The Anti-Inflammatory Activity Of The Black Turmeric (Curcuma Caesia Roxb.) Ethanol Extract In Carrageenan Induced Male Wistar Stream Rats

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Abstract

The high risk of side effects caused by the use of modern medicines has resulted in people turning to the use of traditional medicines. Currently, the treatment of inflammation still relies on conventional drugs, especially non-steroidal anti-inflammatory drugs (NSAIDs), the continuous use of these drugs will certainly cause various adverse side effects. This research aims to determine the anti-inflammatory activity of ethanol extract of Curcuma caesia Robx in carrageenan-induced male rats. In this research, Curcuma caesia Robx was extracted using 80% ethanol solvent, then the viscous extract obtained was made into suspension preparations with concentrations of 32.5mg/ml, 65mg/ml, 97mg/ml, 130mg/ml, and 195mg/ml. The extract was tested for anti-inflammatory testing using the Paw edema method induced by carrageenan 1% w/v to the soles of the rats' feet. Changes in the volume of the rat's feet were observed at 0, 1, 2, 3, 4 and 5. The Kruskal-Wallis test was used to analyze the data, followed by a post hoc Mann-Whitney test with a 95% confidence level. The results of the anti-inflammatory activity test of the ethanolic extract of Curcuma caesia Robx showed a significant inhibition of inflammation ($p < 0.05$) both at doses of 250 mg/kgBW, 500 mg/ kgBW, 750 mg/kgBW, 1000 mg/kgBW, and 1500 mg/kgBW at the 5th hour. This anti-inflammatory activity can be influenced by the content of phytochemical compounds contained in it, including alkaloids, flavonoids, and tannins.

Keywords: Curcuma caesia, Anti-inflammatory, Carrageenan induction method

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INTRODUCTION

Inflammation is a protective response of the body against injury or injury. This condition is not a disease but a manifestation of the disease. This reaction is an effort of the body's defense to eliminate the cause of injury (Pringgoutomo et al., 2002). When an injury occurs, substances such as histamine, bradykinin and prostaglandins as well as serotonin are released. The release of these Substances causes vasodilation and increased permeability of the capillary walls (cells). Blood flow to the site of injury increases, phagocytic cells migrate to the site of injury to destroy substances that are considered harmful. If excessive phagocytosis will actually increase inflammation which is characterized by redness, swelling, heat, pain, and loss of function (Priyanto 2008).

Inflammation treatment is currently still dependent on synthetic drugs class of Non-Steroid Anti-Inflammatory Drugs and corticosteroids. However, long-term use of NSAIDs can cause various side effects such as ulcers and gastrointestinal bleeding, nephrotoxicity, and hepatotoxicity. Steroids can suppress the immune system and trigger erectile dysfunction, manic depression, hypertension, cramps, dizziness, the appearance of active diabetes, skin atrophy, decreased bone density, stomach ulcers with possible gastric wall

perforation, irregular menstruation, vision and allergy problems and reduce healing. wounds (Katzung 2013). So to avoid the side effects caused by the use of NSAIDs, it is necessary to have alternative treatments to reduce the adverse effects of using these drugs.

The rhizomes are usually used for anorexia, dyspepsia, chronic ulcers, pruritus, gout, and inflammations (Thokchom, S. S., and Phucho, 2015). Curcuma caesia Robx. or known as black turmeric is a plant that can be used as an anti-inflammatory. Plants belonging to the Zingiberaceae family are annual plants belonging to the genus Curcuma which are distributed throughout the tropical and subtropical regions of the world. In India this plant is found in the Northeast and Central parts and is also rarely found in Papi Hills in East Godavari, West Godavari and Khammam Districts in East Godavari Andhra Pradesh (Das et al., 2013). Curcuma caesia is widely used in India as an anti-inflammatory and antiasthmatic in Ayurvedic medicine (Arulmozhi D, Sridhar N, Veeranjaneyulu A, 2006). The rhizome EO of Curcuma caesia from south India was composed mainly of 1,8-cineole (30.1%) followed by camphor, ar-curcumene, and camphene (Angel, 2014) while the oil from central India has camphor (28.3%), followed by ar-turmerone, (Z)-β-ocimene, ar-curcumene, and 1,8-cineole (27.0%) (Pandey, A.K.; Chowdhury, 2003), camphor (16.8%) (Behura, S.; Srivastava, 2004), (Saito, 2004), (Moteki, 2002). In Indonesia itself, the popularity of the benefits of black turmeric is also quite well known. Black turmeric is usually used as a herbal medicine to treat stomach pain, increase appetite, increase stamina and overcome coughs. It is also used as a blood purifier, black turmeric is also used as a remedy for rashes, ringworm and scabies to help treat itchy skin. This is because the content in black turmeric is capable of being anti-inflammatory so that in addition to overcoming itchy skin, turmeric can also be used as an anti-inflammatory on the itchy skin (Dinas Kesehatan DI Yogyakarta, 2021).

