Study of antiinflammation activity in ethanol extract from coriander leaf (*Coriandrum sativum* L.) induced by carrageenan in male rats

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Abstract. Coriander (*Coriandrum sativum* L.) is a popular spice plant in Indonesia. This plant has benefits as traditional herbal medicine plants, especially in the leafy part of which are useful as anti-inflammatory. The objective of this research is to determine the anti-inflammatory effects of ethanol extract of coriander leaves on the soles of male rats induced by λ-carrageenan. The method used in this study is the paw edema method using a digital plethysmometer with the principle of measurement based on Archimedes' law and carried out experimentally by dividing 25 mice into 5 groups. Group 1, 2, and 3 were given ethanol extract of coriander leaves (EECL) with each doses of 100, 200, and 400 mg/kgbw, group 4 as negative control, group 5 as positive control given diclofenac sodium, then the volume is measured. The results of this study from tukey test results on inflammation showed that EECL doses of 100, 200, and 400 mg/kgbw didn’t differ significantly (p> 0.05) with diclofenac sodium dose of 2.25 mg/kgbw at 240 minutes to 360 minutes, but significantly different from CMC Na 0.05% (p<0.05). In inhibition percent of inflammation EECL doses of 100, 200 and 400 mg/kgbw didn’t differ significantly (p> 0.05) with diclofenac sodium dose of 2.25 mg/kgbw at 30 minutes to 360 minutes. The Conclusion of this study is EECL doses of 100, 200, and 400 mg/kgbw have anti-inflammatory activity in carrageenan-induced male rats. EECL dose of 400 mg/kgbw has the best average inflammation inhibitory activity.

1. Introduction

Inflammation is linked to certain diseases that can be found in the community, usually characterized by swelling, pain, redness, and heat. The inflammation can be caused by a normal protective response to tissue injury from physical trauma, hazardous chemicals, or microbiological agents. Inflammation is a response of the body's efforts to activate or destroy invading organisms, remove irritants and prepare phase of the tissue repair process. Steroid and non-steroid anti-inflammatory drugs have many side effects so that many anti-inflammatory developments are made from natural ingredients, especially in plants. Plants that are scientifically proven to have antiinflammatory characteristic, one of which is from zingiberaceae family, such as kencur, leaves of tamarind, etc. [1].

Coriander plant is one of the spice traditionally plants which is often used as a food flavoring ingredient that has the potential to be developed as a medicinal or nutritious food ingredient. It is a plant that is traditionally used for the treatment of various diseases including inflammation [2]. According to research conducted by Tansos, (2019) explains that coriander leaves have secondary
metabolites, namely alkaloids, tannins, saponins, flavonoids and steroids. While ethylacetate extract only contains a number of secondary metabolites, namely flavonoids (quercetin), saponins and steroids. Traditionally, coriander isused for the treatment of antiseptic, gastrointestinal complications, antidiabetes, and antihyperglycemic\cite{11,3}. The aim of this studyis to investigate the anti-inflammatory effects of ethanol extract of coriander leaves in carrageenan-induced male rats.

2. Materials and Methods

2.1 Materials
The materials were coriander leaves (Coriandrum sativum L.), distilled water, ethanol 96% (distillate yield), 0,9% NaCl solution and propanolyzed quality ingredients (E. Merck): α-naphthol, concentrated nitric acid, acetic acid anhydride, concentrated hydrochloric acid, concentrated sulfuric acid, iron (III) chloride, ethyl acetate, iodine, isopropanol, potassium iodide, chloroform, methanol, sodium hydroxide, sodium chloride, n-hexane, mercury (II) chloride, magnesium powder, simvastatin, lead (II) acetate, choral hydrate and toluene, Na-CMC (sodium carboxy methyl cellulose).

2.1.1 Sample Collection. Sampling is done purposively, without comparing with the same brand from other regions. The sample used in this study was coriander leaves from Berastagi, Medan, North Sumatra Province.

