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Antioxidant and Anti-inflammatory Activities of Extract Ethanol *Curcuma zedoaria*

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Abstract

BACKGROUND: Inflammation is a response of the body to injury or infection. When an injury occurs, the body will try to neutralize and eliminate harmful agents from the body and prepare for tissue repair. White turmeric is known to have secondary metabolites that have pharmacological activities such as antioxidants, anti-hyperlipidemia, antidiabetic, and others.

AIM: This study aims to determine the anti-inflammatory, antioxidant, and total phenol and flavonoid activity of white turmeric ethanol extract.

MATERIALS AND METHODS: This study used carrageenan as an inflammatory inducer. This study was divided into five groups, namely, the normal group, the CMCNa group, the EEKP group at a dose of 300 mg/kg, the EEKP group at a dose of 600 mg/kg, and 900 mg/kg. And in this study, measurements of IC_{50}, total flavonoids, and total phenol of white turmeric ethanol extract were carried out. The results showed that the ethanol extract of white turmeric at doses of 300, 600, and 900 mg/kg bw had anti-inflammatory activity by decreasing the percentage of inflammation and increasing the percentage of inflammation inhibition. The dose of 600 mg/kg bw had no statistically significant difference ($p > 0.05$) with the diclofenac sodium group.

RESULTS: Analysis of the results of inflammatory infiltration in gastric histopathology in Groups P1, P2, P3, and P4 showed mild inflammatory infiltration compared to Groups P(5) and K(+). The description of the acinar glands on gastric histopathology showed that the P2 group gave a better picture of the acinar gland repair than the K(+), P2, P3, P4, and P5 groups.

CONCLUSION: It can be concluded that white turmeric ethanol extract has anti-inflammatory and antioxidant activity.

Introduction

Inflammation is a response of the body to injury or infection. When an injury occurs, the body will try to neutralize and eliminate harmful agents from the body and make preparations for tissue repair. The presence of an inflammatory process is characterized by characteristic features, namely, the appearance of redness, swelling in the area of inflammation, burning sensation, and the onset of pain [1], [2], [3]. Ferdhyanti stated that an increase in the number of leukocytes (leukocytosis) occurs physiologically and pathologically. Physiologically, there is an increase in the number of neutrophil cells and lymphocytes in the circulation. While pathologically, active leukocytes against microorganisms can increase the total number of leukocytes in the circulation. The number of leukocytes in each individual can reach high values, under the following conditions: Stress, physiological activity, nutrition, and age. According to Armansyah, types of leukocytes consist of neutrophils, basophils, eosinophils, monocytes, and lymphocytes. The five types of leukocytes can be increased (leukocytosis) or decreased (leukopenia). In the blood of white rats, it is normal to have an average leukocyte count of $6.1–10.5×10^3$/mm of blood [4], [5], [6]. Leukocytes will increase if the adrenal glands are stimulated as a physiological response such as stress. Turmeric or *Curcuma zedoaria* is a rhizome plant that has been widely known by the world, both on a household scale and on an industrial scale. Curcumin contained in turmeric rhizome is useful as an antitumor and anti-inflammatory. Saponins are efficacious as antineoplastic (anticancer) while beta-carotene, polyphenols, and flavonoids function as antioxidants [7], [8], [9], [10].

White turmeric or *C. zedoaria* is recommended to treat inflammation or inflammation, high cholesterol, stomach pain, menstrual disorders, wounds, eczema, jaundice, inflammation, symptoms of cancer, and as a blood purification activity [11], [12]. The part that is often used is the rhizome. Turmeric rhizome can be used as an anticoagulant, lowering blood pressure, malaria medicine, anthelmintic, bactericide, stomachache medicine, increasing breast milk, fungicide, stimulant, treating sprains, bruises and rheumatism, asthma medicine, diabetes mellitus, appendicitis, tonsillitis, thrush, add blood, remove acne and black spots on the face, protect the heart, inflammation of the nose, reduce fever, relieve itching, cure seizures, treat wounds, and

cure liver disease. Apart from being a medicine, turmeric is widely used as a kitchen spice. This study aims to determine the antioxidant activity and anti-inflammatory effects of white turmeric (*C. zedoaria*).

