

Arthritis Assay on Combination of Red Ginger (*Zingiber Officinale*) and Secang Wood (*Caesalpinia Sappan*) Extract Towards Rat Oedema Induced by Complete Freund's Adjuvant

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ABSTRACT

Rheumatoid arthritis is a systemic chronic autoimmune disease associated with multiple inflammatory mediators leading to joint destruction, synovial and cartilage inflammation. Treatment often used for rheumatoid arthritis is DMARD which causes dangerous effects, such as gastrointestinal bleeding, nausea, dyspepsia, and impaired kidney function. The side effects of this treatment have caused many rheumatoid arthritis sufferers to turn into herbal medicines that have milder side effects. The purpose of this study was to determine the anti-arthritis effect of red ginger and secang wood combination ethanol extract based on decrease of oedema volume and the percentage of oedema inhibition. The method used was adjuvant-induced arthritis by subplantar induction of Complete Freund's Adjuvant. Animal model used was 35 Wistar rats divided into 7 groups. The results showed that the suspension of red ginger and secang wood combination in 0.5% CMC at the F2 (red ginger extract 14 mg/200 g BW + secang wood extract 102 mg/200 g BW) had the best anti-arthritis effect with the percentage of inhibition of reducing oedema by 76.09% and having a significant anti-arthritis effect ($p < 0.05$) compared to the negative control in terms of decreasing the volume of oedema.

Keywords: Rheumatoid arthritis, Red ginger, Secang wood, Edema volume.

1. INTRODUCTION

Rheumatoid arthritis is a systemic chronic autoimmune disease associated with several inflammatory mediators that cause joint and bone damage, synovial and cartilage inflammation [1]. Rheumatoid arthritis generally accompanied by pain and swelling in the fingers, knees, and wrists. The disease does not cause death but can reduce the quality of life. The incidence increases with age, especially in women due to hormonal balance factors. The peak incidence of the disease is the age of 40-60 years.

Treatment often used to treat rheumatoid arthritis is DMARD (Disease Modifying Anti Rheumatic Drugs), namely methotrexate, leflunomide, and sulfasalazine [2]. The use of these drugs can cause side effects and toxicity. Drugs of this class are strong anti-inflammatory and are often combined with non-steroidal anti-inflammatory drugs to strengthen their effects, but the use of these drugs can cause quite dangerous effects, which can cause gastrointestinal bleeding, nausea, dyspepsia, and

impaired kidney function [3]. The side effects of these drugs have caused many rheumatoid arthritis sufferers to turn to alternative medicine, namely herbal medicines that have milder side effects.

One of the plants that can be used as traditional medicine is the secang wood (*Caesalpinia sappan*). Part of the plant used as traditional medicine is stem wood. Secang wood contains phenolic components with various structural subtypes including xanthenes, coumarins, flavones, homoisoflavonoids, and brazilin. Brazilin has potential bioactivity including antioxidant, antibacterial, anti-inflammatory, anti-aging, hypoglycemic, vasorelaxant, and hepatoprotective [4].

Empirically, flavonoid compounds can act as an anti-inflammatory [5]. The mechanism of anti-inflammatory flavonoids is through inhibition of the release of pro-inflammatory cytokines such as IL-6, inhibition of cyclooxygenase or lipoxygenase. Flavonoids are also a trigger for the activation of the immune system [6].

On the other hand, the red ginger plant is no longer denied its usefulness that red ginger extract is also an antioxidant and anti-inflammatory [7]. The main constituents of red ginger are sesquiterpenoids, with (-)-zingiberene. Sesquiterpene lactones (SLs) are natural products that are responsible for their anti-inflammatory activity [8]. In addition, red ginger also has active compounds that are responsible for anti-inflammatory, namely gingerol with its derivatives and 6-shogaol which act as an anti-inflammatory with the mechanism of action of inhibiting the formation of PGE₂ [9].

Based on the pre-clinical test related to the anti-inflammatory activity of the two plants, a combination of the secang plant and red ginger can be used as a herbal medicine for rheumatoid arthritis. Anti-arthritis assay was carried out in vivo on male Wistar rats induced with Complete Freund's Adjuvant (CFA). CFA provides a clinical picture similar to that of arthritis and the pathogenesis of naturally occurring arthritis conditions.

Based on the description above, the authors are interested in examining the anti-arthritis activity test of the combination of ethanol extract of red ginger and secang wood on the volume of oedema in the legs of male Wistar rats induced by CFA.