2.1.2 Preparation of Samples. Fresh coriander leaves are collected and then cleaned, then washed under running water several times until clean, drained and spread on parchment until evenly distributed while aerated until the water is absorbed, then put in a drying cabinet at a temperature of ± 40 ° C until the leaves are dry and easily brittle. Simplicia is mashed and stored in plastic containers to prevent the influence of moisture and other impurities. The resulting simplicia was divided into two parts, namely for phytochemical screening and 96% ethanol extract of coriander\cite{11}.

2.2 Preliminary Phytochemical Screening
The crude extract of EELC was screening by using the standard protocol to know the presence of phytochemical compounds\cite{4,5}.

2.3 Preparation of Ethanol Extract Of Coriander Leaves (EECL).
Extract of coriander leaf was prepared by the ethanolic solvent by using maceration method for 5 days. About 300 g of the fine powder coriander leaf was extracted with 96% ethanol by using maceration for 5 days at room temperature. The ethanol extract of coriander leaf (EECL) solution part was evaporated by using rotary evaporator to get the crude extract\cite{6}.

2.4 Simplicia and Extract Characterization Examination.
Simplicia characterization includes macroscopic examination, microscopic examination, determination of water content, determination of water soluble extract content, determination of ethanol soluble extract content, determination of total ash content and determination of acid insoluble ash content. Extract characterization includes determination of water content, determination of total ash content and determination of acid insoluble ash\cite{7}.

2.5 Preparation of 0.5% Na-CMC Suspension
A total of 0.5 g Na-CMC was sown in a mortar filled with hot distilled water. Leave for 15 minutes, then crushed to obtain a transparent mass, then crushed until homogeneous, diluted with distilled water, homogenized and put into a 100 ml flask, volume sufficient with distilled water up to 100 mL\cite{8}.
2.6 Doses of Ethanol Extract of Coriander Leaves (EECL)
The doses of EECL given to animals are 100, 200, 400 mg/kg of weight orally. Amounts of 100, 200, and 400 mg of EECL were weighed and put into mortars and added 0.5% Na-CMC suspension, and then the volume was sufficient to 10 mL.

2.7 Preparation of Diclofenac Sodium Suspension
Weighed 20 diclofenac sodium tablets taken, crushed, then weighed in total. The weight of the active ingredient is diclofenac sodium in 1 tablet of diclofenac sodium which is 25 mg. The total active ingredient content in 20 tablets is 500 mg. Weigh the tablet equivalent to 2.25 mg/kg bw, then put into a mortar and add a 0.5% w/v Na-CMC suspension little by little while crushed until homogeneous, the volume is sufficient to 10 mL [8].

2.8 Preparation of λ-carrageenan 1%
Weighed 100 mg λ-carrageenan, then crushed until homogeneous with 0.9% NaCl solution and then put into a 10 ml volumetric flask, adequately with 0.9% NaCl solution to the marked line. Incubated at 37°C for 24 hours [9].

2.9 Anti-inflammatory Activity Test
Before testing, the rats were fasted for 18 hours while still being given in drinking water. Rats were grouped into 5 groups, namely the negative control group (Na-CMC suspension), positive control (diclofenac sodium) and the ethanol extract group of coriander leaves (EECL) with doses of 100; 200; and 400 mg/kg bw.

- Group I: 0.5% Na-CMC Suspension
- Group II: diclofenac sodium suspension 2.25 mg/kgbw
- Group III: Suspension EECL dose of 100 mg/kgbw
- Group IV: Suspension EECL dose of 200 mg/kgbw
- Group V: Suspension EECL dose of 400 mg/kgbw

Rats were marked on their left foot, then foot volume was measured before treatment using a plethysmometer. Each animal was given a test preparation orally according to the group. After 1 hour, each rat was induced 0.1 mL λ- carrageenan 1% sub-plantar. Volume measurement was performed every 30 minutes for 6 hours after induction of carrageenan [10].

