saponins. The content in tamarind leaves can provide benefits, especially for health. This tropical plant is used in traditional medicine for inflammation, stomach pain, rheumatism, and sore throats. Additionally, this plant is used in the treatment of wound healing, diarrhea, sore eyes, constipation and as an aphrodisiac (Mun'im, 2009; Komakech, 2019).

The inflammatory response, or inflammation, is a physiologic response to tissue injury and infection. The inflammatory response to these harmful agents can be caused by tissue injury, infection, and allergic reactions (Kowalak, 2011). Characteristics of inflammation include pain (dolor), heat (calor), redness (rubor), and swelling (tumor). These dangerous agents will cause the release of inflammatory mediators such as prostaglandins, prostacyclin, bradykinin, histamine, cytokines, and leukotriene C4 (Frederickson, 2018).

Long-term use of anti-inflammatory drugs can carry the risk of dangerous side effects. NSAIDs (Non-Steroidal Anti-Inflammatories) have side effects such as stomach ulcers and AIS (Anti-Inflammatory Steroids) have side effects such as decreased immunity. Currently, a lot of research is being carried out to find alternative treatments that can treat inflammation with minimal side effects.

Several studies have been carried out on the *Tamarindus indica* L tamarind plant, namely that infusion of tamarind leaves is effective as an anti-inflammatory at concentrations of 10, 20, and 30% w/v in mice induced by carrageen (Pramana, 2008). According to Akor, *et al* (2015), testing the water extract of *Tamarindus indica* L leaves has antinociceptive and anti-inflammatory activity at a dose of 400 mg/kgbb in albino mice. The results of research on anti-inflammatory activity with carrageenan induction in male Wistar rats showed that hydroethanol extract of tamarind leaves at a dose of 1000 mg/kg bw provided a maximum inhibitory effect of 73.63% (Bhadoriya SS, 2012). There have been limited studies concerned on leaves tamarind Therefore, this research intends to testing the ethanol extract of tamarind leaves as an anti-inflammatory.

II. Research Method

II.1 Sampling

The fresh tamarind leaf samples obtained were wet sorted and cleaned using running water. Dry by airing indoors for 14 days. The drying results are then sorted dry to obtain leaves that are suitable for use. After that, it is crushed to obtain a coarse powder.

II.2 Extraction Process

The extraction method to obtain tamarind leaf extract is the maceration method. A total of 500 g of coarse powdered tamarind leaves is put in 5 L of 96% ethanol in a maceration container until the simplicia powder is completely submerged. The container was closed tightly and left for 3x24 hours at room temperature protected from direct sunlight, stirring occasionally. The filtrate was filtered and filtered three times. The filtrate obtained was collected and evaporated using a rotary evaporator to obtain a thick extract (Hanani, 2015)

II.3 In Vivo Anti-Inflammatory Activity Testing

The rat test animals were acclimatized for 1 week, then weighed to determine the volume given to each test animal. Rats were divided into 5 groups, namely group I was given Na. CMC (negative control). Group II was given diclofenac sodium suspension at a dose of 5.136 mg/kgBW, groups III, IV, and V were given ethanol extract of tamarind leaves at a dose variant of 250 mg/kgBW; 500 mg/kgBW; and 1000 mg/kgBW. The initial volume of the rat's paw (leg) was measured before initial volume treatment (V0). Measurement of the volume and thickness of rat paw edema using a peltysnometer and calipers. Test animals were treated with the test preparation orally once a day. Next, one hour later the rats were induced with 0.1 mL of 1% carrageenan intraplantarly. The volume and thickness of the rat's paw edema were measured for 7 hours at 1 hour intervals, represented as the final volume (Vt) (Winter, 1962; Samodra, 2019).

II.4 Data Analysis

The research quantitative data is in the form of AUC (Area Under Curve) values from the curve of average edema volume and thickness against time and percent anti-inflammatory power (% DAI). The AUC value is the average area under the curve which is the relationship between the average volume and thickness of edema per unit time using the formula (1).

$$AUC^{tn}_{tn-1} = \frac{V_{tn-1}+V_{tn}}{2}(t_n - t_{n-1}) \quad (1)$$

Description Vtn-1: Edema volume one hour before, Vtn: hour edema volume

The percentage of edema and thickness of the rat's paws were calculated. The percentage of edema and the average (mean) percentage of edema inhibition are calculated using the following formula (2).

$$\%DAI = \frac{AUC_k - AUC_p}{AUC_k} \times 100\% \quad (2)$$

Description % DAI: Percent anti-inflammatory inhibition, AUCk: Average for negative controls, AUCp: Average for treatment group (Pramitaningastuti, 2017)

The AUC value obtained was tested statistically using the one-way Anova test then followed by the LSD test with a significance level of 0.05