From a study it is known that the methanol extract of black turmeric extract is known to contain chemical compounds such as alkaloids, flavonoids, phenols, curcuminoids, terpenoids, tannins and proteins (Krup et al., 2013)(Itokawa, H.; Shi, Q.; Akiyama, T.; Morris-Natschke, S.L.; Lee, 2008). In various studies, it is known that some plant secondary metabolites are able to act as anti-inflammatory. Some of these secondary metabolites, one of which is flavonoids, flavonoids are compounds that have various bioactivities, including anti-inflammatory (Miranda & Buhler, 2003). Flavonoids are substances that can inhibit the inflammatory process, flavonoid compounds are said to have anti-inflammatory, antioxidant, and antimicrobial effects (Ilavarasan et al., 2005). Flavonoids are able to protect lipid membranes against damaging reductions (Robinson, 1995). Flavonoids can also inhibit the release of inflammatory mediators such as histamine and prostaglandins (Jayasekara et al., 2002).

This research was conducted to isolate and identify secondary metabolites of black turmeric rhizome ethanol extract. The extraction method used is maceration using ethanol as a solvent. The advantage of using ethanol as a solvent is its low boiling point so that it is easy to evaporate and separate, so that concentrated methanol extract can be obtained through the evaporation process and tends to be safe. Ethanol is also non-toxic and dangerous, besides that ethanol also has a high polarity making it easy to dissolve resin compounds, fats, oils, fatty acids, carbohydrates and other organic compounds (Munawaroh & Handayani, 2010).

Based on this background description, researchers are interested in conducting research on the anti-inflammatory activity of the ethanolic extract of black turmeric rhizome (Curcuma caesia Robx). The test was carried out on carrageenan-induced inflammatory mice.

MATERIAL AND METHODS

3.1 Material

The materials used in this study were black turmeric extract, chloroform, ammonia, sulfuric acid (H2SO4) 2N, dragendrof, ethanol 96%, Mg plate powder, concentrated HCL, 25% NaOH, anhydrous acetic acid, methanol, 1% ferricchloride, CMC Na 0,5%, diclofenac sodium, carrageenan, 0.9% Nacl and aquadest.

3.2 Tools

The tools used in this study were analytical balance (Acis), Blender (Myako), filter paper, porcelain cup, 100 mL glass beaker (pyrex), test tube (Herma), glass funnel, rat cage, water bath, hot plate, rod stirrer, pletismometer, buncher funnel, 100 ml measuring cup, glass jar, syringe.

3.3 Research design

This study is an experimental study with a randomized pre-test and post-test with control group design using 7 test groups. This study was conducted to examine the anti-inflammatory activity of black turmeric extract (Curcuma caesia Robx.) against carrageenan-induced male rats (Rattus norvegicus).

3.4 Variables and Variable Operational Definitions

3.4.1 Research Variables

In this study there are three types of variables:

1. Independent variable, the independent variable is a variable that is applied to the treatment group so that the dependent variable arises. In this study, the independent variable was the ethanol extract of black turmeric which was made in this study.
2. The dependent variable, the dependent variable is the variable caused by the treatment of the independent variable on the subject. In this study, the dependent variable was the volume of inflammation in rat paws induced by carrageenan.
3. Control variables, control variables are variables that are made the same. In this study, the control variable was the experimental animal used, namely male Wistar strain rats aged ± three months and male.

