ABSTRACT

Objectives: The aim of this study is to investigate anti-inflammatory activity of β-glucan (laminaran) of brown algae, *Sargassum crassifolium* which was extracted by using two different methods, β-glucan laminaran acid extract (LAE) and laminaran modified extract (LME).

Methods: β-glucan (laminaran) of brown algae, *S. crassifolium* have been extracted using two different methods, namely, β-glucan LAE and LME. The edema reducing activity of the extracts was investigated with carrageenan-induced leg edema male of *Rattus norvegicus* as a model.

Results: The swelling of edema was strongly inhibited by the treatment of LME with a percentage of 81.62% after 3 hrs. It also found that the size of edema were inhibited by all treatments with the value ranging from 25.49% to 81.68%. Smallest edema size was found on LME treatment, with a size of 0.68±0.09 mm where the control was about 0.92 mm. Furthermore, all β-glucan-treated mice showed lower edema size compared to control.

Conclusion: It was obvious that all type of laminaran had anti-inflammatory activity. LME is the best method for production of β-glucan (laminaran) with high anti-inflammatory activity. Hence, in this study, we reported a new anti-inflammatory agent derived from *S. crassifolium*.

Keywords: β-glucan (laminaran), Edema, *Sargassum crassifolium*, Carrageenan, Edema.

INTRODUCTION

Exploration of marine natural resources have been attracting scientist attention. Many resources, such algae, microorganism, wait to be explored [1,2]. One of the potential algae is brown algae, *Sargassum* sp. *Sargassum* grouped in order of Fucales. Many species are distributed over tropical regions, including Indonesia. This algae is a source of β-glucan (laminaran), linear polysaccharide composed of β-(1,3) -linked glucose in its main chain, with β - (1, 6) -linked -chain branch differed depending on the variant of the isolated species, as well as the predicted environmental factors directly affect the biological properties [3,4]. By far, exploration of brown algae *Sargassum* sp. for its uses as anti-inflammatory, anti-infective, antimicrobial, anti-cholesterol, antioxidant, antiinflammatory, and antiaging agents has attract many marine biologist as well as marine biotechnologist [5,6].

Inflammation is a local response of living mammals’ injured tissue. It is a defense reaction of the body to eliminate or limit the spread of harmful agents [7]. Complex occurrences and mediators involved in the inflammatory reaction could cause or worsen [8] the condition. There are various components to inflammatory reactions contributing to the symptoms associated with tissue injury. Edema, leukocyte infiltration, and granuloma formations are components representing inflammatory condition [9].

In this study, we investigate the potency of β-glucan (laminaran) derived from brown algae, *S. crassifolium* for anti-inflammatory agent through edema reducing activity.

METHODS

Materials and equipment

Materials used in this study were *S. crassifolium*. They harvested from Talango Island, Madura, Indonesia. All chemicals agents such λ carrageenan, phenyl butazone (Sigma Aldrich, USA), and ethylphenyl propiolate (EPP) (Phka, Japan) including other materials such as rivanol and alcohol170% are analytical grades.

Preparation of β-glucan (laminaran) extract

The brown alga, *S. crassifolium*, was sun dried. The samples chopped into pieces and then filtered to obtain powder. Algae was extracted using Yvin et al. and Mohan et al. [10,11] method with modification. Using two different extraction tools, the hot plate magnetic stirrer (HPMS) and ultra-sonication. β-glucan (laminaran) generated from HPMS with sulfuric acid as a solution called laminaran acid extract (LAE). For the result of the ultrasonic method with distilled water as solution, the extract called laminaran modified extract (LME).

Test animal

40 male rat (20 for paw edema and 20 Wistar rats for ear edema experiments) with the weight of 180-200 g used as test animals. Each of them placed in individual cages. The wistar rat kept under standard environmental conditions and had free access to diet and water (*ad libitum*). All of these animals acclimatized for a week before testing period. All experimental procedures conducted by following the guidelines of Brawijaya University Animal Ethics Committee.

Anti-inflammatory activity test with carrageenan hind paw edema

20 rat divided into 4 groups, each of 5 wistar rat, treated with the administered extracts a similar concentration of 375 mg/sample, while a control was left without treatment. Edema was induced by 100 μL subplantar injection containing 1% solution of carrageenan suspended in fresh 0.9% NaCl solution. The solution injected into the subplantar tissue of the right rear leg of Wistar rat. Linear foot circumference measured at intervals of 1, 3, and 5 hrs. Anti-inflammatory activity measured as the percentage reduction of edema relative to controls after topical medications [12].

Mouse ear swelling assays (MESA) with EPP

The ear swelling test conducted using a method modified from Frenkel [13], in which the ears of wistar rat subcutaneously injected
with EPP for hypersensitivity test Nualkaew et al. [14]. Suggested that that before the use of EPP, ear of test animals should be measured in advance using a caliper EPP was used as an inflammatory agent, which was injected subcutaneously at a dose of 1 mg/20 μL/ear. LAE and LME extracts were individually dissolved in 5% dimethyl sulfoxide in acetone at a concentration of 375 mg/ml in a similar volume of 0.5 ml/100 g of animal weight, which was then administered topically to the surface of the inner and outer ear. Results of observation showed that the height of swelling was 4 hrs after induction. Ear thickness was measured. Effects of β-glucan extract (laminaran) on ear edema in both control and treatment groups were compared, and the percentage inhibition was then calculated.

RESULTS AND DISCUSSION

Carrageenan-induced paw edema

Edema of hind paw induced by carrageenan has been extensively used as a model of inflammation in the search for new anti-inflammatory drugs. Edema-reducing activity was evaluated using a method, which is based on rat leg edema induced by carrageenan, modified from Winter et al. and Adeyemi et al. [15,16]. The results of this work were presented in Table 1.

