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Activity of Tokulo (*Kleinhovia hospita* L.) as Anti Rheumatoid Arthritis and Anti-inflammatory in White Rats Induced by Complete Freud Adjuvant (CFA)

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Abstract

Tokulo (*Kleinhovia hospita*) leaves are commonly used by Moronene people (Southeast Sulawesi) to treat headaches. This is supported scientifically from research which states that tokulo leaves have analgesic and anti-inflammatory activity. The NSAID group is included in the anti-rheumatoid arthritis therapy. To determine the anti-RA activity of this plant, a study was carried out on CFA-induced rats. Based on the results of the study, it is known that the ethanol extract of tokulo leaves has anti-RA and anti-inflammatory activity in CFA-induced rats.

Keywords: Kleinhovia hospita, Rheumatoid Arthritis, Inflammation

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1. Introduction

In several regions in Indonesia, tokulo is widely used empirically to treat pneumonia, eye wash, jaundice [1] While the Moronene people who inhabit Tobu Hukaea-Laea usually use this plant as a headache remedy [2]. This is supported by the results of preliminary research which prove that there is analgesic activity in the ethanol extract of tokulo leaves. Other studies have also shown that the ethanol extract of tokulo leaves has anti-inflammatory activity comparable to diclofenac sodium [3]

Tokulo is also known by the names of guest tree (America), boha (India), mahar (South Kalimantan), tengkele (Sunda), katimanga (Java). This plant belongs to the Magnoliophyta division, the Magnoliopsida class, the Marvales,

the Sterculiaceae tribe, the Kleinhovia genus, and the Kleinhovia hospita L. species.

Tokulo is a type of wood that is traditionally considered nutritious. The leaves of this plant are widely used in traditional medicine or as a food ingredient. The people of Southeast Sulawesi have used this plant as a medicine that can cure liver disease [4]. The water produced from the decoction of tokulo leaves can be drunk to treat liver disease, jaundice and hepatitis [5]. The activity test on the ethanol extract of tokulo leaves using the rat paw edema method with carrageenan inducers proved that the leaves of this plant have anti-inflammatory activity [6].

Rheumatoid Arthritis (RA) is a progressive autoimmune disease with chronic inflammation that attacks musculoskeletal system. RA can involve organs and body systems as a whole, characterized by swelling, joint pain and destruction of synovial tissue accompanied by movement disorders followed by premature death [7]

Rheumatoid arthritis is an autoimmune disease that requires long-term medication and control. The cause of RA disease is still not known with certainty. Suspected viral, bacterial infection as the initial initiator of RA. In the process of RA, there are various interrelated roles, including the role of genetics, infection, autoantibodies and the role of cellular immunity, humoral, the role of cytokines, and various inflammatory mediators. The inflammatory process due to autoimmune processes in RA, shown by laboratory tests in the presence of RF (Rheumatioid Factor) and anti-CCP in the blood. At present RF and anti-CCP are important diagnostic means of RA and reflect disease progression [8].

In pathophysiology of RA, B cells, T cells, and pro-inflammatory cytokines also play an important role. The result of T cell differentiation stimulates the formation of IL-17, a cytokine that stimulates the formation of synovitis (inflammation of the synovial membrane). B cells play a role through the formation of antibodies, function to bind pathogens, then destroy them. Joint damage begins with an inflammatory reaction and the formation of new blood vessels in the synovial membrane. This event causes the formation of pannus (granulation tissue consist of proliferating fibroblasts, microvascular and various types of inflammatory cells). In the initial state there is microvascular damage, edema in tissue under the synovium, mild proliferation of synovial, PMN infiltration, and blockage of blood vessels by inflammatory cells and thrombus. In RA which is clinically clear, it will be seen macros that the synovium will experience edema and protrude into the joint space with villi formation. (no deletion yet, forgot)

2. Experimental section

2.1 Tools and Materials

Tools and materials used in this study were Electric scale, maserator, rotary vacuum evaporator, water bath, plesthysmometer, tokulo leaves simplicia, 70% ethanol, Complete Freud Aduvant (CFA), Na.CMC, aquadest, 0.9% physiological salt solution,

2.2 Sample Preparation

Tokulo leaves were obtained from Tobu Hukaea-Laea, Bombana, Southeast Sulawesi. The sample obtained is washed under running water to remove adherent impurities. Samples were dried in an aerated manner and protected from direct sunlight. After drying, the sample is chopped.

2.3 Extract Preparation

The chopped tokulo leaves sample was put into a macerator and then immersed in 70% ethanol until the entire surface of the sample was immersed. This maceration process is carried out 3x24 hours by stirring it occasionally, where the replacement with a new solvent is carried out every 24 hours. The obtained maserate was then filtered using filter paper and then evaporated using rotary evaporator vacuum then followed by

evaporating the sample until its weight is constant with at a temperature below 40°C.