Operational definition

1. The Black Turmeric Extract in this study was an extract of black turmeric rhizome simplicia obtained from extraction using 96% ethanol as solvent.
2. The inflammation referred to in this study is inflammation of the paws of rats induced by carrageenan.
3. Anti-inflammatory efficacy of black turmeric ethanol extract is the effect in suppressing the volume of inflammation in the soles of rats induced by carrageenan and measured with a plethismometer.
4. The rats used in this study were male wistar rats aged 2-6 months.

3.5 Population and sample

3.5.1 Population

The population used in this study was white rats (Rattus norvegicus) Wistar strain aged 2-6 months.

3.5.2 Sample

The sample used in this study was a male Wistar strain white rat obtained from the pharmacy faculty of Mahasaraswati University which had the following criteria:

The sample criteria are divided into three, namely inclusion, exclusion and drop out. Inclusion is a general characteristic of research subjects from the target population who are eligible and will be studied, exclusion is a general characteristic of research subjects from the target population who do not meet the requirements. While drop out is eliminating or removing subjects who meet the inclusion criteria from the study for various reasons. The inclusion and exclusion criteria in this study are:

1. Inclusion criteria

a. Male sex
b. Body weight 100-200 grams
c. 2-6 months old
d. Healthy is characterized by clear eyes, clean hair and active movement
e. No anatomical defects

2. Exclusion criteria

a. Mice are sick, characterized by inactive movements, dull fur, not clear eyes
b. Rats are dead

3. Drop out criteria

The drop out criteria in this study were mice that died during the test.

3.5.3 Sample Calculation

Calculation of the sample that will be used in this study is calculated using the Federer formula.

Federer formula; $(n-1)(t-1) \geq 15$
Source: Federer, W, 1963
Description: n = Number of samples in each treatment group
t = Number of treatment groups
With this formula the number of samples used:
Is known: t = 7
Cause of that, $(n-1)(t-1) \geq 15$

$$(n-1)(7-1) \geq 15$$
$$(n-1)(6) \geq 15$$
$$n-1 \geq 15/6$$
$$n \geq 2.5$$
$$n \geq 3$$

Based on the results of the calculations above, the sample size used in this study was 3 individuals for each group. To avoid drop out, 10% was added to the sample so that the number of samples was 2.5 which were rounded up to 3 rats for each group.

3.6 Research Location and Time

3.6.1 Research Site

Research on Phytochemical Tests and Anti-Inflammatory Activity of Black Turmeric (Curcuma caesia Robx.) Ethanol Extract on Carrageenan Induced Wistar Male Rats was carried out at the Pharmacology and Natural Materials Laboratory, Faculty of Pharmacy, Mahasaraswati University Denpasar, Jalan Cambodja No. 11A Denpasar.

3.7 Procedures

3.7.1 Making Simplicia

Fresh black turmeric rhizome that has been obtained, washed with water until clean and drained. Black turmeric rhizome is cut to a size of 2-3mm. After that, the rhizomes were dried by aerating and covered with black cloth and then dried in an oven at 40ºC until dry. After drying, powdered by means of a blender.

3.7.2 Preparation of Black Turmeric Rhizome Extract

Black turmeric extract was prepared using the maceration method. Each black turmeric powder was weighed as much as 50 g, dissolved in 250 ml of 96% ethanol solvent, and put in 500 ml Erlemayer. The mixture of turmeric powder with solvent was then shaken 2 times for 5 minutes and macerated for 2 x 24 hours. The solution was filtered using whatman paper no. 42. The filtrate obtained was then evaporated using a rotary vacuum evaporator with the aim of evaporating the solvent mixed with the material during the extraction process.