The inflammation percentage can be calculated using the formula below [12]:

\[
\text{Percent of inflammation (\%) } = \frac{V_t - V_0}{V_0} \times 100
\]

Note: 
- \(V_t\) = The volume of rat feet at time \(t\)
- \(V_0\) = The initial volume of rat feet

Percent of inflammation inhibition can be calculated by the formula below:

\[
\text{Percent of inflammation inhibition (\%IR) } = \frac{a - b}{a} \times 100
\]

Note: 
- \(a\) = The inflammation percent of the average control
- \(b\) = The inflammation percent of the average treatment group or comparative drug

2.10 Data Analysis
Data were tested for normal distribution and homogeneity of variants (p> 0.05), then data were analyzed using ANOVA. Contains a number of secondary metabolites, namely flavonoids (quercetin), saponins and steroids.
3. Results And Discussion

3.1 Preliminary Phytochemical Screening
Preliminary phytochemical screening was tested by standard procedure on the EECL. The result of phytochemical screening of simplicia and coriander leaf extract can be seen Table-1.

| No. | Screening   | Results Simplisia | Extract |
|-----|-------------|--------------------|---------|
| 1   | Flavonoids  | Positive           | Positive |
| 2   | Alkaloids   | Positive           | Positive |
| 3   | Saponins    | Positive           | Positive |
| 4   | Tannins     | Positive           | Positive |
| 5   | Glycosides  | Positive           | Positive |
| 6   | Steroids    | Positive           | Positive |

From the Table 1 can be seen that the data obtained showed that flavonoids, alkaloids, saponins, tannins, glycosides, and steroids are present in simplicia and EECL. All of these phytochemical compounds are predicted to have a beneficial pharmacological effect to us.

3.2 The Characteristics of Simplisia Coriander Leaves
The characteristics of simplicia coriander leaves was tested by standard procedure. The result of characterization of coriander leaves simplicia can be seen Table-2.

| No | Parameter                  | Results   |
|----|---------------------------|-----------|
| 1  | Water content             | 9.66 %    |
| 2  | Water-soluble extract     | 41.75 %   |
| 3  | Soluble essence in ethanol| 17.02 %   |
| 4  | Total ash content         | 11.86 %   |
| 5  | Ash content does not dissolve in acids | 0.93 % |

From the Table 2 can be seen that all the data obtained showed that analysis of water content, water soluble extract, soluble essence in ethanol, total ash content, and ash content does not dissolve in acidshave met the permitted standards [13].

3.3 Anti-Inflammatory Effects of EECL
In this experiment, the animals tested were grouped into 5 groups. The experimental group consisted of a negative control group (0.5% Na CMC suspension), a positive control group (diclofenac sodium suspension), and the dose test groups with varying doses of 100 mg/kg, 200 mg/kg and 400 mg/kg. The measurement of anti-inflammatory effect test is carried out using a digital plethysmometer with measurements based on Archimedes' law. Inflammation induction was done chemically using intraplantar λ-carrageenan 1% solution on the soles of rat feet. Carrageenan induction is the possible method to be analyzed after given a single dose of nontoxic in one day. Besides, this method is the most widely used as it is simple, easy, and suitable for testing the anti-inflammatory activity of a compound in small amounts.
3.4 The inflammation volume
Induction of carrageenan can increase inflammation volume in the rats which can be seen in inflammation volume before and after administration of carrageenan. The result of the injection of \( \lambda \)-carrageenan solution is marked by inflammation of the feet of mice, marked by a change in the volume of the plethysmometer called the inflammation volume. The average data of Inflammation volume for each group can be seen on Figure 1.

From the Figure 1 can be seen that The diclofenac sodium group showed that the drug can suppress inflammation caused by \( \lambda \)-carrageenan, namely by indicating that at 360 minutes the volume of the rats' feet decreased almost the same as V0. In the 0.5% Na CMC group there was a very significant increase in leg volume compared to the other groups, because the Na CMC group was the comparison to other groups. It can be seen that at the 360 minutes the volume of the rats' feet did not decrease significantly.

The EECL group with dose of 100 mg/kg bw, showed that the volume of the rats' legs did not decrease significantly. It can be seen that at the 360 minutes, the mouse foot volume is still larger than the initial volume. The EECL group with dose of 200 mg/kg bw, The volume of mouse feet at minute 360 has shown a decrease. While The EECL group with dose of 400 mg/kg bw shows that the volume of the mouse feet at the 360 minutes shows a decrease that is almost close to V0.

