ANTI-INFLAMMATORY ACTIVITY TEST OF PURPLE EGGPLANT PEELS EXTRACT (Solanum melongena L.) ON WISTAR STRAIN WHITE RATS (Rattus norvegicus) INDUCED BY CARRAGEenan

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ABSTRACT

Inflammation is the human body’s defense to prevent tissue damage due to different kinds of factors. The purple eggplant peel (Solanum melongena L.) may have the potential to have anti-inflammatory properties due to containing nasunin, which is a phenolic, flavonoid anthocyanin substance by inhibiting cyclooxygenase pathway. This research was done to prove the anti-inflammatory effect of purple eggplant peel extract in rats. The research method was conducted by true experimental post-test only control-group design. 25 rats were divided into 5 groups with different treatments which are: positive control, negative control, purple eggplant peel extract with a dose of 0.3 mg/gBW, 0.6 mg/gBW and 0.9 mg/gBW. The data measured was the decrease of edema volume induced by carrageenan at hours-0 1, 2, 3, 4, 5, and 6. Data analysis was performed at the 4th hour using ANOVA and post-hoc LSD tests. The results of the study found that there were significant differences between the negative control group and the positive control group and the entire treatment group. The conclusion of this study was that purple eggplant peel extract had anti-inflammatory activity with the best effectiveness at a dose of 0.9 mg/gBW.

Keywords: Eggplant, Solanum melongena L., anti-inflammation, carrageenan.

INTRODUCTION

Inflammation is an immune response to maintain tissue homeostasis during infection or injury caused by harmful stimuli. Therefore inflammation can be divided into acute inflammation that is relatively short ranging from a few minutes to days and chronic inflammation that occurs weeks to months or even years. Diseases with the highest mortality rates such as coronary heart disease (1.5%) and cancer (1.8%) are caused by inflammation. Inflammation also causes diseases with the most cases such as acute respiratory infections (9.3%), joint diseases (7.3%), asthma (4.8%), pneumonia (2%), diabetes mellitus (1.5%), hepatitis (0.4%) and other autoimmune diseases.

Anti-inflammatory drugs in the form of Non-Steroidal Anti-Inflammatory Drugs (NSAIDs) are often used as therapy. This type of drug is relatively easy to obtain in Indonesia because it is usually sold over the counter without using a doctor's prescription. Based on the results of the national socio-economic survey (SUSENAS) in 2020, the population who practiced self-medication due to health problems reached 62.74%. Prolonged use of NSAIDs may result in various side effects such as gastrointestinal lesions, peptic ulcers, and risk of cardiovascular disorders. Therefore, in modern times, many people tend to turn to herbal remedies such as the use of plant, vegetable or fruit extracts. Some natural ingredients that have been widely used to treat inflammation include mahkota dewa fruit, mangosteen fruit, and kencur rhizome because most of them contain active compounds in the form of flavonoids that can inhibit inflammatory activity. The use of drugs from natural ingredients is expected to reduce the side effects of treatment.

Another natural ingredient that has potential as an anti-inflammatory is purple eggplant (Solanum melongena L.). Purple eggplant is rich in phenolic compounds and flavonoids, and from the purple eggplant skin, anthocyanins in the form of nasunin are also found, which have potential as antioxidants and anti-inflammatories. Purple eggplant has been categorized as a family medicinal plant that can treat diabetes, cancer, cholesterol and prevent atherosclerosis. The highest phenolic content of purple eggplant is found in its peel, therefore further research needs to be conducted on the potential of ethanol extract of purple eggplant peels (Solanum melongena L.) as an anti-inflammatory agent. Therefore, this study aims to prove the anti-inflammatory effect of purple eggplant peels extract.

RESEARCH MATERIALS AND METHODS

This study was conducted at the Integrated Biomedical Laboratory, Faculty of Medicine, Udayana University, Denpasar, Bali. The method used was true experimental post-test only control-group design. The research subjects

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were 25 Wistar male white rats (Rattus norvegicus) with inclusion criteria of healthy rats, body weight 100-250 grams, active movement, and willing to be fed. As for the exclusion criteria, the rats were sick, less active, standing fur, eyes not clear, and difficult to feed.

