The anti-inflammatory activity of *Stichopus horrens* Selenka on male white rats after carrageenan induced

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Abstract
Research conducted for the anti-inflammatory activity of sea cucumber, Stichopus horrens Selenka. Inflammation is a pathophysiological response of living tissue to injuries leading to the local accumulation of plasmic fluid and blood cell. The complex events and mediator involved in the inflammatory reaction may induce, maintain or aggravate many diseases Sea cucumber had been used empirically as anti-ibflammatory. This study aimed to determine the anti-inflammatory effect of ethanol extract of Stichopus horrens with dosage range from 250 mg/kg BW, 500 mg/kgBW and 1000 mg/kgBW to male White rats with Winter method by induced carrageenan as well as to find out the best dosage of antiinflammatory effect.

Data were analyzed usingone-way analysis of variance (ANOVA) and Student Newman–Keuls. The test result showed the highest percentage of anti-inflammatory effects occurs at a fifth hour for dosage of 250 mg/kgBW was 42,27%, the dosage of 500 mg/kg BW was 53,70% and dosage of 1000 mg/kg BW for 78,90%. Statiistical analysis showed that ethanolic extract provides anti-inflammatory effect is comparable to Sodium Diclofenac.

Keywords: *Stichopus horrens* Selenka, antiinflammatory, carrageenan.

Introduction

Inflammation is one of the body's defense mechanisms against foreign bodies (antigens) characterized by symptoms of redness, swelling, heat and pain. The antigen is present in various forms and in considerable amounts such as dust, chemicals and microbes. Therefore, inflammation has a fairly high number of events.[11]

The current inflammatory treatment poses many problems. Common drugs such as non-steroidal anti-inflammatory agents (AINS) cause irritation of the gastrointestinal tract and disruption of blood cells; Steroid drugs have more complex and dangerous side effects[7]. Therefore, new anti-inflammatory agents are required that have relatively low side effects, one of which is derived from natural ingredients. The use of natural materials as a traditional medicine has been done hereditary to overcome health problems. Traditional medicine is considered safer due to its relatively mild side effects than synthetic drugs.[10]

Indonesia is an archipelagic country with a coastal length of about 81,000 km. The natural and climatic conditions have not changed much throughout the year so Indonesian waters are many types of economic biota[6]. Marine, resources, plant or animal-based materials have potential to explored more in the pharmaceutical field. One of them is the sea cucumber[4]. Sea cucumbers are marine biota from Echinodermata phyla used as food and export commodities[9]. In addition to food, sea cucumbers are empirically believed to treat diseases such as hypertension, treat wounds including relieving arthritis[2]. Previous studies of sea cucumbers indicate the presence of antibacterial activity[3], vitality enhancer[8] and hypertension drugs.[5]

Sea cucumbers contain useful bioactive ingredients in the pharmaceutical and health fields because of its high nutritional and nutritional content. From the results of research nutrition of dried sea cucumber, protein is as much as 82%, fat 1.7%, water 8.9% and carbohydrates 4.8%[6]. One of the most popular sea cucumber in terms of utilization as a food or disease treatment is *Stichopus horrens* Selenka. Based on several studies of another sea cucumber species *Stichopus japonicus* which showed the presence of anti-inflammatory activity, research on anti-inflammatory activity of sea cucumber *Stichopus horrens* extract is necessary.

Material and Methods

Materials and equipments: Animals used are white male Wistar rats aged 10 weeks, weighing 170-200 grams. Sea cucumber, *Stichopus horrens* obtained from Karimun Jawa, Jepara, Central of Java, Indonesia., ethanol (Wika), carrageenan (Wika), PGA (Sigma), diclofenac sodium (Novartis) and distilled water; Analytical balance (Mettler Toledo, AL204), maceration tool, rotary evaporator (IKA®, RV 10), mortar and stamper, beaker glasses, water bath (SMIC), pletismometer, stopwatch, scales mouse, 1 mL syringe, oral sonde, gloves.

Methods

1. Sea Cucumber Collection and Determination: 10 kg of sea cucumber are collected from Karimun Jawa island, Central of Java Province and determined at the Laboratory of Animal Taxonomy, Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Padjadjaran. The results of determination indicate that the Sea Cucumber used is *Stichopus horrens* Selenka.

2. Sample Preparation: *Stichopus horrens* is cleaved and remove the abdomen contents to eliminate the presence of impurities that may affect the extraction results, then dry in

drying cabinet at temperature of 70°C. The dried material is then cut until a smaller form is obtained.

3. Extraction: Two kilograms dried *Stichopus horrens* are extracted with 96% ethanol solvent by using maceration method which lasted for 3x24 hours. The solvent was removed by evaporation at temperature of 50° C. The yield of the ethanolic extract obtained is 34.6 g.