From the Figure 1 can be seen that EECL with dose of 400 mg/kg bw known that can giving the best effects, because Because at the 360 minutes show that the volume of the rats feet is almost close to the initial volume of the rats feet (V0). This shows that EECL dose of 400 mg/kg bw is equivalent to diclofenac sodium dose of 2.25 mg/kg bw.

![Figure 1](image)

**Figure 1.** The average percentage of change on inflammation volume of the rat feet by giving 0.5\% CMC Na, diclofenac-Na 2.25 mg/kgbw, EECL doses of 100, 200, and 400 mg/kgbw every minute for 360 minutes.

3.5 The Percent of inflammation
The percent of inflammation group which is smaller than the negative control group shows that there is a test preparation that is able to suppress inflammation caused by \( \alpha \)-carrageenan. Results inflammation percent on average for each group can be seen on Table 3 and Figure 2.
Table 3. Average percentage results of rat foot inflammation with 0.5% CMC Na, diclofenac-Na 2.25 mg/kgbw, EECL 100, 200, and 400 mg/kgbw every minute for 360 minutes (Mean ± SEM).

| Measurement (minute) | Percentage of Rat Foot Inflammation After Giving a Test Group Every Minute for 360 minutes |
|----------------------|------------------------------------------------------------------------------------------------|
|                      | Na CMC 0.5% | Diclofenac Sodium | EECL 100 mg/kgbw | EECL 200 mg/kgbw | EECL 400 mg/kg bw |
| 30                   | 5.73±1.96  | 2.30±0.45         | 4.31±0.80         | 3.73±0.34         | 2.95±0.55          |
| 60                   | 9.48±1.56  | 5.62±0.79         | 8.33±1.11         | 7.59±1.26         | 6.89±1.02          |
| 90                   | 15.16±2.20 | 10.18±1.62        | 12.08±1.11        | 11.75±1.47        | 11.1±1.05          |
| 120                  | 20.46±1.53 | 14.61±1.45        | 18.40±1.41        | 17.54±2.06        | 15.98±1.36         |
| 180                  | 24.28±1.70 | 17.75±1.32        | 21.18±1.37        | 20.52±2.19        | 19.34±2.11         |
| 240                  | 30.11±2.23 | 12.16±2.07*       | 16.33±2.22*       | 15.29±2.11*       | 13.66±0.71*        |
| 300                  | 35.85±3.83 | 6.72±1.53*        | 10.42±2.08*       | 9.49±0.69*        | 7.57±2.07*         |
| 360                  | 43.01±3.50 | 2.14±0.42*        | 3.77±1.32*        | 2.98±0.58*        | 2.50±0.43*         |

Note: * = Significantly different from Na CMC 0.5% (p > 0.05)

Based on Table 3, show that EECL with dose of 100 mg/kgbw at the 30th minute to 180 minutes indicates that there was no significantly differences Na-CMC group (p > 0.05), but at the 240th minutes until to 360th minute showed that there were a significantly differences with the Na-CMC group (p <0.05), and the EECL group with dose of 100 mg/kgbw at the 30th minute to the 360th minute showed that there was no significant differences with the diclofenac sodium group (p > 0.05). This result shows that the EECL group with dose of 100 mg/kgbw at the 30th minute to the 360th minute already had anti-inflammatory activity.

The EECL group with dose of 200 mg/kgbw at the 30th minute to the 180th minute showed that there was no significantly differences with the Na-CMC group (p > 0.05), but at the 240th minute to the 360th minute showed that there was significant differences with the Na CMC group (p <0.05), and the EECL group with dose of 200 mg/kgbw at the 30th minute to the 360th minute shows that there was no significant differences with the diclofenac sodium group (p > 0.05). This shows that the EECL group with dose of 200 mg/kgbw at the 30th minute to the 360th minute already has anti-inflammatory activity.