Materials and Tools

The research materials used were purple eggplant obtained from vegetable plantations in the Bogor area, as well as other research materials such as distilled water, citric acid powder, Na-CMC powder, carrageenan powder, NaCl 0.9%, diclofenac sodium 50 mg, and mercury.

The tools needed include a stainless steel knife, oven, blender, analytical balance, glass jar, glass stirring rod, funnel, filter paper, rotary evaporator, plethysmometer, injection syringe, oral syringe, measuring cup, measuring flask, and laboratory tools in general.

Preparation of Purple Eggplant Peels Extract (PEPE)

Purple eggplant (Solanum melongena L.) was washed and peeled to separate the peels from the pulp then heated at ±50°C for 36 hours and crushed into powder. Purple eggplant peels powder was then extracted using maceration procedure by mixing the powder into a mixture of distilled water solvent and citric acid with pH ± 2 for 3x24 hours while stirring every 1x24 hours. The extract was then filtered using filter paper and the filtrate obtained was processed in a rotary evaporator to obtain purple eggplant peels extract (PEPE) with doses of 0.3 mg/gBW, 0.6 mg/gBW, and 0.9 mg/gBW.

The maceration technique is used to avoid the heating process because anthocyanins can be damaged at 60°C, besides that this technique is quite simple and cost-effective. Acidic solvents are used because acids function to denature plant cell membranes, so that anthocyanin pigments can escape from cells and dissolve in solvents, and also can prevent flavonoid oxidation. 13,14

Phytochemical Test Procedure

The extracts obtained were subjected to phytochemical screening to determine the chemical compounds contained therein. The test procedure is as follows: 15,16

1. Alkaloid Test
   The extract was mixed with 2N hydrochloric acid and distilled water, then divided into 3 parts and each was added to the reagent as follows:
   a. Added Mayer reagent, resulting in a white/yellow precipitate
   b. Added Bourchardat reagent, resulting in a black brown precipitate
   c. Added Dragendorf reagent, resulting in a brick red precipitate
   Results are positive for alkaloids if a precipitate is produced in at least 2 of the 3 tests above.

2. Flavonoid Test
   The extract was diluted using distilled water then added Mg powder, concentrated HCl and amyl alcohol, then shaken and allowed to separate. Flavonoids are positive if red, yellow, orange color is formed in the amyl alcohol layer.

3. Saponin Test
   The extract was added to distilled water and then shaken vigorously for 10 seconds, then froth or foam will form as high as 1-10 cm for not less than 10 minutes. Furthermore, 1 drop of 2 N hydrochloric acid solution is added, if the foam does not disappear, the extract is positive for saponins.

4. Tannin Test
   The extract was diluted with distilled water until colorless. The dilution results are then added with iron (III) chloride. Positive results contain tannins if a blue or blackish green color occurs.

5. Steroid/Triterpenoid Test
   The sample was macerated with n-hexane for 2 hours, then filtered and vaporized. Next anhydrous acetic acid and concentrated sulfuric acid were added. Positive results contain steroids / triterpenoids when purple or red color appears then turns blue green.

Preparation of 1% Na-CMC Suspension

A total of 1 g Na-CMC was mixed with preheated distilled water, then stirred until a transparent mass was obtained, then the volume was sufficient using distilled water to reach 100 ml.

Preparation of Diclofenac Sodium Suspension

Weigh 10 tablets of diclofenac sodium 50 mg and calculate the average weight of 1 tablet, then grind the tablets. Next, weigh the powder of diclofenac sodium equal to the average weight of 1 tablet and suspend it in 1% Na-CMC until it reaches a volume of 10 ml.

Preparation of 1% Carrageenan Solution

A total of 0.1 g of carrageenan was dissolved with 10 ml of physiological saline solution (NaCl 0.9%).

Research Procedure

The research procedure was carried out by dividing the rats into 5 research groups and adapting to the new environment for one week by giving food and drink ad libitum. On the day of the study, rats were fed 3-4 hours before treatment, then rats in each group were given oral treatment as follows:

Negative control : 1% Na-CMC suspension.
Positive control : Diclofenac sodium suspension.
1st Dose : PEPE 0.3 mg/gBW.
2nd Dose : PEPE 0.6 mg/gBW.
3rd Dose : PEPE 0.9 mg/gBW.