Materials and Methods

White turmeric rhizome, 96% ethanol, filter paper, aluminum foil, 3 ml syringe, 1 ml syringe, distilled water, CMC Na, diclofenac sodium, carrageenan, DPPH, methanol, Vitamin C, gallic acid, Folin–Ciocalteu solution, sodium carbonate, quercetin, aluminum chloride, and sodium acetate were used.

Animal

The experimental study used 30 rats (*Rattus norvegicus*) in good health and weighing between 150 and 200 g. Rats are housed in plastic cages with a humidity level of 40–60% and a 12 h dark/light cycle. In addition, rats were given cratachem producing pellet diet and water *ad libitum*. The University of North Sumatra had granted ethics clearance for this project.

Plant

Sampling was carried out purposively without comparing with the same plants from other areas. The rhizomes were obtained from the Simpang Limun traditional market in Medan, North Sumatra.

Ethanol extract preparation

To begin the maceration process, dissolve the white turmeric in a solvent, particularly ethyl acetate in a 1:10 (w/v) ratio, dissolved in 10 parts ethyl acetate, then poured with 75 parts 96% ethyl acetate. Covered and kept in a dark place for 5 days, stirring occasionally. After 5 days, the solution was filtered, the dregs were squeezed out, and the solution was rinsed with sufficient water to get 100 parts. The juice (Maserati) was transferred to a closed vessel and stored in a cool, shaded area for 2 days. The resulting extract was then evaporated at a temperature of 50°C in an evaporator, dried, and weighed [13].

Ethanol extract preparation

White turmeric extract was made by maceration method using 96% ethanol. As much as, 1000 g of white turmeric powder is put into a glass container then add 75 parts of 96% ethanol (7.5 L), cover and leave for 5 days protected from light while occasionally stirring, strain, squeeze, and wash the dregs with a liquid filter as much as 25 parts (2.5 L) to obtain 100 parts (10 L). Transfer to a closed vessel, leave in a cool place, protected from light for 2 days and then pour or filter. The obtained Maserati was then concentrated with a rotary evaporator at a temperature of ±40°C until an almost thick extract was obtained. Continue the evaporation process on a water bath until a thick extract is obtained [14].

Antioxidant activity of ethanol extract of white turmeric

The principle of testing antioxidant activity using the DPPH method is to look at the ability of the test preparation (white turmeric ethanol extract) in reducing the oxidation process of DPPH free radicals (1,1-diphenyl-2-picrylhydrazyl) which are free radicals in methanol solution (so that the DPPH color changes from purple to yellow) with an IC_{50} value (concentration of the test preparation capable of reducing free radicals by 50%) was used as a parameter to determine the antioxidant activity of the test preparation [15].

Total phenol of the ethanol extract of white turmeric

Weighed 10.5 mg of white turmeric ethanol extract then dissolved with methanol, put into a 10 mL volumetric flask, and made up to the mark line. 0.5 mL of this solution was pipetted and 2.3 mL of distilled water and 0.2 mL of Folin–Ciocalteu solution were added, vortexed for ± 1 min then allowed to stand for 4–8 min, added 2 mL of 20% sodium carbonate solution, and allowed to stand for 70 min and the absorbance was measured at a wavelength of 775 nm. Measurements were carried out with five repetitions. The phenol concentration was calculated from the substitution in the linear equation and expressed as the equivalent number of milligrams of gallic acid in 1 g of the extract [16].

Total flavonoid of the ethanol extract of white turmeric

Weighed as much as 10.5 mg of white turmeric ethanol extract, dissolved in ethanol, put into a 10 mL volumetric flask, and filled with methanol to the mark line. 0.5 mL of this solution was pipetted, added 1.5 mL of methanol, 0.1 mL of 10% aluminum chloride solution, 0.1 mL of 1 M sodium acetate, and 2.8 mL of distilled water and allowed to stand for 30 min then the absorbance was measured at wavelength of 436 nm. Measurements were carried out with five repetitions. The flavonoid concentration was calculated from the substitution in the linear regression equation and expressed as the equivalent number of milligrams of quercetin in 1 g of extract [17].