Antiinflammatory Activity Assay: Twenty Wistar male white rats were quarantined for one week. The rats fasted for ± 18 hours before treatment but drinking water is still given. The rats were randomly divided into 5 groups with each group consisting of 4 rats. The weight of a rat is weighed and given a specific code. Each rat is marked on the ankle with a permanent marker.

In this study, each group was given the following treatment: The negative control group was given 2% PGA, the positive control group was given diclofenac sodium 10 mg / kgBW and the test group was given ethanol extract at 250 mg / kgBW, 500 mg / kg BW and 1000 mg / kgBW orally. The right rear foot of the rats was measured by pleistomomete and noted as starting volume an hour later injected with 2% carrageenan in 0.9% NaCl solution of 0.1 mL subcutaneously to induce inflammation. After that, edemavolume was observed every hour until fifth-hour using pleistomometer. Furthermore, inflammatory volume data is converted to the percentage of inflammatory volume. The percentage volume of inflammation can be calculated as follows:

% Inflammation = (Vt - V0) / V0 x 100%
where, Vt = rat foot volume at time t and V0 = rat foot volume at initial time.

Then calculate the percentage of inflammatory inhibition based on the formula:

% Inflammation Inhibition = (a-b) / b x 100%
where, A = percent of control inflammation and B = percent of flammation assay.

Data Analysis: The results were analyzed statistically by using one-way variance analysis (ANOVA) with 95% confidence degree. If the results obtained are meaningful, proceed with the Student Newman - Keuls (SNK) range test.

Results and Discussion

Antinflammatory Activity Assay: The method used in this anti-inflammatory activity test is the Winter method. Winter method is a common method used in the assay of anti-inflammatory activity that is by the formation of artificial inflammation on the back foot of white male rats induced by carrageenan.[12] The result of observation can be seen in figure 1.

From the graph, it was seen that all of the groups showed anti-inflammatory effect. The mean inflammatory volume of each test extract group was not as high as control inflammation except for a dose of 1000 mg/kg BW. From the study of Aliya et al[1], it can be concluded that the time of formation of inflammation resulting from induction of carrageenan consists of two phases.

The first phase (early phase) occurs within 1-2 hours of injections of carrageenan. The second phase (late phase) is after 3 hours of injection of carrageenan. Based on these studies, suspected *Stichopus horrens* ethanol extract works in the second phase (late phase).

Data of Inflammation volume was obtained and was used to calculate the percentage of inflammation. The average yield of inflammatory volume to the group can be seen in figure 2. Percentage of inflammatory obtained was used to calculate the percentage of inflammatory inhibition (figure 3).

From the percentage data of inflammatory inhibition on time it was seen that all treatments, except negative controls showed inflammatory inhibition and in general, the maximum occurred at the fifth hour for positive control and negative control after 2% carrageenan injection, whereas for the extract, occurred at the third hour. This is thought to be due to the slow onset of drug work and the effect of maximum inhibition at fifth-hour. Negative controls do not provide inflammatory inhibition because they are not given anti-inflammatory agents.

The highest inhibition percentage of inflammatory was positive controlled by 87.45% at the third hour, but the activity started to decrease at the fifth hour to 69.19%. The inhibition percentage of ethanolic extract is 250 mg kgBW; at first hour was 27,38%, then decreased at the second and third hour that is 3,53% and 6,44% but increased again 47.43% and 53.70% at the fourth and fifth hour respectively.

The inhibition percentage of ethanolic extract of dose 500 mg/kgBW at first hour was 46,42%, then decreased at second and third to 44,14% and 21,69%. Furthermore, the ethanol extract dose of 1000 mg/kg BW was increased in the first hour of 70.61% and then decreased at second hour by 44.44% and increased from third to the fifth hour from 59.19% to 78.90% respectively

Data Analysis: Based on the ANOVA followed by the Student Newman-Keuls range test for the highest percentage of inhibition at fifth hour, it was found that the dose of the assay group and the positive control group had differed significantly with the negative control. The dose of the assay group did not give significant difference with positive control ($p < 0,05$).