III. Results and Discussion

Inflammation is the body's response to infection and tissue damage at the site of injury to eliminate the cause of injury (for example microbes or toxins) and can cause necrosis in cells and tissues (Kumar *et.al*, 2021). In this study, tests were carried out to determine the potential of tamarind leaf extract (*Tamarindus indica* L) as an anti-inflammatory in Wistar rats induced by carrageenan. This study aims to determine the effect and effective dose of tamarind leaf extract (*Tamarindus indica* L) as an anti-inflammatory. The method used in this study was Rat Hind Paw Edema, namely the formation of artificial edema caused by injecting 0.1 mL of 1% w/v lambda carrageenan intraplantarly. Measurement of edema in rats' feet was carried out by measuring the volume and thickness of the edema using calipers and a pletysnometer

Carrageenan has been widely used as an edema inducer and inflammation inducer. There are two stages of the mechanism by which carrageenan induces inflammation, namely in the first stage the release of histamine, bradykinin and serotonin in the initial phase during the first hour and the second stage due to excessive prostaglandin production in the tissue and associated with the release of bradykinin, protease, lysomal enzymes, cell migration, activation and degranulation which takes place after one hour of carrageenan induction (Singh *et al*, 2014). In the study, a positive control was used, namely diclofenac sodium. Diclofenac sodium is a class of non-steroidal anti-inflammatory drugs which has a mechanism of action, namely inhibiting the cyclooxygenase enzyme in the formation of prostaglandins and other pain mediators, thereby reducing the transmission of nerve pain received by the CNS (Dipiro *et al*, 2020).

Data obtained from measuring the thickness and volume of mouse paw edema at any time in all groups were tabulated and graphed. Next, the measurement values for edema thickness and edema volume are calculated based on AUC. The AUC (Area Under Curve) value is obtained from data on the thickness and volume of edema formed in each test animal at each time. AUC

(Area Under Curve) is a value that describes the volume of edema for each group of test animals at each unit of time. The greater the AUC value indicates that the anti-inflammatory activity of a drug in reducing the volume of edema is smaller. Conversely, the smaller the AUC value indicates the greater the anti-inflammatory activity of the drug (Sutrisna, 2010). The AUC values of test animals at each time were added up and averaged to be used in calculating the percentage of anti-inflammatory power (%DAI) of tamarind leaves. The percentage of anti-inflammatory power shows the percent value of the ability of a test group to provide an anti-inflammatory effect on test animals. This value is inversely proportional to the AUC value, where the greater the DAI percentage, the smaller the AUC value obtained.

Data on decreasing edema volume is used to determine the total AUC (Area Under Curve) value, which is then calculated as the average value of the total AUC and percent anti-inflammatory power (%DAI) in the following Table:

Table 1. Average AUC and percent anti-inflammatory power in rat paw edema volume

Treatments Groups	Average AUC Volume Rat's Paw Edema (mm^3)	% DAI Volume Rat's Paw
Negative control (Na.CMC 1%)	10,71	-
Positive Control (Diclofenc sodium)	9,01	16%
EEDAJ 250 mg/KgBB	11	2%
EEDAJ 500 mg/KgBB	10,36	4%
EEDAJ 1000 mg/KgBB	9,48	12%

Disscussion: Na.CMC= Carboxilmetilcellulose sodium; EEDAJ= Ethanol Extract tamarind leaves; AUC= *Area Under Curve*; DAI= Inhibition of antiinflammatory

Table 1 shows that the positive control group had the lowest average AUC value with a value of 9.01 mm3.hour and had the highest percentage of anti-inflammatory power, namely 16%, which means that diclofenac sodium provided the best anti-inflammatory effect compared to other groups in reducing edema volume. The test preparation group that was best in providing anti-inflammatory effects was EEDAJ 1000 mg/KgBW with a %DAI value of 12% then for the EEBC 500 mg/KgBW and EEBC 250 mg/KgBW groups with %DAI values of 4% and 2% respectively.

The calculation results of the AUC value for the total thickness of rat paw edema that were obtained were statistically analyzed using the One Way Annova test (can be seen in appendix 6, Table 4). The normality and homogeneity test data for all treatment groups met the requirements with a p value of> 0.05. Then proceed with the LSD test to see the differences between treatment groups. The results of statistical analysis of the AUC value of rat paw edema volume in the negative control group (Na.CMC) showed no significant difference with EEDAJ 250 mg/KgBW and EEADJ 500 mg/kgBW with a value of p= 0.318 and p= 0.134 but significantly different from the test group other. This means that the negative control group had the same effect as EEDAJ at a dose of 250 mg/kgBW and EEADJ 500 mg/kgBW in reducing inflammation (edema volume) in the rats' feet. The positive control group showed no significant difference to the EEDAJ 1000 mg/kgBW group with a p value = 0.078. This means that the positive group given diclofenac sodium had the same effect as the EEDAJ 1000 mg/kgBB group as an anti-inflammatory in reducing the volume of rat paw edema. For the extract group, the test showed that EEDAJ at a dose of 1000 mg/kgBW was significantly different from EEDJ 250 mg/kgBW and 500 mg/kgBW with a value of p = 0.000 and p = 0.043. This means that the 1000 mg/kgBB EEDAJ extract has a better effect on reducing the thickness of edema in the legs of mice compared to the other test extract groups.