3.7.3 Phytochemical Screening

Phytochemical screening is carried out in the following way:

a. Alkaloid test

A sample of 2 ml was mixed with 2 ml of chloroform and 2 ml of ammonia then heated, shaken and filtered. The sample was then added with 5 drops of 2 N sulfuric acid (H_2SO_4) then shaken and allowed to stand. The top of each filtrate was taken and tested with Dragendroff's reagent. The formation of an orange precipitate indicates the presence of alkaloids (Sastrawan et al., 2013). If the filtrate is added with Mayer's reagent, the formation of a white or yellow precipitate indicates the presence of alkaloid metabolites.

b. Flavonoid test

A sample of 2 ml was mixed with 2 ml of ethanol, shaken, heated, and then shaken again and then filtered. The sample was then added 0.2 g of Mg and 3 drops of concentrated HCl to each filtrate. The formation of a red color on the ethanol layer indicates the presence of flavonoids (Sastrawan et al., 2013). The second phytochemical screening method is, as much as 3 ml of the test solution is added 0.3 g of Mg powder then 1 ml of chlorhydrate alcohol is added, then 2 ml of amyl alcohol is added then shaken vigorously and allowed to separate, a positive result is indicated by the formation of color in amyl alcohol (red), orange or yellow).

c. Saponin test

Samples of 3-7 drops were put into a test tube then added 5 ml of water (H_2O). The solution was shaken for 30 seconds. The foam formed indicated the presence of saponins. The solution was allowed to stand for several minutes if the foam was still stable between 1-10 cm indicating the presence of saponin compounds (Harbone 1987).

d. Steroid test

A sample of 2 ml was added with 2 ml of chloroform, added 3 drops of concentrated sulfuric acid and 10 drops of anhydrous acetic acid. A blue or green color change indicates the presence of steroids (Sastrawan et al., 2013).

e. Terpenoid test

A sample of 2 ml was mixed with 2 ml of chloroform and 2 ml of concentrated sulfuric acid. The formation of a brownish red color between the surfaces indicates the presence of terpenoids (Sastrawan et al., 2013).

f. Tannin test

A 2 ml sample was added with 1 ml of methanol and a few drops of 1% ferric chloride. The formation of a greenish-brown color indicates the presence of tannins (Sastrawan et al. 2013).

3.7.1 Preparation of Preparations

a. Preparation of black turmeric extract solution:

The maximum fluid volume that can be given orally to white rats weighing 200 grams is 5 ml (Stevani, 2016). Black turmeric extract is made in 5 doses, namely black turmeric extract at a dose of 250mg/kgBB, 500mg/kgBB, 750mg/kgBB, 1000mg/kgBB, 1500mg/kgBB. Each black turmeric extract used in this study was then made into a suspension by adding 0.5% CMC Na solution.

Table 3.2 Procedure for Preparation of Black Turmeric Rhizome Ethanol Extract Suspension

Number	Concentration				
	32,5mg/1ml	65mg/1ml	97mg/1ml	130mg/1ml	195/1ml
Black Turmeric Extract	325 mg	650 mg	970 mg	1300 mg	1950 mg
CMC Na	ad 10 mL	ad 10 mL	ad 10 mL	ad 10 mL	ad 10 mL

b. Manufacturing of Diclofenac Sodium Suspension

The positive control used was 9 mg of diclofenac sodium crushed in a mortar, made into a suspension by adding 0.5% CMC solution ad 20 ml then ground until homogeneous.

3.7.4 Anti-inflammatory Activity Test

Testing of anti-inflammatory activity was carried out on male rats aged 2-6 months and weighing 100-200 g, previously adapted to the research environment for 3 days and fasted to eat for 12 hours before the study but still given water. Prior to the study, each rat was marked with a line with a permanent marker on the left hind leg. Rats were divided into 7 groups consisting of 4 rats. All mice were induced by inflammation by subplantar injection of 0.05 mL of carrageenan in saline (0.5% w/v) solution. Furthermore, after being injected with carrageenan, each rat was given treatment on the soles of the feet induced:

Group P1: Group 1 as a positive control was given the anti-inflammatory drug sodium diclofenac at a dose of 4.5 mg/kgBW
Group P2: Group 2 as a negative control was given 0.5% CMC Na suspension.
Group P3: Group 3 as the control group was given ethanol extract of black turmeric rhizome at a dose of 250mg/kgBW
Group P4: Group 4 as the control group was given ethanol extract of black turmeric rhizome at a dose of 500mg/kgBW
Group P5: Group 5 as the control group was given ethanol extract of black turmeric rhizome at a dose of 750mg/kgBW
Group P6: Group 6 as the control group was given ethanol extract of black turmeric rhizome at a dose of 1000mg/kgBW
Group P7: Group 7 as the control group was given ethanol extract of black turmeric rhizome at a dose of 1500mg/kgBW

The treatment was repeated at 0, 1, 2, and 3 hours after measuring edema. Inflammation volume was measured at 0, 1, 2, 3, and 4 hours, on the left hind leg of the rat. Volume was measured to the limit mark using a plethismometer.

