

TEST OF THE EFFECTIVENESS OF GINGER *EXTRACT (Zingiber officinale)* AS AN ANTIRHEUMATIST AGAINST MICE (*Mus musculus*)

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A B S T R A C T

Background Rheumatism is an autoimmune disease characterized by joint inflammation, pain, and swelling that reduces patients' quality of life. Modern therapies such as NSAIDs and corticosteroids are effective but may cause serious side effects with long-term use. Ginger (*Zingiber officinale*) contains bioactive compounds such as gingerol, shogaol, and paradol with anti-inflammatory and analgesic properties, making it a potential herbal alternative for anti-rheumatic therapy. **Objective** : This study aimed to evaluate the effectiveness of ginger extract as an anti-rheumatic agent in male white mice (*Mus musculus*) induced with carrageenan. **Methods** This experimental laboratory study used 15 mice divided into five groups: negative control (0.5% Na CMC), positive control (sodium diclofenac), and three treatment groups with ginger extract doses of 200 mg/kg BW, 400 mg/kg BW, and 800 mg/kg BW. The main parameter was the reduction of paw edema measured with calipers every 60 minutes for 360 minutes. Data were analyzed using ANOVA. **Results** Ginger extract at 800 mg/kg BW showed the most significant reduction of edema, comparable to sodium diclofenac. The 200 mg/kg BW dose produced the lowest effect. ANOVA results indicated significant differences between groups ($p < 0.05$). **Conclusion** Ginger extract demonstrated effective anti-rheumatic activity, with the optimal dose at 800 mg/kg BW. Ginger has strong potential to be developed as a safe and effective herbal therapy for rheumatism.

INTRODUCTION

Rheumatism is a Autoimmune disease where a person's immune system is damaged which leads to damage to joints, especially in the hands, feet and knees. This disease often causes symptoms of pain, swelling, stiffness, and limited movement, which have a significant impact on the quality of life of the sufferer. In addition to reducing individual productivity, rheumatism is also a considerable economic burden, especially for those who require long-term treatment. The disease is chronic, and although modern medicine has been widely developed, the management of rheumatism often still relies on the use of nonsteroidal anti-inflammatory drugs (OAINS) and corticosteroids (Laughter) *et al.*, 2022)

The main cause of rheumatism is inflammation of the joints, which is caused by an autoimmune reaction, in which the immune system mistakenly attacks its own tissues. In rheumatoid arthritis, for example, the immune system attacks the synovial lining of the joint, causing inflammation and damage to the joints. Meanwhile, in osteoarthritis, damage to cartilage tissue causes friction between bones and inflammation. In addition to genetic factors, environmental factors such as infections, unhealthy lifestyles, poor diet, and lack of physical activity can also contribute to the development of rheumatism (Sasmithae *et al.*, 2023)

Ginger (*Zingiber officinale*) is one of the most widely used spices in the world, especially in Indonesia. In ancient times, ginger was highly valued for its medicinal properties. As a medicinal plant, ginger has various health benefits, especially in dealing with inflammation and pain problems. Modern scientific research reveals that ginger contains active compounds such as gingerol, shogaol, and paradol that have anti-inflammatory, analgesic, and antioxidant properties (Yohana & Dewi, 2024)

Ginger (*Zingiber officinale*) It has many benefits, both on a household and industrial scale, including it can be used as a traditional medicine and modern medicine. Testing *Vitro* ginger extract (*Zingiber officinale*) inhibiting the formation of inflammatory components, as well as administering ginger powder to rheumatic patients is reported to reduce the intensity of pain and swelling (Andy

Setyawan *et al.*, 2013). Ginger also has a lot of ingredients such as essential oils, oleoresins and starches so that it can heal the body (Pairul *et al.*, 2017)), besides ginger has many properties such as antihelmintics (Lightweight *et al.*, 2015).