Furthermore, the rat’s paw was marked at the lateral malleolus to facilitate repeated measurement of edema volume. Body weight and paw volume of rats were

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measured and recorded before carrageenan induction. Then 30 minutes after the above treatment, all rats were induced 0.15 ml of 1% carrageenan subplantarly to form local edema on the soles of the previously marked rat’s paw, then the edema volume was measured at hours-0, 1, 2, 3, 4, 5, and 6. Then the measurement results from each group were recorded for further analysis. Post-research animals were returned to the livestock for re-breeding.

The data that has been collected will be calculated as the percent of inflammation and the percent of inflammation inhibition, then statistical analysis is carried out to see differences between research groups. Percent inflammation is calculated using the formula \( \frac{V_t - V_o}{V_o} \times 100\% \) where \( V_o \) is the initial volume of the rat's paw and \( V_t \) is the volume of the rat's paw at time \( t \) after being induced by carrageenan. Meanwhile, the percent of inflammation inhibition was calculated using the formula \( \frac{a-b}{a} \times 100\% \) with (a) being the percent of inflammation in the negative control group and (b) being the percent of inflammation in the treatment group.

**RESULTS**

### Table 2. Mean ± standard deviation of rat’s paw volume over 6 hours

<table>
<thead>
<tr>
<th>Group</th>
<th>Before induction</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td>NC</td>
<td>31.20 ±</td>
<td>48.00 ±</td>
<td>58.00 ±</td>
<td>63.20 ±</td>
<td>65.20 ±</td>
<td>66.80 ±</td>
<td>60.40 ±</td>
<td>56.80 ±</td>
</tr>
<tr>
<td>PC</td>
<td>2.68</td>
<td>4.24</td>
<td>3.74</td>
<td>10.45</td>
<td>12.05</td>
<td>8.32</td>
<td>7.27</td>
<td>4.60</td>
</tr>
<tr>
<td>1st Dose</td>
<td>31.20 ±</td>
<td>44.40 ±</td>
<td>53.60 ±</td>
<td>38.40 ±</td>
<td>42.40 ±</td>
<td>37.60 ±</td>
<td>34.80 ±</td>
<td>33.20 ±</td>
</tr>
<tr>
<td>2nd Dose</td>
<td>2.68</td>
<td>7.80</td>
<td>5.73</td>
<td>6.23</td>
<td>3.58</td>
<td>4.34</td>
<td>3.63</td>
<td>3.35</td>
</tr>
<tr>
<td>3rd Dose</td>
<td>22.80 ±</td>
<td>35.20 ±</td>
<td>48.00 ±</td>
<td>48.40 ±</td>
<td>47.60 ±</td>
<td>40.00 ±</td>
<td>34.80 ±</td>
<td>33.20 ±</td>
</tr>
<tr>
<td></td>
<td>22.00 ±</td>
<td>35.20 ±</td>
<td>46.00 ±</td>
<td>34.80 ±</td>
<td>36.80 ±</td>
<td>37.60 ±</td>
<td>36.40 ±</td>
<td>31.60 ±</td>
</tr>
<tr>
<td></td>
<td>19.20 ±</td>
<td>32.80 ±</td>
<td>40.00 ±</td>
<td>28.00 ±</td>
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<td>24.80 ±</td>
<td>23.20 ±</td>
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<tr>
<td></td>
<td>3.03</td>
<td>4.38</td>
<td>4.90</td>
<td>3.46</td>
<td>5.10</td>
<td>8.44</td>
<td>8.79</td>
<td>4.60</td>
</tr>
</tbody>
</table>

**Description:**

- **NC:** Negative control
- **PC:** Positive control
- **1st Dose:** PEPE 0.3 mg/gBW
- **2nd Dose:** PEPE 0.6 mg/gBW
- **3rd Dose:** PEPE 0.9 mg/gBW

**Figure 1.** Overview of the average rat’s paw volume during 6 hours of observation.

**Phytochemical Test of Purple Eggplant Peels Extract**

The purple eggplant used in this study was obtained from a vegetable plantation in the Bogor area. Based on the results of phytochemical tests, purple eggplant skin extract was shown to contain flavonoids, tannins, and steroids/triterpenoids (Table 1).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Test Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloids</td>
<td>Negative (-)</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>Positive (+)</td>
</tr>
<tr>
<td>Saponin</td>
<td>Negative (-)</td>
</tr>
<tr>
<td>Tannins</td>
<td>Positive (+)</td>
</tr>
<tr>
<td>Steroids/Triterpenoids</td>
<td>Positive (+)</td>
</tr>
</tbody>
</table>