2. METHODS

The research was carried out at the Pharmacology Laboratory, Faculty of Medicine, Airlangga University. The type of research used is experimental laboratory research using Pre- and Post-Test Only Control Group Design using 35 Wistar rats.

2.1. Extraction

Extraction of red ginger and secang wood was done by using maceration method with ethanol as solvent. In this process, 500 grams of powdered red ginger and secang wood were placed in a covered container with ethanol solvent. The maceration was carried out for 3 days and occasionally stirred. The filtrate obtained then was evaporated using a rotatory evaporator to obtain an ethanolic extract of red ginger and secang wood.

2.2. Preparation of Experimental Animals

The rats used were 150-200 grams of white Wistar strain (*Rattus norvegicus*). The rats were divided into 7 groups consisting of 5 rats in each group. This is based on the results of calculations using the Federer formula [10] as follows:

$$(t-1)(n-1) \geq 15$$

in which:

t: the amount of treatment

n: the amount of repetition of each treatment

in this study, t=7 means n=4, so the minimum number of rats used in each group is 4 rats. Rats were adapted to the environment. During the adaptation, the rats were given standard feed and water ad libitum then the rats were weighed.

2.3. Dose of Ethanol Extract of Red Ginger and Secang Wood

Previous research stated that the effective dose of red ginger as an anti-inflammatory is at a dose of 60 mg/kg BW/day [11]. In this study, the dose of red ginger used was 14 mg/200 g BW in rats, then the dose is made up to 14 mg/200 g BW; 28 mg/200 g BW. While, the effective dose for secang wood as an anti-inflammatory was 56 mg/200 g BW in rats and the dose levels were made up to 56 mg/200 g BW; 102 mg/200 g BW. Thus, in this study, the following dose combinations were used:

- a. F1 = red ginger extract 14 mg/200 g bw + secang wood extract 56 mg/200 g BW
- b. F2 = red ginger extract 14 mg/200 g bw + secang wood extract 102 mg/200 g BW
- c. F3 = red ginger extract 28 mg/200 g bw + secang wood extract 56 mg/200 g BW

2.4. Dose of Diclofenac Sodium

Diclofenac sodium was used as a positive control. The usual dose of diclofenac sodium for humans is 150 mg/day, so the conversion of the human to rat dose was 2.7 mg/200 g suspended in 0.5% CMC.

2.5. Adjuvant-induced Arthritis Test in Rats

On day 1, each rat in all test groups was injected with 0.1 ml of CFA reagent subplantarily on the left hind leg of the rat. The occurrence of arthritis was carried out for 16 days and then observed for swelling that occurred. The arthritis index was determined on the 17th and 31st days. Three groups received rat suspension extract in 0.5% CMC orally with the dose variation. One group was given the comparison diclofenac sodium in 0.5% CMC as a positive control. Diclofenac sodium used as a positive control was 9 mg/200 g BW rats. One group was given 0.5% CMC as a negative control.

Table 1. The treatment group of red ginger extract and secang wood against adjuvant-induced arthritis in rats

Group	Treatment
Positive control	Day-1 injection of 0,1 ml CFA, observed until day-17 Day-18 until 31 injection of diclofenac sodium in CMC 0,5% dose 9 mg/200 g BW
Negative control	Day-1 injection of 0,1 ml CFA, observed until day-17 Day-18 until 31 injection of CMC suspension 0,5%
F1	Day-1 injection of 0,1 ml CFA, observed until day-17 Day-18 until 31 injection of 1 ml red ginger suspension dose 14 mg/200 g BW + 1 ml secang wood suspension dose 56 mg/200 g BW in CMC 0,5%
F2	Day-1 injection of 0,1 ml CFA, observed until day-17 Day-18 until 31 injection of 1 ml red ginger suspension dose 14 mg/200 g BW + 1 ml secang wood suspension dose 102 mg/200 g BW in CMC 0,5%
F3	Day-1 injection 0,1 ml CFA, observed until Day-17 Day-18 until 31 injection of 1 ml red ginger suspension dose 28 mg/200 g BW + 1 ml secang wood suspension dose 56 mg/200 g BW in CMC 0,5%
Secang wood comparison	Day-1 injection of 0,1 ml CFA, observed until day-17 Day-18 until 31 injection of 1 ml secang wood suspension dose 56 mg/200 g BW in CMC 0,5%
Red ginger comparison	Day-1 injection of 0,1 ml CFA, observed until day-17 Day-18 until 31 injection of 1 ml red ginger suspension dose 14 mg/200 g BW in CMC 0,5%