Based on research (Nurfatimah *et al.*, 2024) With the title The Application of Ginger Warm Compress Technique to Control Pain Level with Rheumatoid Arthritis Cases, it shows that there is an effect of ginger warm compress in reducing rheumatoid arthritis pain from pain scale 6 to pain scale 3. Where with 3 doses in the morning for 1 week within 20 minutes can reduce rheumatic pain.

METHOD

This research uses laboratory experimental methods which were carried out from May to July 2025 at the Pharmacy Laboratory of Baubau Polytechnic and the Mandala Waluya Kendari Pharmacy Laboratory.

Pestles, sieves, stirring rods, blenders, petri dishes, beakers, 10 ml measuring cups, cannulas, mice cage, pimples, 1 cc spoits, horn spoons, calipers, analytical scales, handscoons, masks, knives or cutting tools, drip pipettes, and glass jars.

The tools needed in the research are bunsen fires, sieves, basins, stirring rods, belenders, glass funnels, porcelain cups, beaker glasses, measuring cups, scissors, tripods, watch glass, mice cage, cannula, filter paper, parchment paper, coarse cloths, drip pipettes, stretcher spoons, analytical scales, glass jars, *rotary evaporators*, 1 cc spoit and extraction containers. The ingredients used are aquadest, 70% ethanol, carrageenan, diclofenac sodium and 0.5% Na CMC.

The working procedure is the preparation of simplicia, After the ginger rhizome sample is collected, the ginger rhizome sample is washed with running water intended to reduce microbes that can cause decay. Cut into small pieces and then dried by aerating, this process aims to remove the moisture content in the sample. The results of dried simplicia were obtained as much as 1 kg. Then the dried simplisia is blended into a fine powder.

To make the extract, ginger powder is weighed as much as 200 grams, then 200 grams of ginger powder is dissolved in 70% ethanol as much as 2500 mL. Maceration is carried out in a maceration container at room temperature for 3 days by being given occasional stirring, then tapped and poured for 2 days the solution is then filtered using filter paper and all the maserat is collected, the maserat is then evaporated using a *vacuum rotary evaporator* until a thick extract is obtained.

To make 0.5% Na CMC, 0.25 grams of Na CMC are weighed, then put into a beaker containing 50 mL of warm aquadest, stirred until homogeneous.

The test sample was made from ginger extract with different doses (200, 400, and 800 mg/kg BB), while the comparative test solution was made as many as 10 tablets of 50 mg Sodium diclofenac, crushed using a lump and pestle until homogeneous and then weighed Sodium diclofenac which had been crushed by 0.13 mg then put into a beaker containing 0.5% Na CMC suspension as much as 5 mL and stirred until homogeneous.

The experimental animals in the study were randomly divided into five groups, each consisting of three mice. Group 1 was a negative control group that was only given a 0.5% Na CMC suspension of 1 mL. Group 2 as a positive control was given a sodium diclofenac suspension at a dose of 50 mg/kg BB dissolved in 1 mL of 0.5% CMC. Group 3 was given treatment in the form of extract suspension with a dose of 200 mg/kg BB in 1 mL Na CMC 0.5%. Furthermore, group 4 received treatment in the form of a suspension of extract dose of 400 mg/kg BB in 1 mL Na CMC 0.5%, while group 5 was given a dose of extract suspension of 800 mg/kg BB in 1 mL Na CMC 0.5%. (at the Baubau Polytechnic Pharmaceutical Laboratory).

Antirheumatic testing was carried out by weighing the test animals first, induced with carrageenan subplantarly with 0.1 ml of 1% carrageenan solution, the initial volume of leg edema in rats was measured using a caliper after 30 minutes of carrageenan induction, given an oral suspension of the test material according to the group, measurements were taken every 60 minutes for 360 minutes and data were recorded.

RESULTS & DISCUSSION

Research Results

This study is an experimental research using the maceration extraction method with 70% ethanol solvent. then the sample is concentrated using a rotary evaporator with a water bath temperature of 65°C with the aim of preventing the loss of bioactive compounds that are not heat-resistant. The yield of the condensed extract using a rotary evaporator was 18.42 grams, with a yield of 9.21%.