After the carrageenan injection, wistar rat got leg swelling at the first 1 hr, in both control and treatment groups. In control group, increased swelling in control group occurred during early 5 hrs of observation. Whereas in all treatment groups, which rat were treated with β-glucan (laminaran) extracts (LAE, LME), inhibition similarly occurred and peaked at the 3rd hr. Ratheesh and Helen [17] reported that study on Ruta graveolens as an anti-inflammatory also showed maximum inhibition of inflammation after 3 hrs of observation. This is corroborated by a report from Adeyemi et al. [16] which stated that the effects of R. americana extracts on carrageenan-induced edema legs are most prominent in the 3rd hrs. Amdekar et al. [9] demonstrated a slight difference result in the maximum time triggering inflammation, i.e., at 4th hrs, when researching the inhibition effects on inflammation by Lactobacillus casei and L. acidophilus.

The mechanism of edema production by carrageenan is possibility due to release of prostaglandins (PG), which is in line with the release phase of prostaglandin at the 3rd hrs [18]. The development of inflammation or edema in the legs of Wistar rat after injection of carrageenan is due to the release of histamine, serotonin, and PG [17].

LME extracts significantly suppressed the Wistar rat feet edema by 81.62%, followed by LAE extract, and LMEs by 73.47% and 67.35%, respectively. This means that LME extract can function as an anti-inflammatory compound and even with the strongest activity as observed in this study. When compared to results of Ratheesh and Helen [17] using R. graveolens as object of the study, and the introduction of 20 mg/kg dose, results in this study demonstrated that the decrease of mice edema by 90.9% inhibition was still lower than ability of extracts LME to do so. However, the decrease is higher when compared to the results from Voyeran as standard drug (72.72%) [17]. This means that the anti-inflammatory activity of LME extracts is higher than that of Voyeran.

MESA

EPP is a material used to cause edema in addition to arachidonic acid. EPP was usually used on the ear of test animals such as mice. Non-invasive swelling test of mouse ear (MESA) is a delayed hypersensitivity model type used as a promising testing protocol for allergic contact dermatitis (ACD). The formation of ear edema of mice induced by EPP is a model useful for screening and investigating the anti-inflammatory activity of the tested substance in the acute phase of inflammation [19]. MESA allows the identification of potential of ACD for β-glucan (laminaran) at very low concentrations of applications, which could not produce ACD using other techniques.

In this study, using EPP subcutaneously injected into the Wistar rat ears, it turns out the edema response occurred very slowly, where swelling detected just after 8 hrs as shown in Table 2.

<p>| Table 1: Edema reducing activity of β-glucan (laminaran) on Edema-hind paw of Rattus norvegicus |</p>
<table>
<thead>
<tr>
<th>Observation (hour)</th>
<th>Sample</th>
<th>Mean size of edema (cm)</th>
<th>Relative inhibition on control (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 hr</td>
<td>Control</td>
<td>0.38±0.025</td>
<td>28.95</td>
</tr>
<tr>
<td></td>
<td>LAE</td>
<td>0.27±0.13</td>
<td></td>
</tr>
<tr>
<td></td>
<td>LME</td>
<td>0.24±0.16</td>
<td>36.84</td>
</tr>
<tr>
<td>3 hrs</td>
<td>Control</td>
<td>0.49±0.041</td>
<td>73.47</td>
</tr>
<tr>
<td></td>
<td>LAE</td>
<td>0.13±0.08</td>
<td></td>
</tr>
<tr>
<td></td>
<td>LME</td>
<td>0.09±0.08</td>
<td>81.62</td>
</tr>
<tr>
<td>5 hrs</td>
<td>Control</td>
<td>0.51±0.041</td>
<td>25.49</td>
</tr>
<tr>
<td></td>
<td>LAE</td>
<td>0.38±0.13</td>
<td></td>
</tr>
<tr>
<td></td>
<td>LME</td>
<td>0.33±0.13</td>
<td>35.29</td>
</tr>
</tbody>
</table>

R. norvegicus: Rattus norvegicus; LAE: Laminaran acid extract, LME: Laminaran modified extract

The thickness of edema on both right and left ears of wistar rat in the control group on 8 hrs after the injection, reached the highest level (0.92±0.03 mm). LAE, LME extracts with better inhibition intensity better that using another commercial anti-inflammatory drug, phenylbutazone [at a dose of 1 mg/ear]. The lowest thickness of rat ear edema obtained from treatment using LME extract by 0.71 mm ±0.09 meaning that LME extracts generated significant inhibitory activity on edema formation at a dose of 375 mg/ml/ear, compared to other treatments did, also provided slightly higher intensity than phenylbutazone did. Thickness reduction of edema occurred on day 12 of observation, on both the right and left ears of rat.

The occurrence of edema in the ear is caused by the release of inflammatory mediators such as histamine, serotonin, bradykinin, and PG. These mediators are able to promote vasodilation and to increase vascular permeability. Hence, it would synergistically produce edema [20]. It was known that the LME extract had inhibitory effect on edema formation. According Panthong et al. [19], the tested extract may possess anti inflammatory activity by inhibiting the release or synthesis of a variety of inflammatory mediators.

CONCLUSION

β-glucan (laminaran) LME extract exhibited edema-reducing activity which was proved by its inhibition against the right feet of carrageenan induced-edema with a percentage inhibition of 81.62% after 3 hrs treatment. It also inhibits the wistar rat ear swelling by 0.71 mm ±0.09, which was higher than that of control, phenyl butazone. Therefore, β-glucan (laminaran) from S. cruzifolium able to act as edema-reducing agent.

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REFERENCES