The arthritis index was determined on the 17th and 31st days. Rats can be said to have rheumatoid arthritis if the index is 1 and is characterized by swelling, redness, changes in the shape of the fingers and soles of the feet. Arthritis index observation parameters can be seen in the following table:

Table 2. Parameters of arthritis index

Symptoms of Arthritis	Score
Swelling and red on 1 toe	0.25
Swelling and red at least 2 toes	0.50
Swelling on the soles of the feet	0.75
Swelling and redness of the toes and changes in the shape of the soles of the feet	1.00
Swelling and redness of the toes and soles	1.25
Swelling and redness of the toes and a little swelling of the soles and ankles	1.50
Swelling and redness on the fingers and soles of the feet and swelling in almost all the soles and ankles	1.75
Swelling and redness of the toes, soles, and ankles	2.00

The effect of anti-arthritis drugs was assessed from the percentage of inhibition of oedema caused by CFA and calculated by the following formula:

$$\text{oedema volume inhibition} = 1 - \left[\frac{a-x}{b-y} \right] \times 100\%$$

- a: the average volume of the rat's feet after being induced in the group of rats given the drug
- x: the average volume of the rat's feet before induction in the group of rats given the drug
- b: the average volume of the rat's feet after being induced in the group of rats that were not given medication (negative control)
- y: the average volume of rat's feet before induction in the group of rats that were not given the drug (negative control)

3. RESULTS AND DISCUSSION

In vivo anti-arthritis test of red ginger and secang wood extracts using male Wistar strain rats which were divided into 7 groups (n=5), namely positive control, negative control, red ginger comparison, secang wood comparison, and three groups receiving a combination suspension of red ginger extract and secang wood extract. Arthritis induction was carried out by injecting CFA into the left leg of the rat. CFA contains Mycobacterium or bacterial cell walls as an inducing agent. Inflammation due to CFA injection was the first visible sign of disease initiation and injection of CFA in the hind paws of rats resulted in an increase in oedema volume and feet diameter. This is the clinical picture of rheumatoid arthritis.

On the first day, the rats were induced with 0.1 mL of CFA subplantarily on the rat's left leg and left until the 16th day with the aim that the rats had arthritis with an

arthritis index value > 1. If the arthritis index is > 1, then the rats could be said to have arthritis with symptoms of swelling of the feet, redness of the toes, and changes in the shape of the soles of the feet. Observations on the occurrence of arthritis were carried out on days 1 to 17 after being induced by CFA and on days 17-31 to determine the anti-arthritis activity of red ginger and secang wood suspension against CFA-induced rats based on their arthritis index.

expressed in percent [12]. According to Table 3, it is known that the volume of foot oedema of rats in groups F1, F2, F3, red ginger comparison group, secang wood comparison group, and positive control decreased when compared to the negative control group. In the untreated negative control group, arthritis continued to develop due to the inflammatory process after the induction of CFA reagents which caused immune cells to secrete pro-inflammatory cytokines such as IL-1 and TNF- α

Table 3. The average oedema volume

Group	Oedema volume						
	Before CFA Injection	Day-17	Day-20	Day-23	Day-26	Day-29	Day-31
Positive control	3.34 ± 0.51	10.94 ± 1.66	10.35 ± 1.51	9.18 ± 1.12	7.50 ± 1.26	6.68 ± 1.24	5.75 ± 0.76
Negative control	3.55 ± 0.29	9.76 ± 1.23	10.03 ± 1.17	9.52 ± 1.61	9.31 ± 1.39	9.18 ± 0.86	8.18 ± 1.06
F1	3.36 ± 0.27	10.18 ± 1.39	9.61 ± 1.27	8.40 ± 1.11	7.68 ± 1.44	7.24 ± 1.14	6.00 ± 1.06
F2	3.49 ± 0.39	8.85 ± 2.64	8.09 ± 2.03	7.14 ± 2.05	6.52 ± 1.65	5.36 ± 1.34	4.60 ± 1.05
F3	3.74 ± 0.27	9.52 ± 1.59	7.91 ± 1.32	7.04 ± 0.76	6.42 ± 0.56	6.97 ± 1.65	5.87 ± 1.03
Secang wood comparison	3.79 ± 0.67	10.08 ± 0.98	8.13 ± 0.51	7.38 ± 0.47	6.54 ± 0.24	5.71 ± 0.20	5.12 ± 0.26
Red ginger comparison	3.39 ± 0.45	11.23 ± 1.76	10.56 ± 2.12	9.37 ± 2.25	7.90 ± 1.23	6.55 ± 0.70	5.51 ± 0.45