The results of ginger extraction and the results of the yield calculation on the condensed extract are as follows:

Table 1. Results of the Presentation of the Transcript Yield

Weight of simplisia powder	Extract weight	Yields
200 g	18.42 g	9,21 %

Source: Primary Data, 2025

Edema testing in mice's legs for 6 hours using a caliper. The results of edema measurements are shown in Figure 1 through the following table:

Table 2. Measurement results of edema in mice feet

Treatment groups	Mice	Carrageenan induction	After treatment					
			Minute (60)	Minute (120)	Minute (180)	Minute (240)	Minute (300)	Minute (360)
Group I	1	95.23 mm	93.36 mm	90.05 mm	89.42 mm	86.87 mm	84.03 mm	80.26 mm
	2	73.84 mm	70.95 mm	69.92 mm	69.06 mm	68.33 mm	60.48 mm	57.17 mm
	3	82.82 mm	81.07 mm	80.31 mm	83.65 mm	80.86 mm	79.13 mm	70.20 mm
Group II	1	98.39 mm	88.20 mm	75.03 mm	70.24 mm	50.09 mm	39.97 mm	30.01 mm
	2	66.79 mm	57.43 mm	50.16 mm	46.48 mm	24.54 mm	20.32 mm	15.25 mm
	3	88.41 mm	74.30 mm	62.05 mm	57.63 mm	40.58 mm	23.63 mm	19.21 mm
Group III	1	70.62 mm	67.45 mm	60.37 mm	54.29 mm	40.53 mm	30.79 mm	26.32 mm
	2	69.97 mm	61.13 mm	56.78 mm	42.40 mm	39.18 mm	30.00 mm	21.15 mm
	3	90.76 mm	90.00 mm	83.20 mm	78.1 6 mm	60.22 mm	56.43 mm	44.07 mm
Group IV	1	65.80 mm	56.80 mm	48.30 mm	37.00 mm	30.12 mm	25.10 mm	20.64 mm
	2	92.75 mm	90.14 mm	85.22 mm	78.32 mm	60.98 mm	57.25 mm	43.70 mm
	3	73.90 mm	70.65 mm	63.48 mm	56.16 mm	45.89 mm	38.53 mm	25.34 mm
Group V	1	71.98 mm	60.50 mm	49.66 mm	35.96 mm	22.00 mm	19.72 mm	10.21 mm
	2	82.74 mm	74.61 mm	61.35 mm	46.48 mm	25.55 mm	16.74 mm	07.90 mm
	3	77.96 mm	62.51 mm	43.98 mm	34.47 mm	30.12 mm	17.51 mm	04.03 mm

Source: Primary Data, 2025

The results of the mouse leg edema volume test were obtained by the average edema volume of each treatment group was recorded and compared between the negative control group, positive control, and ginger extract group with different doses. The results of the average edema volume test are shown in figure 2 through the following table:

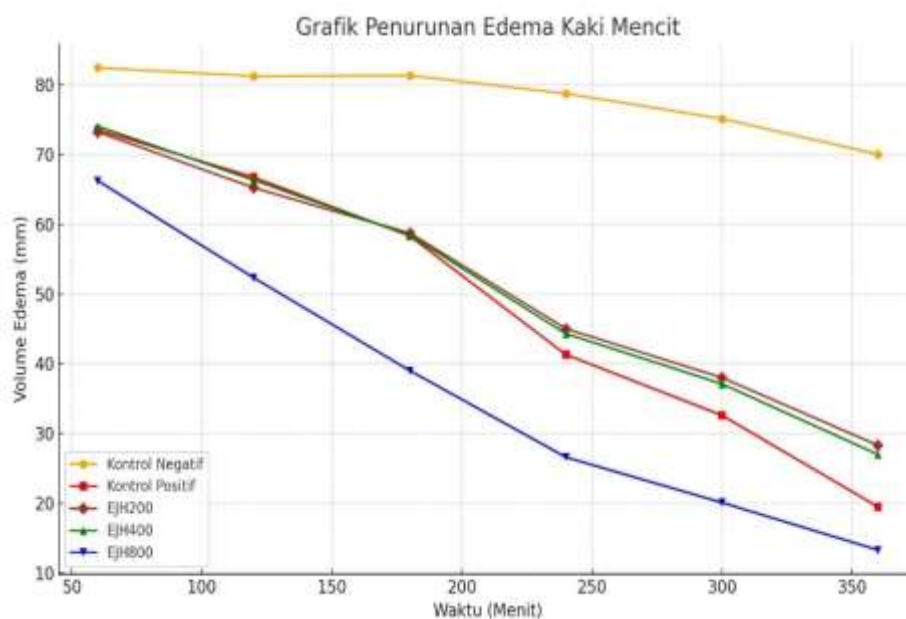
Table 3. Average test results of mouse foot edema