The experimental animals used were male Wistar rats aged 8 weeks and weighing 150-200 grams. The experimental animals were acclimatized to the experimental conditions for 1 week. The cage condition was maintained at a temperature of 28°-32°C and a dark and light cycle of 12 hours each. The experimental animals were fed with standard diet pellets and drinking water *ad libitum*.

2.4 Anti-Rheumatoid Arthritis Test

The tested animals were divided into 6 groups with each group consisting of 5 rats. Before being treated, the left ankle behind the rats was marked with picric acid and then the initial volume of the rats' feet was measured using plesthysmometer. After the initial volume was being measure, the rats' left hind leg was induced by CFA. 16 days after CFA induction, the test animals were treated according to the determined group division. The division of the test animal groups was as follows:

Normal group	:	(not induced by CFA + normal saline).
Negative control group	:	(CFA-induced + Na-CMC 0.5%,
Positive control group	:	(CFA-induced + Acetosal),
Dosage group I	:	(CFA-induced + extract dose of 245 mg / kg BW)
Dosage group II	:	(CFA-induced + extract dose of 490 mg / kgBW)
Dosage group III	:	(CFA-induced + extract dose 980 mg / kgB)

Edema volume measurement was carried out for 34 days starting from the treated animal. The arthritis index was set on day 17 to day 31.

To determine whether the rats have rheumatoid arthritis or not, the symptoms that arise are expressed as an arthritis index. Rats can be called rheumatoid arthritis if the index that occurs is ≥ 1 and is usually characterized by swelling, redness, and deformities in the fingers and soles of the feet (Smit, 2000). On day 1 to day 16, the rats were induced with CFA and had not received treatment with the aim of making the mice arthritis [9]

Table 1. Scoring table of arthritis assessment [9]

Symptoms	Score
swelling and redness of 1 toe	0.25
swelling and redness of at least 2 toes	0.50
swelling of foot pad	0.75
swelling and redness of toes and swollen foot pad	1.00
swelling and redness of toes and foot pad	1.25
swelling and redness of toes and minor swelling of foot pad and ankle	1.50
swelling and redness of toes and major swelling of foot pad and ankle	1.75
swelling and redness of toes, foot pad, and ankle	2.00

Mice were considered to have arthritis when Arthritis Index was ≥ 1.

3. Results and Discussion

One of the characteristics of RA disease is joints (usually the hands and feet) become inflamed, causing pain and can cause damage to the inner joints. One of the RA treatment options is the use of steroids and Non-Steroidal Anti-Inflammatory Drugs (NSAIDs). Steroids and NSAIDs are a class of drugs used as anti-inflammatory and analgesic. Based on previous research, it is known that tokulo (K. hospita) leaves have anti-inflammatory and analgesic activity [3][2].

The choice of the test method in this study was because according to several approaches that have been developed, the use of RA animal models is a suitable method to be used in studying the pathogenesis and inflammatory process of arthritis and for developing anti-rheumatic drugs suitable for the treatment of rheumatism. In addition, RA in mice can also be easily induced by CFA [10], [11 From several research models, testing using a test animal model to study the pathogenesis of disease and treatment is a potential testing model for anti-rheumatism [12]. Adjuvant-induced arthritis is the best testing model for studying the effects of

arthritis and is still widely used in preclinical testing [13].

3.1 Arthritis Index Measurement

Based on the results of research, the arthritis index before therapy and after therapy can be seen in table 2

Table 2. Arthritis Index Mean Before and After Therapy

Group of Treatment	Arthritis Index	
	Before therapy ± SD	After Therapy ± SD
Normal	0.00±0.00	0.00±0.00
Negative control (Na.CMC 1%)	1.45±0.35	1.50±0.35
Positive control (Acetosal 5g/kgBB)	1.47±0.11	1.50±0.18
Dosage 1 (EETL 245 mg/kgBB)	1.48±0.55	0.75±0.18
Dosage 2 (EETL 490 mg/kgBB)	1.48±0.00	0.65±0.29
Dosage 3 (EETL 980 mg/kgBB)	1.47±0.33	1.25±0.31

*EETL : Ethanol Extract of Tokulo Leaves

From table 2, we can see that before the treatment all test groups except the normal group which was not induced by CFA had Rheumatid Arthritis, this is indicated by the arthritis index value ≥1. After treatment, the arthritis index score in the dose groups 1,2, and 3 decreased. The greater the arthritis index value, the more severe the arthritis suffered.