2.8 Data Processing and Analysis

The data obtained were tested statistically with the SPSS 22 for windows program, using the analysis of variance (ANOVA) method with a 95% confidence level followed by a Tukey posttest. If the distribution of data is not normal or does not meet the requirements of the ANOVA test, the data is tested using the Kruskal-Wallis test with Mann-Whitney post hoc.

RESULT AND DISCUSSION

4.1 Determination Test Results

In this study, the sample used was black turmeric extract. The black turmeric plant used has been determined at LIPI-UPT Plant Conservation Center "Eka Karya" Botanical Gardens located in Bedugul Bali. The results obtained are as follows:

Kingdom: Plantae (Plants)
Subkingdom: Tracheobionta (vascular plants)
Super division: Spermatophyta (Produce seeds)
Division: Magnoliophyta (flowering plants)
Class: Liliopsida (Plants with monocots)
Subclass: Commelinidae
Order: Zingiberales
Tribe: Zingiberaceae
Clan: Curcuma
Species: Curcuma caesia Roxb.

4.2 Phytochemical Screening Test Results

After obtaining the ethanolic extract of black turmeric from the extraction process by maceration, then phytochemical screening was carried out on the ethanolic extract of black turmeric. Phytochemical screening was carried out to determine the content of phytochemical compounds contained in the ethanolic extract of this black turmeric rhizome. Phytochemical screening carried out includes tests for Alkaloids, Flavonoids, Steroids, Terpenoids, Saponins and Tannins. The results of this phytochemical screening can be seen in Table 4.1.

Table 4.1 Phytochemical Screening Results

No	Compound	Reactor	Results
			Black turmeric extract
1	Alkaloid	Dragendroft	A brown precipitate is formed (+)
2	Flavonoid	Powder Mg + HCL + Ammil alkohol	The top layer is orange (+)
3	Steroid/Triterpenoid	Libermanburcard	Brown color (-)
4	Tannin	$FeCl_3$ 5%	Formation of blackish green color (+)
5	Saponin	Aquades + shuffling 1 minute	No stable foam is formed (-)
6	Kuinon	NaOH 1N	Purple Color Formed (-)

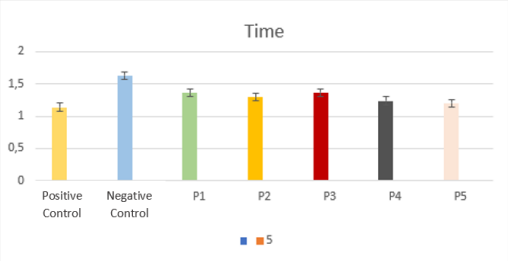
Description: + : identified, - : not identified

The high risk of side effects caused by the use of modern medicines has resulted in people turning to the use of traditional medicines. Currently, the treatment of inflammation still relies on conventional drugs, especially non-steroidal anti-inflammatory drugs (NSAIDs), the continuous use of these drugs will certainly cause various adverse side effects. To overcome these problems, it is necessary to create a treatment innovation to reduce dependence on conventional drugs. Curcuma caesia Robx. or known as black turmeric is a plant that has the potential to be used as an anti-inflammatory. This is due to the presence of secondary metabolites that have anti-inflammatory activity such as alkaloids, flavonoids, phenols, curcuminoids, terpenoids, tannins and proteins (Krup et al., 2013). To determine the potential anti-inflammatory activity of this plant, it is necessary to conduct a preliminary study to prove the anti-inflammatory properties of this plant.

The black turmeric used in this study was first extracted using 96% ethanol as solvent. Ethanol 96% is an organic solvent that is not toxic and relatively safe if used in moderate amounts. In addition, ethanol also has a high polarity so that it is easy to dissolve resin compounds, fats, oils, fatty acids, carbohydrates and other organic compounds (Munawaroh & Handayani, 2010). According to Markham 1988, flavonoid aglycones are polyphenols that have chemical properties of phenolic compounds. The presence of a number of hydroxyl groups, flavonoids are also polar and therefore quite soluble in polar solvents such as ethanol.