Group	60 minutes	120 minutes	180 minutes	240 minutes	300 minutes	360 minutes
Negative Control	81.79 mm	80.09 mm	80.71 mm	78.69 mm	74.55 mm	69.21mm
Positive Control	73.31 mm	62.41mm	58.12 mm	38.40 mm	27.97 mm	21.49 mm
EJH200	72.86 mm	66.78 mm	58.28 mm	46.64 mm	39.07 mm	30.51mm
EJH400	72.53 mm	65.67 mm	57.16 mm	45.66 mm	40.29 mm	29.89 mm
EJH800	65.87 mm	51.66 mm	38.97 mm	25.89 mm	17.99 mm	7.38 mm

Source: Primary Data, 2025

In Figure 3. showed that the negative control had the smallest decrease in edema, while the positive control showed a greater decrease. The ginger extract groups of 200 and 400 mg/kg BB had moderate effects, while the 800 mg/kg BB dose showed the highest reduction in edema up to the 360th minute. It can be seen as follows:

Figure 4. Decreased edema in mice's feet



Source: Primary Data, 2025

The results of ANOVA analysis can be seen in figure 4. The value of $F_{cal} = 16.68$ was greater than $F_{krit} = 3.478$ with $p\text{-value} = 0.0002$ ($p < 0.05$), thus showing a significant difference between treatment groups on the volume of mouse foot edema. Thus, ginger extract at different doses provides a significantly different effect than the positive control in Figure 4, while the detailed results are shown in the following table:

Table 5. Anova Test Results (*Analysis of Variance*)

Source of Variation	ANOVA					
	SS	Df	MS	F	P-value	F crit
Between Groups	5515,30	4	1378,83	16,68	0,0002	3,478
Within Groups	826,40	10	82,64			
Total	6341,70	14				

B. Discussion

Ginger or *Ginger* It is a type of spice plant that has long been known for its benefits and efficacy, including as herbal medicine or medicine, raw materials for the food industry, beverages, perfumes and cosmetics. Ginger contains many chemical compounds such as Gingerol, Zingerone, Flavonoids, Potassium and Essential oils which have benefits to treat various diseases such as Hypoglycemia, Osteoarthritis, Gout, Rheumatoid Arthritis, Migraine, diseases of the gastrointestinal system, Cardiovascular and hepatoprotective (Nadia, 2020)

The use of natural ingredients as a source of active compounds for medicines has great potential in treating chronic diseases such as rheumatoid arthritis. One of the natural ingredients that has the potential to be a drug for RA (*Rheumatoid arthritis*) is ginger (*Zingiber officinale*) Because ginger has several active compounds as anti-inflammatory agents, namely gingerol. Gingerol is the most abundant ingredient in ginger. Gingerol has anti-inflammatory and antirheumatic effects that have the potential to be an RA drug (*Rheumatoid arthritis*). The mechanism of action of gingerol, first. Gingerol has a powerful antioxidant that can reduce oxidative stress and tissue damage associated with RA (*Rheumatoid arthritis*). (Zhang *et al.*, 2018). second. Modulation of Osteoclast Activation: Gingerol may inhibit the differentiation and activation of osteoclasts, which plays a role in bone erosion in RA (Hwang *et al.*, 2018)

Arthritis or rheumatic disease is a disease that affects the musculoskeletal system and joints, involving several organs and systems of the body as a whole, which is characterized by swelling and pain of the joints. Where arthritis often occurs in adults (Hidayat & Jalil, 2021).

