**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 (Rheumatoid 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, plesthyssometer, 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 maseate was then filtered using filter paper and then evaporated using rotary evaporator vacuum then followed by...
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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 ples-thysmometer. 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:

| Group               | Description                                                                 |
|---------------------|-----------------------------------------------------------------------------|
| Normal group        | (not induced by CFA + normal saline).                                       |
| Negative control    | (CFA-induced + Na-CMC 0.5%,                                                  |
| Positive control    | (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)                                   |

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

| Group of Treatment | Arthritis Index Before Therapy ± SD | After Therapy ± SD |
|--------------------|-----------------------------------|--------------------|
| Normal             | 0 ± 0                             | 0 ± 0              |
| Negative control (Na.CMC 1%) | 1.45 ± 0.35             | 1.50 ± 0.35       |
| Positive control (Acetosal 5g/kg BB) | 1.47 ± 0.11              | 1.50 ± 0.18       |
| Dosage 1 (EETL 245 mg/kg BB) | 1.48 ± 0.55             | 0.75 ± 0.18       |
| Dosage 2 (EETL 490 mg/kg BB) | 1.48 ± 0.00             | 0.65 ± 0.29       |
| Dosage 3 (EETL 980 mg/kg BB) | 1.47 ± 0.33             | 1.25 ± 0.31       |

Table 2. Arthritis Index Mean Before and After Therapy

From Table 2, we can see that before the treatment all test groups except the normal group which was not induced by CFA had rheumatoid 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 ≤ 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 ≥ 0.05).

In the positive control group and dose 3 group, there was no significant difference with the negative control group (p ≥ 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

| Group of Treatment | Presentement of Rat Paw Edema (%) Before Therapy ± SD | After Therapy ± SD |
|--------------------|------------------------------------------------------|--------------------|
| Normal             | 0.200 ± 0.00                                         | 0 ± 0.00           |
| 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/kg BB | 0.640 ± 0.055                                   | 157 ± 0.252       |
| Dosage 2 EETL 490 mg/kg BB | 0.640 ± 0.089                                   | 209 ± 0.381       |
| Dosage 3 EETL 980 mg/kg BB | 0.620 ± 0.084                                   | 194 ± 0.395       |

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

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.

Based on the Mann Whitney test, almost all test groups had a significant difference (p ≤ 0.05), except for the dose groups 2 and 3 (p ≥ 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 anti-rheumatoid 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/kggb has the lowest percentage of anti-inflammatory inhibition, at this dose it has anti-AR abilities comparable to the dose of 245 mg/kgbw

Acknowledgement

Thanks are conveyed to various parties at Mandala Waluya University for their support so that this research can be carried out.

Conflict of Interest

No conflict of interest is associated with this study.

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