Data on decreasing edema thickness is used to determine the total AUC (Area Under Curve) value, which is then calculated as the average value of the total AUC and percent anti-inflammatory power (%DAI) in the following Table:

Table 2. Average AUC and percent anti-inflammatory power in rat paw edema thickness

Treatments Groups	Average thickness Rat's Paw Edema (mm.jam)	% DAI Thickness Rat's Paw
Negative control (Na.CMC 1%)	43.4	-
Positive Control (Diclofenc sodium)	40.93	6%
EEDAJ 250 mg/KgBW	42.8	1%
EEDAJ 500 mg/KgBW	42.58	2%
EEDAJ 1000 mg/KgBW	41.8	4%

Disscussion: Na.CMC= Carboxilmetilcellulose sodium; EEDAJ= Ethanol Extract tamarind leaves; AUC= *Area Under Curve*; DAI= Inhibition of antiinflammatory

Table 2 shows that the positive control group had the lowest average AUC value with a value of 40.93 mm.hour and had the highest percentage of anti-inflammatory power, namely 6%, which means that diclofenac sodium provided the best anti-inflammatory effect compared to other groups in reducing the width of edema. The test preparation group that was best in providing anti-inflammatory effects was EEDAJ 1000 mg/KgBW with a %DAI value of 4% then for the EEBC 500 mg/KgBW and EEBC 250 mg/KgBW groups with %DAI values of 2% and 1% respectively.

The calculation results of the AUC value for the total thickness of rat paw edema that were obtained were statistically analyzed using the One Way Annova test. The normality and homogeneity test data for all treatment groups met the requirements with a p value of> 0.05. Then proceed with the LSD test to see the differences between treatment groups. The results of statistical analysis of the AUC value of rat paw edema thickness in the negative control group (Na.CMC) showed no significant difference with EEDAJ 250 mg/KgBW with a p value = 0.108 but was significantly different from the other test groups. This means that the negative control group had the same effect as EEDAJ at a dose of 250 mg/kgBW in reducing inflammation (edema thickness) in the rats' feet. The positive control group showed a significant difference to all groups of test preparations with a p value <0.05. This means that the positive group given diclofenac sodium had a good anti-inflammatory effect. For the extract group, the test showed that EEDAJ at a dose of 1000 mg/kgBW was significantly different from EEDJ 250 mg/kgBW and 500 mg/kgBW with a p value = 0.014 and 0.043. This means that the 1000 mg/kgBB EEDAJ extract has a better effect on reducing the thickness of edema in the legs of mice compared to the other test extract groups

Based on the results of this research, it can be concluded that the ethanol extract of tamarind leaves has an anti-inflammatory effect with an effective dose in carrageenan-induced test animals of 1000 mg/KgBW. EEBC at a dose of 1000 mg/KgBW had a good anti-inflammatory effect and had the highest % DAI among the other tested extract doses in terms of volume and thickness of rat paw edema at 12% and 4%. The effectiveness of the ethanol extract of tamarind leaves as an anti-inflammatory is thought to be due to the presence of flavonoids, alkaloids, and tannins. The mechanism of flavonoids as anti-inflammatory is through direct inhibition of cyclooxygenase and lipoxygenase enzyme activity which causes inhibition of prostaglandin and leukotriene biosynthesis (Nijveldt *et al*., 2001). Alkaloid compounds act as anti-inflammatories through the mechanism of inhibiting the cyclooxygenase 2 (COX-2) enzyme thereby reducing PGE2 synthesis (Li *et al*, 2020). Apart from that, tannin also plays an anti-inflammatory role by inhibiting NO and prostaglandin-E2 (PGE2) (Wijesinghe, 2013).

IV. Conclusions

Ethanol extract of tamarind leaves (*Tamarindus indica* L) has anti-inflammatory effects with an effective dose of 1000 mg/kgBW.

V. Acknowledgment

The research team would like to thank the Faculty of Pharmacy, UMI, for making this research possible.