Carrageenan is a mucopolysaccharide obtained from Irish red seaweed (*Chondrus crispus*). Carrageenan plays a role in the formation of uedema in acute inflammatory models (Singh A; Malhotra S; Subban R, 2008). Carrageenan is a foreign substance (antigen) that when it enters the body will

stimulate the release of inflammatory mediators such as histamine so that it causes inflammation because the body's antibodies react to the antigen to fight its effects (Necas & Bartosikova, 2013).

Na CMC or sodium carboxymethyl cellulose is an important additive in pharmaceutical, food, cosmetic fields and is widely used in the field of oral or topical formulations in pharmaceuticals, such as tablet binders, disintegrants and emulsion stabilizers (Yolansari et al., 2022)

This study aims to determine the effect of giving ginger extract with different doses on size change by looking at edema in the legs of mice measured using calipers. The use of calipers is carried out to obtain accurate and precise measurement results on the observed parts of the body of mice, both before induction, after induction, and after treatment.

The extraction process of ginger powder using the maceration method with 70% ethanol solvent has been carried out. The ginger sample was weighed as much as 200 grams, then put in a glass container and added 70% ethanol as much as 2500 mL. The maceration process is carried out for 3 days with occasional stirring to ensure that the active substance can be extracted to the maximum. After the maceration process is complete, the solution is filtered using filter paper or a white cloth to separate the filtrate and the mafiber. The collected maserrate is then evaporated using a rotary evaporator to remove the solvent and produce a viscous extract. The extraction results showed that the condensed extract obtained was 18.42 grams of grams with a yield of 9.21%. This yield shows that the extraction process has succeeded in taking about 9.21% of the initial sample weight, but further analysis is needed to determine the chemical composition of the extracts obtained.

This study used 15 mice with 5 treatment groups, each group consisting of 3 mice. Before treatment, the mice test animals were first fasted for 8 hours but still given water with the intention of avoiding absorption disturbances due to the influence of food. Then antirheumatic effectiveness testing was carried out using a carrageenan solution with a concentration of 1% which can give edema to the feet of mice, injection was carried out subplantar as much as 0.1 ml. Furthermore, treatment was given according to their respective groups, namely negative control using Na CMC 0.5%, positive control using sodium diclofenac 50 mg/20gBB, extract dose group namely 200 mg/20gBB extract in mice, 400 mg/20gBB in mice and 800 mg/20gBB in mice. The result was the amount of edema in mice for 6 hours. It can be seen in table 4.2.

In group 1 (negative control) 0.5% Na CMC suspension was given for 6 hours. At the 60th minute, the volume of edema was quite high (93.36 mm, 70.95 mm, 81.07 mm) and did not show a significant decrease until the 360th minute, with the final averages of about 80.26 mm, 57.17 mm and 70.20 mm. This is due to the absence of active substances in the negative control group treatment in reducing edema. Na CMC only functions as a carrier substance and has no effect on the reduction of edema. Edema persists as a result of carrageenan induction, which triggers the release of histamine and prostaglandins.

In group II, positive control was given a diclofenac sodium suspension. This group showed a sharp and consistent decrease in edema. At the 60th minute, the edema started at 88.20 mm, 57.43 mm, and 74.30 mm, then decreased significantly at the 360th minute to 30.01 mm, 15.25 mm, and 19.21 mm. This proves that sodium diclofenac as a non-steroidal anti-inflammatory drug (NSAID) works effectively inhibiting the synthesis of prostaglandins through the cyclooxygenase (COX) pathway, so that edema can be significantly suppressed.