EECL group at a dose of 400 mg/kgbw at the 30th minute to the 180th minute showed that there was no significantly differences with the Na CMC group (p > 0.05), but at the 240th minute to the 360th minute showed that there were significantly differences with the Na CMC group (p <0.05), and the EECL group with dose of 400 mg/kgbw at the 30th minutes to the 360th minutes shows that there were no significantly differences with the diclofenac sodium group (p > 0.05). This showed that EECL with dose of 400 mg/kgbw at the 30th minutes until 360th minutes already has the activity of anti-inflammatory. The average of inflammation Percent graph at mouse feet can be seen on Figure 2.
From Figure 2 can be seen that the lowering of inflammatory percent at the group of diclofenac sodium, EECL with doses of 100, 200, and 400 mg/kgbw from 240 minutes to 360 minutes. The control positive group (diclofenac sodium) had the lowest percentage of inflammation followed by the EECL group with doses of 400, 200, and 100 mg/kgbw. So that the higher the doses of EECL given, then the lower the percent of inflammation in rats induced foot carrageenan and approaches with diclofenac sodium.

The ANOVA and Kruskal Wallis statistical tests, the EECL group doses of 100, 200, 400 mg/kgbw have didn’t significantly differences with the Na-CMC group (p> 0.05) at the 30th minute until to 180th minute, but significantly differences with the Na-CMC group (p <0.05) at the 240th minute to the 360th minute and the EECL group with doses of 100, 200, 400 mg/kgbw have didn’t significantly differences from diclofenac sodium (p> 0.05) at the 30th minute until to the 360th minute. Based on the results can be concluded that EECL at doses of 100, 200, 400 mg/kgbw has anti-inflammatory activity and has the same activity with diclofenac sodium.

3.6 The Percentage of Inflammation Inhibition

The percentage of inflammation inhibition of the rat foot were smaller than the negative control (Na-CMC) showed that diclofenac sodium and EECL have capable to inhibit inflammation in rat foot ceased by λ-carrageenan. This ability to inhibit inflammation is called inflammation inhibition. The inhibition percent of diclofenac sodium as a positive control becomes a standard reference in seeing the potential of drug compounds in suppressing inflammation that will occur after test animals are induced with carrageenan. The results of the percentage of inflammation inhibition can be seen in Table 4.

Table 4. The average Measurement of the Percent Inhibition of Inflammation of Rat Foot which given diclofenac sodium, EECL with Doses of 100, 200, and 400 mg/kgbw Every Minute for 360 Minutes (Mean ± SEM).

| Measurement (minute) | The Percentage Inflammation of the Rat Foot after Giving a Test Group Every Minute for 360 minutes |
|---------------------|---------------------------------------------------------------------------------------------------|
|                     | Diclofenac Sodium 100 mg/kgbw | EECL 100 mg/kgbw | EECL 200 mg/kgbw | EECL 400 mg/kgbw |
| 30                  | 50.12±10.22                | 70.13±29.60       | 49.83±22.01       | 56.39±11.81       |
| 60                  | 41.89±10.47                | 28.04±9.22        | 56.03±5.14        | 48.80±10.03       |
| 90                  | 35.01±11.96                | 40.91±4.63        | 38.22±3.08        | 41.55±11.18       |
| 120                 | 26.50±10.48                | 21.87±7.36        | 19.59±5.57        | 22.84±6.40        |
Based on Table 4 can be seen that the EECL group with dose of 100 mg/kgbw at the 30th to 360th minutes showed that there were no significant difference with the diclofenac sodium group (p>0.05). EECL group with dose of 200 mg/kgbw at the 30th until 360th minutes showed that there were no significantly differences with the diclofenac sodium group (p>0.05). EECL group with dose of 400 mg/kgbw at the 30th up to 360th minutes shows that there were no significantly differences with the diclofenac sodium group (p>0.05). Based on these data, the EECL group with a dose of 100, 200 mg/kgbw showed activity in suppressing inflammation but almost closer to the value of diclofenac sodium, but the EECL group with dose of 400 mg/kgbw have a good activity in suppressing inflammation because it is able to approach the value of diclofenac sodium.

4. Conclusion
The Conclusion of this study is EECL doses of 100, 200, and 400 mg/kgbw have anti-inflammatory activity in carrageenan-induced male rats. The EECL dose of 400 mg/kgbw has the best average inflammation inhibitory activity.

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