4.3 Results of Anti-inflammatory Activity Test of Black Turmeric Rhizome Ethanol Extract Against Carrageenan Induced Rats.

Anti-inflammatory activity test of black turmeric ethanol extract with concentrations of 32.5mg/ml, 65mg/ml, 97mg/ml, 130mg/ml and 195mg/ml were performed on rats induced with carrageenan in 0.5% w/v saline solution. The anti-inflammatory activity test was carried out using 7 test groups, namely, a positive control group, a negative control group, a black turmeric ethanol extract treatment group at a dose of 250mg/kgBW, 500mg/kgBW, 750mg/kgBW, 1000mg/kgBW and 1500mg/kgBW. Each consists of 4 samples. From the test results from the results of the anti-inflammatory activity, the average value of the relative volume of rat foot inflammation at the 1st hour in the positive control group, negative, ethanol extract of black turmeric rhizome 250mg/kgBW, 500mg/kgBW, 750mg/kgBW, 1000mg/kgBW and 1500mg/kgBW are 1.03±0.010, 1.32±0.041, 1.26±0.086, 1.19±0.056, 1.32±0.072, 1.21±0.122, and 1.24±0.122. At the second hour the volume of inflammation of the feet of rats in the positive, negative control group, the ethanol extract of black turmeric rhizome doses of 250mg/kgBW, 500mg/kgBW, 750mg/kgBW, 1000mg/kgBW and 1500mg/kgBW respectively were 1.19± 0.076, 1.49±0.83, 1.37±0.050, 1.32±0.038, 1.39±0.041, 1.26±0.094, and 1.26±0.120. At the 3rd hour the volume of rat foot inflammation in the control group was positive, negative, the ethanol extract of black turmeric rhizome doses of 250mg/kgBW, 500mg/kgBW, 750mg/kgBW, 1000mg/kgBW and 1500mg/kgBW respectively were 1.21± 0.074, 1.52±0.078, 1.41±0.074, 1.39±0.066, 1.43±0.056, 1.31±0.082, and 1.29±0.109. Meanwhile, at the 4th hour the volume of rat foot inflammation in the control group was positive, negative, the ethanol extract of black turmeric rhizome doses of 250mg/kgBW, 500mg/kgBW, 750mg/kgBW, 1000mg/kgBW and 1500mg/kgBW respectively were 1, 18±0.071, 1.56±0.073, 1.40±0.070, 1.37±0.059, 1.41±0.055, 1.27±0.082, and 1.27±0.112. Meanwhile at the 5th hour the volume of rat foot inflammation in the control group was positive, negative, the ethanol extract of black turmeric rhizome doses of 250mg/kgBW, 500mg/kgBW, 750mg/kgBW, 1000mg/kgBW and 1500mg/kgBW respectively were 1, 14±0.071, 1.62±0.067, 1.36±0.070, 1.29±0.074, 1.36±0.056, 1.23±0.085, and 1.19±0.096. The average value of the relative volume of rat paw inflammation at the 5th hour can be seen in Figure 4.1



Picture. 4.1 Bar chart average value of relative volume of rat paw inflammation at 5 hours