Anti-inflammatory activity of extract

The anti-inflammatory effect was tested using a Digital Plethysmometer with Archimedes' law principles. Before testing the animals were fasted for 18 h but were still given water. The tail and left leg of the rats were marked and the weight of each rat was weighed. Rats were grouped into six groups, namely:

- Group I: Normal group (without treatment)
- Group II: The group with 0.5% CMC Na suspension + 0.1 ml carrageenan induction intraplantar.
- Group III: The group was given 0.1 ml of 0.1 ml of diclofenac sodium suspension + 1% carrageenan induction intraplantar.
- Group IV: The group given EEKP suspension at a dose of 300 mg/kg bw + 0.1 ml carrageenan induction intraplantar.
- Group V: The group given EEKP suspension at a dose of 600 mg/kg bw + 0.1 ml carrageenan induction intraplantar.
- Group V: The group with the EEKP suspension at a dose of 900 mg/kg bw + 1%carrageenan induction intraplantar.

Clean the mouse's feet with a tissue from the dirt that sticks to the feet. The volume of the left leg of each rat was measured by inserting the marked rat's paw into the cell on a Digital Plethysmometer containing a special liquid until the marking line on the rat's foot then holding the pedal, the numbers were recorded on the monitor. The number is recorded as the initial volume (V0), which is the volume of the rat's feet before being treated. Each rat was treated according to the group by orally. 0.1 ml of 1% carrageenan solution was injected intraplantarly into the left foot of each rat after 60 min of treatment. The rat's paw volume was measured after 30 min of injection of 1% carrageenan. The numbers are recorded on the Plethysmometer monitor screen. Changes in fluid volume that occur are recorded as the volume of the rat's paws at a certain time (Vt). Measurements were made every 30 min for 6 h and each time the measurement of the solution in the cell was still filled between the two red lines [18], [19].

Leukocytes counts

As many as 30 test animals that will be given treatment are acclimatized first, fasted, capillary blood is taken through the tail end, inserted into a polytube that already contains EDTA for leukocyte count (early rat leukocytes). Each rat was given treatment according to the treatment group. Capillary blood was taken from the tail end at 6 h after treatment, put into a polytube tube containing EDTA for the calculation of the leukocyte count of each rat (final leukocyte count). The total and type of leukocytes were counted in the UPT, Regional Health Laboratory of North Sumatra Province [20].

Data analysis

Statistical Package for the Social Sciences (SPSS) program 21 was used to analysis of the data. Data are expressed as mean SEM. Comparison for more than 2 groups using one-way ANOVA followed by *post hoc* Tukey. Statistical significance was set at $p < 0.05$.

Results

Antioxidant result

The antioxidant activity of EEKP was obtained from the measurement of DPPH absorbance with the addition of test solutions with the concentrations of 50 g/mL, 100 g/mL, 200 g/mL, and 400 g/mL and concentrations of 2 g/mL, 4 g/mL, 8 g/mL, 16 g/mL, and 32 g/mL for the ethanol extract of white turmeric, which were compared with the DPPH control (without the addition of the test solution). The data are shown in Table 1.

Table 1: IC_{50} of the extract

Samples	Regression equation	IC_{50} (ppm)
Vitamin C	Y = 17.54872X+2.7315	2.456789099
Extract	Y = 3.76X+0.955	14.98765893

In Table 1, the results of the ability of white turmeric showed that the ability of white turmeric to reduce radicals from DPPH with an IC_{50} value of 14.98 when compared to Vitamin C, namely, IC_{50} of 2.456. This shows that EEKP has a very strong antioxidant, which is below the IC below 50.

Total phenol and total flavonoid of extract

The results of total phenol and total flavonoid of the extract are shown in Table 2.

Table 2: Total phenol and total flavonoid of the extract

Sample	Total flavonoid	Total phenol
Extract	41.35	81.31

In Table 2, the results showed that the levels of flavonoids showed 41.53 levels equivalent to quercetin while in the phenol group, it was 81.31 equivalent to gallic acid.

Percent of inflammation and percent inhibition of inflammation

The percent of inflammation is shown in Figure 1 and the percent inhibition of inflammation is shown in Figure 2.