In this study, the occurrence of rheumatoid arthritis was seen on the 8th day marked by swelling and redness on the soles and ankles of rats. After CFA injection, the onset of pain in rheumatoid arthritis indicates that CFA acts as an antigen that triggers macrophages to produce pro-inflammatory cytokines, such as TNF- α , IL-1, and IL-6. These pro-inflammatory cytokines will stimulate the immune system response by over-expressing PGE₂, causing inflammation [8]. The arthritis index was determined on the 17th and 31st days to observe arthritis symptoms after 14 days of treatment. On day 17, all treatment groups had arthritis index > 1, indicating that the rats in each group had Rheumatoid Arthritis.

resulting in pain, swelling, and joint damage. The release of various inflammatory mediators in the joint causes synovial fluid to expand and spread to form inflammation then fibrosis appears. Fibrosis causes loss of joint mobility so that the synovial membrane becomes thickened and cartilage is eroded. This synovial membrane invades the space between joints, causing swelling and pain in the affected joint.

The percentage decrease in oedema volume was obtained from the calculation of oedema volume data on the 17th to the 31st day which is presented in table 4. It showed that the combination of red ginger extract and secang wood at F1, F2, and F3 showed an increasing percentage of oedema inhibition until the 31st day it

Table 4. Percentage of oedema inhibition (%)

Group	Percentage of oedema Inhibition (%)					
	Day-17	Day-20	Day-23	Day-26	Day-29	Day-31
Positive control	-22.30	-8.34	2.18	27.68	40.66	47.91
F1	-9.78	3.40	15.54	24.97	31.00	42.90
F2	13.87	28.99	38.86	47.48	66.90	76.09
F3	6.95	35.50	44.66	53.42	42.58	53.95
Secang wood comparison	-1.29	32.94	39.83	52.17	65.98	71.39
Red ginger comparison	-26.13	-10.71	-0.20	21.74	43.89	54.25

Arthritis testing can be observed from the percentage of oedema volume inhibition, namely the magnitude of the extract's effectiveness in reducing oedema volume

reached 42.90% for F1, 76.09% for F2, and 53.95% for F3. The average percentage of oedema inhibition in F2 (combination of red ginger extract 14 mg/200 g BW + 1 ml secang wood extract 56 mg/200 g BW) had the

greatest value when compared to other formulations from day 17 to day 31 in a row 13.87%; 28.99%; 38.86%; 47.48%; 66.90%; 76.09%, respectively.

The decrease in the percentage of oedema showed that the suspension given had an antiarthritis effect on rats. The pharmacologic effect shown from the suspension of red ginger and secang wood extracts came from the compounds contained in each extract. Secang wood contains phenolic compounds such as brazilin and brazilin which have anti-inflammatory activity. Meanwhile, red ginger contains gingerol and shogaol compounds, both of which are phenolic compounds that have anti-inflammatory properties. The suspension consisting of red ginger extract and secang wood extract at F2 showed the best percent reduction in oedema volume on the 31st day. This showed that polyherbal formulations or combinations of extracts have pharmacological activities that can work together dynamically to produce maximum therapeutic benefits with minimal side effects [13]

Statistical analysis was carried out using non-parametric statistical tests with the Kruskal-Wallis method followed by the Mann-Whitney test to determine the differences between the treatment groups because the data obtained were not normally distributed. In this study, significant value if the analyzed variable has a p-value <0.05. Statistical analysis showed a significant decrease of oedema volume compared to negative control group. Based on the Kruskal Wallis statistical test, there was a significant difference between the treatment groups on day 31 (p=0.015). The measurement of the volume of oedema showed that all treatment groups had significant differences when compared to the negative control group (p<0.05).

4. CONCLUSION

Based on the results of the anti-arthritis test using the adjuvant-induced arthritis method, it is known that the suspension of the combination of red ginger and secang wood ethanol extract in 0.5% CMC at the F2 (red ginger extract 14 mg/200 g BW + secang wood extract 102 mg/200 g BW) given orally had the best anti-arthritis effect with a percentage of inhibition of decreasing rat foot oedema by 76.09% and having a significant anti-arthritis effect (p<0.05) compared to the negative control in terms of decreasing oedema volume.

AUTHORS CONTRIBUTION

All authors conceived and designed this study. All authors contributed to the process of revising the manuscript, and at the end all authors have approved the final version of this manuscript.

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