Anti-inflammatory activity is the ability of a drug to reduce or suppress the formation of an inflammatory response. A simple anti-inflammatory activity test can be done using the paw edema method, this test is carried out based on changes in the volume of inflammation from experimental animals after an inflammatory stimulant is induced. Testing the Anti-inflammatory activity of the ethanolic extract of black turmeric rhizome was carried out using the paw edema method where the parameter observed in this test was the change in the volume of inflammation in the rat's feet which was measured using a pletismometer where this tool has a working mechanism that applies the principle of Arcimedes' Law. To form inflammation in the feet of rats where previously rats were induced using carrageenan. Carrageenan is an irritant that can be used as an inflammatory agent. Carrageenan is a foreign substance which when it enters the body will stimulate the release of inflammatory mediators such as histamine, causing inflammation due to the body's antibodies reacting to these antigens to counter their effects (Necas & Bartosikova, 2013). Carrageenan-induced rat paw edema model is a suitable test for evaluating anti-inflammatory drugs, which has frequently been used to assess the antiedematous effect of the drug. Carrageenan is a strong chemical use for the release of inflammatory and proinflammatory mediators (prostaglandins, leukotrienes, histamine, bradykinin, TNF-α, etc. (Amdekar S, Singh V, Singh R, Sharma P, Keshav P, 2011) Carrageenan was chosen because carrageenan is an irritant that can cause inflammation by secreting prostaglandins. Therefore, the induction of inflammation with carrageenan is very suitable for testing the anti-inflammatory effect of drugs related to the inhibition of prostaglandin formation. There are two phases of inflammation induced by carrageenan induction, the first phase from 0-1 hours is the histamine and bradykinin release phase and the second phase from 1-6 hours is the prostaglandin release phase.

From the results of the anti-inflammatory activity test, it was found that the average relative volume of rat foot inflammation at the 1st hour in the positive, negative control group, ethanol extract of black turmeric rhizome at a dose of 250mg/kgBW, 500mg/kgBW, 750mg/kgBW, 1000mg/ kgBW, and 1500mg/kgBW were 1.03±0.010, 1.32±0.041, 1.26±0.086, 1.19±0.056, 1.32±0.072, 1.21±0.122, and 1, 24±0.122. At the 2nd hour the volume of rat foot inflammation in the control group was positive, negative, the ethanol extract of black turmeric rhizome doses of 250mg/kgBW, 500mg/kgBW, 750mg/kgBW, 1000mg/kgBW, and 1500mg/kgBW respectively were 1.19 ±0.076, 1.49±0.83, 1.37±0.050, 1.32±0.038, 1.39±0.041, 1.26±0.094, and 1.26±0.120. At the 3rd hour the volume of inflammation of the feet of rats in the positive, negative control group, the ethanol extract of black turmeric rhizome at a dose of 250mg/kgBW, 500mg/kgBW, 750mg/kgBW, 1000mg/kgBW, and 1500mg/kgBW, respectively, was 1, 21±0.074, 1.52±0.078, 1.41±0.074, 1.39±0.066, 1.43±0.056, 1.31±0.082, and 1.29±0.109. Meanwhile, at the 4th hour the volume of rat foot inflammation in the positive, negative control group, the ethanol extract of black turmeric rhizome doses of 250mg/kgBW, 500mg/kgBW, 750mg/kgBW, 1000mg/kgBW, and 1500mg/kgBW, respectively were 1,18±0.071, 1.56±0.073, 1.40±0.070, 1.37±0.059, 1.41±0.055, 1.27±0.082, and 1.27±0.112. Meanwhile, at the 5th hour the volume of rat foot inflammation in the control group was positive, negative, the ethanol extract of black turmeric rhizome doses of 250mg/kgBW, 500mg/kgBW, 750mg/kgBW, 1000mg/kgBW, and 1500mg/kgBW were 1.14±0.071, 1.62±0.067, 1.36±0.070, 1.29±0.074, 1.36±0.056, 1.23±0.085, and 1.19±0.096.

4.1 Data Analysis Results

4.4.1 Result of test of normality

Based on the data obtained from the results of the anti-inflammatory activity test of the ethanolic extract of black turmeric rhizome, further analysis was carried out to see the anti-inflammatory effect of the ethanolic extract of black turmeric rhizome. Before conducting statistical analysis to see the difference in the relative volume of inflammation between groups. So first, a normality test is carried out on the data obtained to determine

what tests are suitable for the data held. From the results of the normality test, it is known that there are data that have a p value <0.05, which means that there are data that are not normally distributed, for that the data obtained cannot be tested by parametric tests. As an alternative, a non-parametric test was carried out with the Kruskal-Wallis test.

Kruskal-Wallis test

The data obtained on testing the anti-inflammatory activity of the ethanol extract of black turmeric rhizome on carrageenan-induced rat paws were then tested using the Krusikal-Wallis statistical method. Based on the results of the Kruskal-Wallis statistical test, it is known that the anti-inflammatory activity test at 5 hours has a p value = 0.036 so it can be concluded that there is a significant difference in the relative inflammation volume between one group and another.