Furthermore, based on the results of the ANOVA test followed by the LSD test, it was found that there was a significant difference ($p \leq 0.05$) between the groups of dosage 1 and 2 with negative control group, in both of the two groups (dosage 1 and 2) had arthritis index score ≤ 1, it was indicate that both of these groups had no arthritis. This shows that the Ethanol Extract of tokulo leaves has anti RA activity at the dosage groups of 1 and 2 with comparable abilities (($p \geq 0.05$).

In the positive control group and dose 3 group, there was no significant difference with the negative control group ($p \geq 0.05$) where the arthritis index of these two groups was ≥1, which means that both groups still had arthritis. This is in line with the use of aspirin in RA therapy, where aspirin only reduces symptomatic symptoms (pain and inflammation) but does not affect the course of the disease or prevent joint damage [14].

3.2 Anti-Inflammatory Activity

The results of the mean percentage of rat foot edema both at initial, induction and therapy can be seen in Table 3

Table 3. Persentation of Rat Paw Edema Mean Before and After Therapy (%)

Group of Treatment	Persentation of Rat Paw Edema (%)	
	Before Therapy ± SD	After Therapy ± SD
Normal	0.200±0.00	0±0.000
Negative control Na-CMC 1%	0.600±0.071	247±0.273
Positive control Acetosal	0.600±0.100	186±0.482
Dosage 1 EETL 245 mg/kgBB	0.640±0.055	157±0.252
Dosage 2 EETL 490 mg/kgBB	0.640±0.089	209±0.381
Dosage 3 EETL 980 mg/kgBB	0.620±0.084	194±0.395

In Table 3, it can be seen that on the 17th day after induction edema had formed except in the control group that was not induced with CFA. An increasing of inflammation percentage indicates the ability of CFA to induce inflammation. The ability of CFA induce inflammation due to administration of it will stimulate phagocytosis, secretion of cytokines by mononuclear phagocytosis, so that various types of proinflammatory cytokines such as TNF-α, IL-1, IL-6, IL-8, PGE-2, NO, MMP and other mediators will released. TNF-α works synergistically with the Receptor Activator Of Nuclear Factor -kβ Ligant (RANKL) and causes osteoclastogenesis via osteoclast precursors. Receptor Activator Of Nuclear Factor-kβ (RANK) will trigger cell differentiation into osteoclasts. TNF-α can also develop chronic inflammatory arthritis, with synovial hyperplasia, inflammatory infiltrates in joints, formation of pannuses, cartilages and bone destruction. In addition, IL-1 and IL-6 are

involved in the pathophysiology of rheumatoid arthritis [15].

To find out the amount of inhibition power (percent inflammation inhibition) can be seen in Figure 1, where the highest percentage of inflammation inhibition is found in the group of test animals given EETL dose 1. Furthermore, to determine the significant difference between each group, the Kruscal Wallis test was carried out followed by Mann Whitney test.

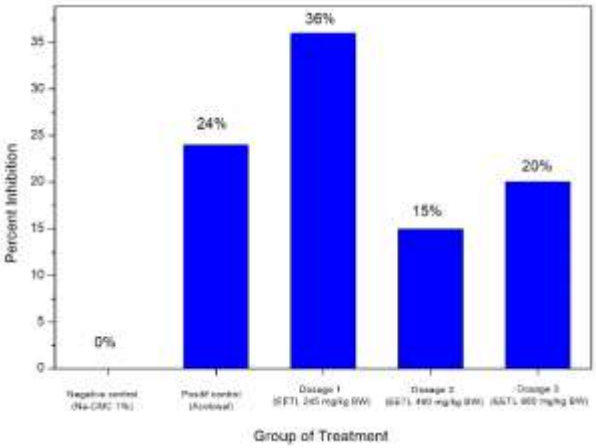

Figure 1. Inhibition percentation of anti-inflammatory of tokulo leaves ethanol extract.
*EETL : Ethanol Extract of Tokulo Leaves

Based on the Mann Whitney test, almost all test groups had a significant difference ($p \leq 0.05$), except for the dose groups 2 and 3 ($p \geq 0.05$). This shows that the greatest ability to inhibit edema is in the dose 1 group followed by the positive control group. Meanwhile, dose groups 2 and 3 had comparable ability to inhibit edema, although it did not exceed positive control.

The ability of tokulo leaves as an antirheumatoid arthritis is thought to be due to the presence of a class of flavonoid compounds that are known to play a role in anti-inflammatory activity, these compounds have an anti-inflammatory mechanism by inhibiting the cyclooxygenase enzyme so as to inhibit the formation of proinflammatory cytokines [16].