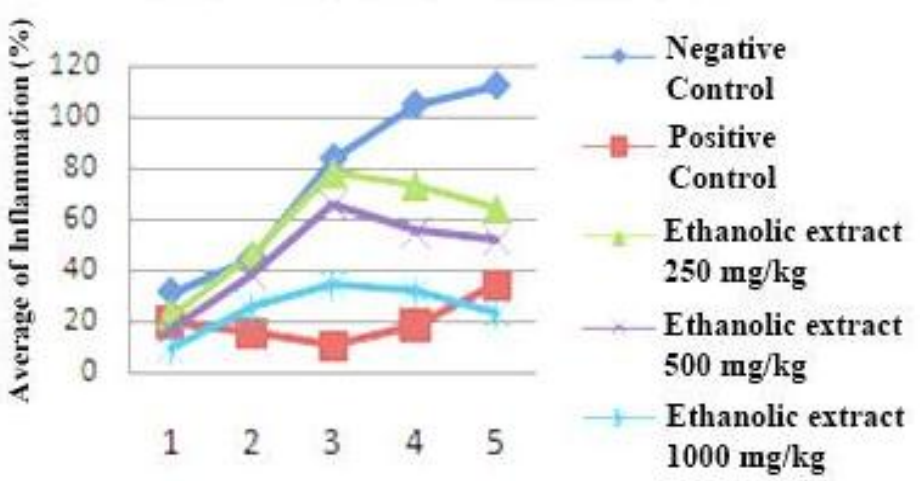

Figure 1: Graph of percentage averages Volume of inflammation with time

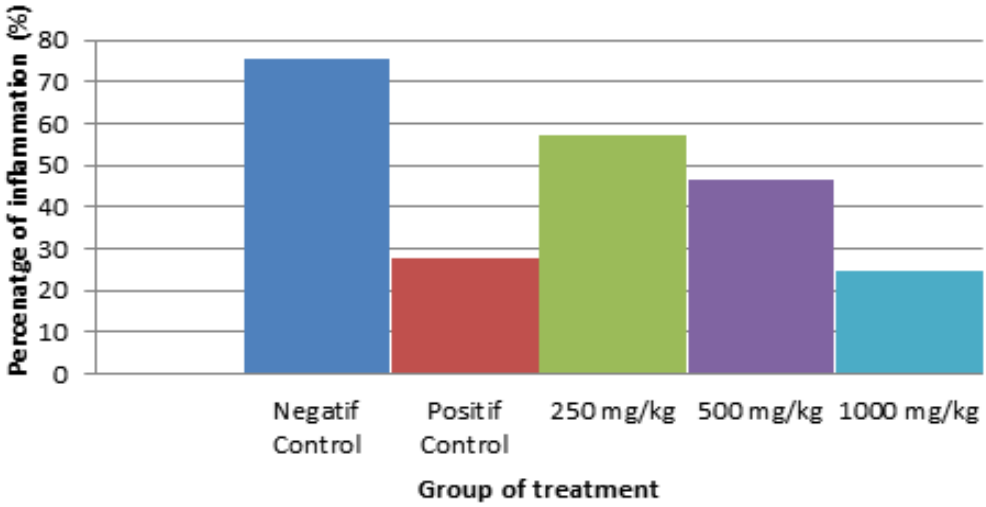

Figure 2: Bar chart of average inflammation volume for 5 hours against the group treatment

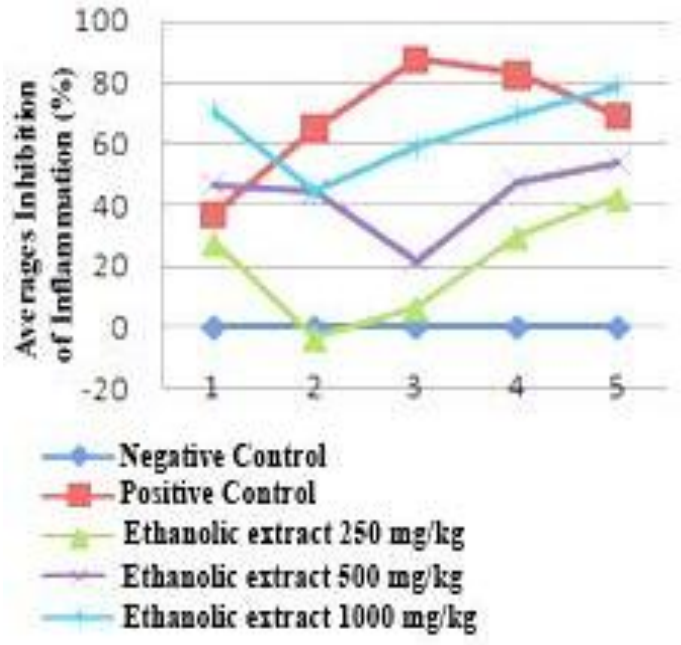

Figure 3: Graph of percentage averages Inhibition of inflammation against time

Conclusion

Based on the results of the research, it can be concluded that ethanolic extract of *Stichopus horrens* has anti-inflammatory activity. The best anti-inflammatory activity is 1000 mg / kgBW

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