In the group given 0.5% Na CMC suspension without any active compounds in it, a very significant

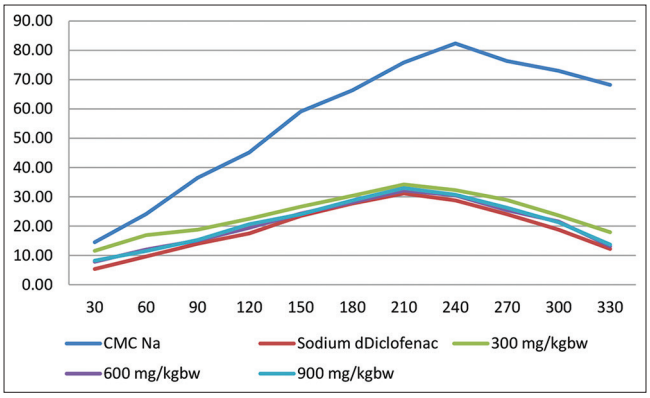

Figure 1: Percent of inflammation

increase in leg volume was seen with a much larger volume than the other test groups. This negative group becomes a reference in comparing the results achieved by other groups. The test group with significantly different results from the negative control group showed the effect of the compound or drug substance given to the test animals. The group that was given the treatment had the effect of decreasing the percentage of inflammation and had a significant difference ($p < 0.05$) against the CMCNa group. In the group given EEKP 300 mg/kg, there was an increase in the percentage of inflammation from the 60th min and a decrease in the 240th min. In the group given EEKP 600 mg/kg bw, there was also an increase in the percentage of inflammation, namely, at the 60th min and a decrease in the percentage of inflammation in the 240th min. At the EEKP dose of 900 mg/kg bw, it also increased at the 60th min and decreased at the 240th min.

Figure 2 shows the ability of each group to suppress inflammation, which is then called the percent inflammation inhibition. In the group that was given diclofenac sodium 25 mg/kg bw and 600 mg/kg bw as an anti-inflammatory agent, the ability to suppress inflammation was very significant ($p<0.05$) when compared to the group that was only given EEKP at a dose of 300 mg/kg bw and given EEKP at a dose of 900 mg/kg bw, there was also an increase in the ability to inhibit inflammation.

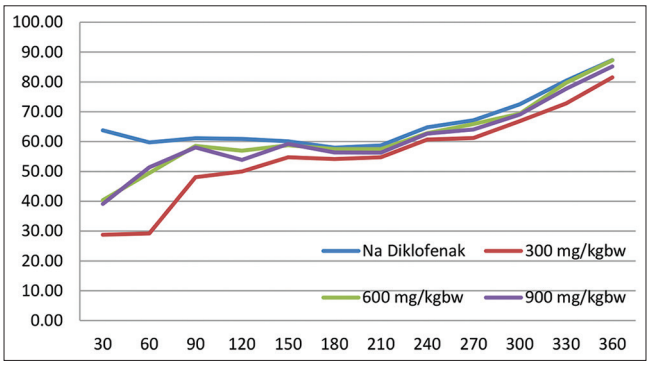

Figure 2: Percent inhibition of inflammation

White blood cells count

White blood cells of each groups are shown in Table 3.

Table 3: White blood cells count

Groups	Neu (%)	Lym (%)	Monosit (%)	EOS (%)	BAS (%)
Normal	49.00	32.43	4.40	0.73	14.90
CMCNa	26.57	56.70	7.83	1.30	7.60
Sodium diclofenac	41.07	41.33	9.87	1.60	6.13
300 mg/kg bw	38.53	35.93	11.63	0.87	13.03
600 mg/kg bw	30.17	48.73	10.80	1.27	9.03
900 mg/kg bw	59.10	19.30	12.93	0.67	8.00