In group III with the administration of EJH200 (Ginger Extract 200 mg/kg BB) In this group, a decrease in edema occurred but was not very significant. From the 60th minute (67.45 mm, 61.13 mm and 90.00 mm), there was only a moderate decrease until the 360th minute, namely 26.32 mm, 21.15 mm and 44.07 mm. This indicates that at low doses (200 mg/kg BB), ginger extract has not provided optimal antirheumatic effects. Although there are active compounds such as gingerol, it is likely not strong enough to suppress inflammation at this dose.

Group IV with the administration of EJH400 (Ginger Extract 400 mg/kg BB). This group showed a more pronounced decrease in edema. From the 60th minute the volume of initial edema was in the range of 56.80 mm, 90.14 mm and 70.65 until at the 360th minute it decreased to 20.64 mm, 43.70 mm and 25.34. This suggests that moderate doses of ginger extract are starting to provide noticeable anti-inflammatory effects, although they are not yet as effective as the positive control group. The effects tended to be stable and consistent throughout the observation time.

Group V with a dose of EJH800 (Ginger Extract 800 mg/kg BB). This group showed the strongest reduction in edema among all ginger extract groups. At the 60th minute, the edema started at 60.50 mm, 74.61 mm and 62.51 mm, and continued to decrease sharply until it became 10.21 mm, 07.90 mm and

04.03 mm at the 360th minute. This suggests that high doses of ginger extract are highly effective in lowering inflammation, most likely due to the higher content of gingerol and shogaol, which are capable of inhibiting the production of prostaglandins and leukotrienes, as well as having antioxidant effects that strengthen the anti-inflammatory response.

Based on previous research conducted by (Sentat *et al.*, 2018), Analgesic Effectiveness Test of Red Lemongrass Leaf Ethanol Extract (*Cymbopogon nardus*) Doses of 100 mg/kg BB, 300 mg/kg BB, and 600 mg/kg BB indicate that the best dose is at a concentration of 600 mg/kg BB, with the analgesic effect beginning to be seen at the 25th minute and reaching its peak at the 40th to 60th minute. These results are in line with this study, which tested the effectiveness of ginger extract (*Zingiber officinale*) as an anti-inflammatory in mice with doses of 200 mg/kg BB, 400 mg/kg BB, and 800 mg/kg BB. The results showed that the highest dose (800 mg/kg BB) produced the strongest and most stable anti-inflammatory effect, with a decrease in the volume of edema that began to be seen from the 60th minute and persisted consistently until the 360th minute.

The results of the ANOVA (*Analysis of Variance*) test that have been carried out show that the calculated F value is greater than the critical F ($16.68 > 3.478$), in addition, the p-value = 0.0002 which is much smaller than $\alpha = 0.05$. so that H_0 is rejected and H_1 is accepted. This means that there is a significant difference between the average volume of edema in each treatment group. From the results of this ANOVA test, it can be seen that ginger extract is able to provide anti-inflammatory effects, especially at the highest dose (800 mg/kg BB), which shows the most significant decrease in edema volume. This corroborates that ginger has pharmacological activity as an antirheumatist, allegedly due to the content of active compounds such as gingerol and shogaol that are able to inhibit inflammatory processes.

COVER

Based on the results of the study carried out, ginger extract (*Zingiber officinale*) showed antirheumatic effectiveness which was characterized by a volume of reduced edema in the legs of carrageenan-induced mice, with the best dose being 800 mg/kg BB mice, this is because the higher the dose of ginger extract, the higher the effect in reducing the symptoms of rheumatic inflammation in mice.

This study only focused on the effectiveness test of ginger extract in reducing edema in mice, so it did not include toxicity testing or clinical trials to ensure the safety of long-term use.

For further research, it is recommended to conduct toxicity tests and effectiveness tests on other animal models or clinical trials, in order to strengthen the scientific evidence and ensure the safety and effectiveness of ginger extract as an alternative to antirheumatic therapy.

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