4. Conclusion

1. The ethanol extract of tokulo leaves at doses of 245 mg/kgBW and 490 mg/kgbw has comparable anti-rheumatoid arthritis activity against CFA-induced rats.
2. Tokulo leaf ethanol extract which is effective as an anti-RA as well as anti-inflammatory which is formed due to CFA induction in white rats. Is at a dose of 245 mg/ kgbw.
3. Although the ethanol extract of tokulo at a dose of 490 mg/kgbb has the lowest percentage of anti-inflammatory inhibition, at this dose it has anti-AR abilities comparable to the dose of 245 mg/kgbw

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Conflict of Interest

No conflict of interest is associated with this study.

References

[1] Paramita Swandari. 2016. Tahongai (*Kleinhovia hospita* L.): A Review of Herbal Medicine from East Kalimantan. *ResearchGate*. Vol. 9. No. 1
[2] Siharis FS., dan Fidrianny I. 2016. Etnofarmakologi dan uji aktivitas salah satu tumbuhan yang ditemukan di suku Moronene Tobu Hukaea Laea Kabupaten Bombana Sulawesi Tenggara. *Indonesia Natural Research Pharmaceutical Journal*, 1(1): 36-42.
[3] Sari T.P., Rijai La Ode, Gama S.I. 2016. Potensi Antiinflamasi Ekstrak Daun Tahongai(Kleinhovia hospita L.). Prosiding Seminar Nasional Kefarmasian Ke-4. Samarinda
[4] Raflizar, Sihombing M. 2009. Dekok daun paliasa (*Kleinhovia hospita* linn.) sebagai obat radang hati akut. *J Ekol Kesehat*. 8(2):984–993
[5] Tayeb R., Wahyudin E., Alam G., Pakki E., dan Lukman L. 2014. Preclinical Study: Hepatoprotective Effects of "Paliasa Tea Bag" on Paracetamol-Induced Liver Damage in Rats. *The*

2nd International Congress of Naturopathic Medicine. Paris.

[6] Solihah Indah, Herlina, Oktia Charmila. 2017. Uji Aktivitas Antiinflamasi Ekstrak Etanol Daun Tahongai (Kleinhovia hospita L.) Menggunakan Metode Rat Paw Edem. *Jurnal Permata Indonesia*. Vol. 8. No. 2. p1-11.

[7] McInnes, I.B., Schett, G. 2011. The Pathogenesis of Rheumatoid Arthritis. N Engl J Med,vol. 365, pp. 2205-19

[8] Putra,T.R., Suega,K., Artana,I.G.N.B. 2013. *Pedoman Diagnosis dan Terapi Ilmu* Penyakit Dalam. Bagian/SMF Ilmu Penyakit Dalam Fakultas Kedokteran. Universitas Udayana/RSUP Sanglah

[9] Smith, F. (2000). Picrorhiza Scrophulariiflora from Traditional Use to *Immunomodulatory*, (Dissertation). Rijksuniversiteit Utrech. Utrecht.

[10] Geboes Lies., Klerck B.D., Balen M.V., Kelchtermans H., Mitera T. Boon L., Peeters C.D.W., Matthys P. 2007., Freund's Complete Adjuvant Induces Arthritis in Mice Lacking a Functional Interferon-_ Receptor by Triggering Tumor Necrosis Factor _–Driven Osteoclastogenesis. *Arthritis & Rheumatism.* Vol.56. No. 8, 1. American College of Rheumatology

[11] Bendele AM. 2001. Animal models of rheumatoid arthritis. *J Musculoskel Neuron* Interact:377–385

[12] Vingsbo C, Sahistrand P, Brun JG et al. 1996. *Pristane induced arthritis in rats*. Am J Pathol 149:1675–1683

[13] Colpaert FC. 1987. Evidence that adjuvant arthritis in the rat is associated with chronic pain. Pain 28:201–2223

[14] Perhimpunan Reumatologi Indonesia. 2004. Diagnosis dan Pengelolaan Artritis Reumatoid, Perhimpunan Reumatologi Indonesia, hal :13

[15] Plenge, R., Cotsapas, C., Davies, L., Price, A., Bakker, P., Maller, J., Pe'er, I., Burtt, N., Blumenstiel, B., DeFelice, M., Parkin, M., Barry, R., Winslow, W., Healy, C., Graham, R., Neale, B., Izmailova, E., Roubenoff, R., Parker, A., & Altshuler, D. 2007. Two independent alleles at 6q23 associated with risk of rheumatoid rthritis. *Nature Genetics*, 39, 1477–1482. https://doi.org/10.1038/ng.2007.27

[16] Sativa, O., Sulstri, E., & Yuliet. (2014). Uji aktivitas antiinflamasi gel ekstrak buah kaktus (Opuntia elatior Mill.) pada tikus (Rattus norvegicus) yang diinduksi lamda karagen. Jurnal of Science and Technology, 3(2), 79-94.