Discussion

In this study, the levels of neutrophils, lymphocytes, eosinophils, and basophils were measured. At the initial measurement, the levels of neutrophils, lymphocytes, eosinophils, and basophils were normal. Meanwhile, after induction of carrageenan as an inflammatory agent, it was seen that there was an increase in the levels of neutrophils, lymphocytes, and monocytes, a decrease in eosinophils and basophils in the group that was only given CMCNa as a negative control group, whereas in the group given diclofenac sodium, there was an increase in neutrophils and monocytes while there was a decrease in the levels of lymphocytes, eosinophils, and basophils. In the group that was given the extract, the 300 mg/kg bw extract showed an increase in neutrophils and monocytes, while there was a decrease in the levels of lymphocytes, eosinophils, and basophils. For the group given 600 mg/kg bw extract, there was an increase in neutrophil levels, while there was a decrease in lymphocyte, eosinophil, basophil, and monocyte levels. In the group given 900 mg/kg bw extract, there was an increase in the levels of neutrophils and monocytes, while there was a decrease in the levels of lymphocytes, eosinophils, and basophils. Monocytes are white blood cells that resemble heterophils, are phagocytic, namely, the ability to attack foreign materials, such as bacteria [21]. The average number of monocytes produced was above normal, namely, 31.75 × $10^3/\mu L$, this was due to monocytes playing a role in regulating immune response by releasing monokine regulatory glycoproteins such as interferons and interleukin I, F and pharmacologically active substances such as prostaglandins and lipoproteins. Monocytes are leukocytes that make up about 3–8% of the total leukocytes. The nucleus is usually eccentric with a deep groove in the shape of a horseshoe. Monocytes have a higher number of primary lysosomes but smaller in size. The number of endoplasmic reticulum, ribosomes, and polyribosomes is small, while the mitochondria are many. The Golgi apparatus is well developed, microfilaments and microtubules are found in the nuclear indentation area. Monocytes are found in the blood, connective

tissue, and body cavities. Monocytes are mononuclear phagocytic and have receptor sites on their membrane surfaces. Monocytes circulate through the blood stream and penetrate the capillary walls into the connective tissue. Monocytes in the tissue react with lymphocytes and play an important role in the recognition and interaction of immunocompetent cells with antigens [22]. Monocytes are known as macrophages after leaving the bloodstream and entering the tissue [23]. An increase in monocytes indicates infection, inflammation, tissue necrosis, or leukemic neoplasia. In addition, trauma and stress, both emotional and physical, can increase leukocyte values. In the setting of infection, especially sepsis, the leukocyte value will usually be very high. This phenomenon is referred to as a leukemoid reaction and will improve quickly if the infection is successfully treated. The decrease in the number of monocytes after oral treatment with white turmeric ethanol extract was due to the action of flavonoids. Flavonoids are polyphenolic compounds that act as antioxidants, which in blood cells can act as a reservoir for hydroxyl radicals and superoxide so as to protect membrane lipids. Antioxidants can protect certain substances (especially fatty ones) from oxidation, including attacks from free radicals [24].

The anti-inflammatory effect is thought to be due to the activity of secondary metabolites contained in the ethanol extract of white turmeric, namely, flavonoids, steroids, and tannins. This is supported by the results of phytochemical screening tests which indicate the presence of these groups of compounds [25].

The mechanism of flavonoids in inhibiting the process of inflammation is done in two ways, namely, by inhibiting capillary permeability and inhibiting arachidonic acid metabolism and lysosomal enzyme secretion from neutrophil cells and endothelial cells. Flavonoids mainly act on the microvascular endothelium to reduce the occurrence of hyperpermeability and inflammation. Several flavonoid compounds can inhibit the release of arachidonic acid and the secretion of lysosomal enzymes from the membrane by blocking the cyclooxygenase pathway. Inhibition of the cyclooxygenase pathway can have a wider effect because the cyclooxygenase reaction is the first step in the pathway to eicosanoids such as prostaglandins and thromboxane. In addition to flavonoids, it is known that triterpenoid and saponin compounds are thought to play an anti-inflammatory role [26].

The anti-inflammatory activity of saponins from various plants has been widely reported, but not much is known about the exact anti-inflammatory mechanism carried out by saponins. Saponins consist of steroids or triterpene groups (aglycones) which have a detergent-like action. The most likely anti-inflammatory mechanism is that saponins are thought to be able to interact saponins as anti-inflammatory, saponins are thought to interact with many membrane lipids, such as phospholipids which are precursors of prostaglandins and other inflammatory mediators [27].

Conclusion

It can be concluded that white turmeric ethanol extract has anti-inflammatory and antioxidant activity.

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