References

Akor, T.S., Wampana, B.O., Sodipo, A. (2015) 'Antinociceptive and anti-inflammatory activities ofthe aqueous leaf extract of Tamarindus indica L. in albino rats', *JPS*:4, http://dx.doi.org/10.5539/jps.v4n2p4

Bhadoriya, S.S., Mishra, V., Raut, S., Ganeshpurkar, A., Jain, K.S. (2012) 'Anti-inflammatoryand antinociceptive activities of a hydroethanolic extract of Tamarindus indica leaves', *Scient Pharmaceut*, 80, 685–700.42. https://pubmed.ncbi.nlm.nih.gov/23008815/

Dipiro, J.T et. al. (2020) *Pharmacotherapy A Pathophysiologic Approach* (11th Ed). United States : by McGraw Hill

Frederickson, N.F and David, M. (2018) Introduction to Immunity and Inflammation cis Brunton, LL., Hilal-Dandan R, Knollman BC. *Goodman & Gilman's, Manual of Pharmacology and Therapeutics* (13th Ed). United States. McGraw-Hill Education

Hanani, E. (2015) *Analisis Fitokimia*, Penerbit Buku Kedokteran EGC, Jakarta, hh. 6-11

Komakech, R., Goo Kim, Y., Matsabisa, GM., Kang, Y. (2019) 'Anti-inflammatory and analgesic potential of Tamarindus indica Linn. (Fabaceae): a narrative review', *Integrative Medicine Research*, 8, 181–186

Kowalak, J.P.,Welsh, W., dan Mayer B. (2011) *Buku Ajar Patofisiologi*. Penerbit Buku Kedokteran EGC, Jakarta, h. 58

Kumar, V., Abbas, A.K., Aster, J.C., Deyrup, A.T. (2021). *Robbins Essential Pathology*. Philadelphia by Elsevier.

Li, S., Liu, X., Chen, X.R and Bi, L. (2020). 'Research Progresson Anti-Inflammatory Effects and Mechanisms of Alkaloids

from Chinese Medical Herbs', Evidence-Based Complementary and Alternative Medicine, Article ID 1303524, 10 pages https://doi.org/10.1155/2020/1303524

Mun'im, A., Hanani, E., Rahmadiah. (2009). 'Karakterisasi Ekstrak Etanolik Daun Asam Jawa (Tamarindus indica L)', Majalah Ilmu Kesehatan Kefarmasian, Vol.1,Hal. 44

Nijveldt, R.J., Nood, E.V., Hoorn, D.E.C., Boelens P.G., Norren, K.V., dan Leeuwen, P.A.M. (2001) 'Flavonoids: A Review of probable mechanisms of action and potential application', *Am J Clin Nutr., 74,*418-425.

Pramana, J.D. (2008). *Uji Efek Antiinflamasi Infus Daun Asam Jawa (Tamarindus indica L). Pada Tikus Putih Jantan*. Fakultas Farmasi. Universitas Katolik Widya Mandala. Surabaya

Pramitaningastuti, A.S., Anggraeny, A.N. (2017) 'Uji Efektivitas Antiinflamasi Ekstrak Etanol Daun Srikaya (*Annona squamosa.* L) Terhadap Udema Kaki Tikus Putih Jantan Galur Wistar', *Jurnal Ilmiah Farmasi*, 13(1), 8-13 ISSN: 1693-8666, http://journal.uii.ac.id/index.php/JIF

Samodra, G., Febrina D.(2019) 'Anti-Inflammatory Effects of Kaempferia galanga L. Rhizome Extract in Carrageenan-Induced Female Rats', A*dvances in Health Sciences Research*, 20, 1-27.

Singh, S., Kaur, M., Singh, A and Kumar, B. (2014) 'Pharmacological Evaluation of Anti-Inflammatory and Anti-Ulcer Potential of Heartwood of Santalum Album in Rats', A*sian Journal of Biochemical and Pharmaceutical Research*, 1(4), ISSN: 2231-2560, 140-153, Researchgate.net/publication/264166755_Pharmacological_Evaluation_of_Anti-Inflammatory_and_AntiUlcer_Potential_of_Heartwood_of_Santalum_Album_in_Rats/link/53d0e76d0cf25dc05cfe70fa/download

Sutrisna, E.M., Widyasari, D.F., dan Suprapto. (2010) 'Uji efek anti inflamasi ekstrak etil asetat buah semu jambu mete (*Anacardium occidentale* L.) terhadap udema pada telapak kaki tikus putih (*Rattus norvegicus*) jantan yang diinduksi karagenan', *Biomedika, 2*(1), 33-37.

Wijesinghe, W., Ahn, G., Lee, W.W., Kang, M.C., Kim, E.A., Jeon, Y.J. (2013) 'Anti-inflammatory activity of phlorotannin-rich fermented Ecklonia cava processing by-product extract in lipopolysaccharide stimulated RAW 264.7 macrophages', *J Appl Phycol*. 25:1207–13. doi: 10.1007/s10811-012-9939-5

Winter, C.A., Risley, E.A., and Nuss, G.W. (1962). 'Carrageenan induced oedema in hind paw of the rats as an assay for anti-inflammatory drugs', *Proc. Soc. Exp. Bio Med*, 111, (544-547).