To find out which data have significant differences, a post hoc test was conducted using the Mann-Whitney test. From the results of the Mann-Whitney test, it is known that there is a statistically significant difference at the 5th hour between the negative control group and the positive control group (p = 0.021); negative control group with 250mg/kgBW extract treatment group (p = 0.043); negative control group with 500mg/kgBW extract treatment group (p = 0.043); negative control group with extract treatment group 750mg/kgBW (p = 0.043); negative control group with 1000mg/kgBW extract treatment group (p = 0.043); negative control group with extract treatment group 1500mg/kgBW (p = 0.021).

Based on the results of the Kruskal-Wallis statistical test, it is known that the anti-inflammatory activity test at 5 hours has a p value = 0.036 so it can be concluded that there is a significant difference in the relative inflammation volume between one group and another. To find out which data have significant differences, a post hoc test was conducted using the Mann-Whitney test. From the results of the Mann-Whitney test, it is known that there is a statistically significant difference at the 5th hour between the negative control group and the positive control group (p = 0.021); negative control group with 250mg/kgBW extract treatment group (p = 0.043); negative control group with 500mg/kgBW extract treatment group (p = 0.043); negative control group with extract treatment group 750mg/kgBW (p = 0.043); negative control group with 1000mg/kgBW extract treatment group (p = 0.043); negative control group with extract treatment group 1500mg/kgBW (p = 0.021). Meanwhile, if each treatment was compared with the positive control group, there was no significant difference in the volume of inflammation between the groups (P>0.05). This shows that the ethanol extract of black turmeric rhizome at doses of 250mg/kgBW, 500mg/kgBW, 750mg/kgBW, 1000mg/kgBW, and 1500mg/kgBW have anti-inflammatory activity which is relatively the same as the positive control.

The anti-inflammatory activity of the five variations in the concentration of black turmeric rhizome ethanol extract is relatively the same even though it has different concentrations, this shows that the ethanol extract of black turmeric rhizome at a dose of 250mg/kgBW is more effective than black turmeric extract at a dose of 500mg/kgBW, 750mg/kgBW, 1000mg/kgBW, and 1500mg/kgBW. According to Amir Syarif et al., (2016), the intensity of the effect of a drug is directly proportional to the fraction of receptors it occupies or binds to, and the intensity of the effect reaches a maximum if all receptors are occupied by the drug. This means that when all receptors have been occupied by the drug, increasing the dose of the drug cannot increase its effect.

The results of the phytochemical screening test showed that this extract contained several phytochemical compounds such as alkaloids, flavonoids, and tannins. The presence of these compounds is responsible for the anti-inflammatory activity of this plant. The anti-inflammatory mechanism carried out by flavonoids can be through several pathways such as inhibiting the activity of COX enzymes and lipoxygenase directly which causes inhibition of the biosynthesis of prostaglandins and leukotrienes which are the end products of the COX and lipoxygenase pathways. This can inhibit leukocyte accumulation and neutrophil degranulation, thereby directly reducing the release of arachidonic acid by neutrophils, as well as inhibiting histamine release. Derivatives of flavonoid compounds such as quercetin have been proven to have anti-inflammatory properties. Quercetin has been shown to inhibit the

production of cyclooxygenase (COX) and lipoxygenase (LOX) which are normally induced by inflammation. Quercetin downregulates the production of COX-2, Nuclear Factor-kappa B (NF-κB), and Nitric Oxid (NO) (Shuang, 2016). In addition to flavonoids and alkaloids, it was also explained that the alkaloids have anti-inflammatory activity. 2017). Based on the results of this study, the ethanolic extract of black turmeric rhizome has potential as an oral anti-inflammatory drug so that it can be used as a product that can be used daily.

CONCLUSION

Based on the results of the research that has been carried out, it can be concluded that the ethanolic extract of black turmeric rhizome has anti-inflammatory activity in carrageenan-induced rats. The content of compounds contained in the ethanol extract of turmeric rhizome such as Alkaloids, Flavonoids, Terpenoids and Tannins.

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