**Anti-Inflammatory Activity Test**

The inflammatory indicator to be observed is edema of the rat’s paw, therefore the volume of the rat’s paw is measured every 1 hour for 6 hours to determine the trend of increasing and decreasing edema volume after carrageenan induction. The results of paw volume measurement can be seen in Table 2 & Figure 1.

In the negative control group, the volume of rat's paw continued to increase until the 4th hour and only began to decrease at the 5th hour of observation. The positive control group, 2nd dose (0.6 mg/gBB) and 3rd dose (0.9 mg/gBB) showed a decrease in the volume of rat's paw starting at the 2nd hour, while the 1st dose group (0.3 mg/gBB) only experienced a decrease in volume starting at the 4th hour.

The difference in body weight of the rats in each research group led to different sizes and volumes of rat's paw from the beginning. The results of the volume of the rat's paw formed were then calculated the percent of inflammation at each hour to more easily compare and determine how much edema was formed in each experimental group (Table 3).
The increase in edema in the negative control reached a peak at the 4th hour, so for data analysis will be taken from one point at the 4th hour percent inflammation (Figure 2) as a comparison between all study groups.

The results of the normality and homogeneity tests showed that the data had a normal and homogeneous distribution, then the One-Way ANOVA test was carried out. The One-Way ANOVA results showed a significance value of 0.000 (p-value ≤ 0.05) so it was concluded that there were significant differences in the data, then analysis of the LSD post-hoc results was carried out to see the significance between research groups.

The results of the post-hoc test (Table 4) showed significant differences between the negative control group and the positive control, 1st dose (0.3 mg/gBW), 2nd dose (0.6 mg/gBW), and also 3rd dose (0.9 mg/gBW) groups. The difference shows that in all treatment groups there is anti-inflammatory activity, but there is a significant difference between the positive control group with 1st dose (0.3 mg/gBW) and 2nd dose (0.6 mg/gBW), so the effectiveness of the two doses is not as good as the positive control. At dose 3 the results were not significantly different from the positive control, so it can be concluded that the effectiveness is close to the positive control group.

Table 3. Percent inflammation of rat's paw

<table>
<thead>
<tr>
<th>Group</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td>NC</td>
<td>86.34 ± 9.78</td>
<td>101.60 ± 16.84</td>
<td>108.24 ± 28.12</td>
<td>113.82 ± 15.36</td>
<td>93.65 ± 17.50</td>
<td>82.49 ± 13.57</td>
</tr>
<tr>
<td>PC</td>
<td>72.64 ± 22.99</td>
<td>23.51 ± 21.54</td>
<td>37.03 ± 20.28</td>
<td>20.36 ± 5.91</td>
<td>11.47 ± 4.87</td>
<td>6.45 ± 6.31</td>
</tr>
<tr>
<td>1st Dose</td>
<td>111.54 ± 28.03</td>
<td>111.38 ± 62.76</td>
<td>111.44 ± 47.83</td>
<td>77.82 ± 32.84</td>
<td>54.74 ± 31.36</td>
<td>47.41 ± 35.24</td>
</tr>
<tr>
<td>2nd Dose</td>
<td>111.75 ± 35.96</td>
<td>59.31 ± 26.71</td>
<td>69.80 ± 30.67</td>
<td>73.24 ± 27.23</td>
<td>68.35 ± 32.85</td>
<td>44.52 ± 26.07</td>
</tr>
<tr>
<td>3rd Dose</td>
<td>110.00 ± 18.78</td>
<td>47.55 ± 18.81</td>
<td>47.73 ± 32.14</td>
<td>48.09 ± 23.60</td>
<td>27.68 ± 30.93</td>
<td>20.59 ± 10.80</td>
</tr>
</tbody>
</table>

Description : * = there are significant differences

The anti-inflammatory effect of purple eggplant peels extract can be seen through the calculation of the percent inhibition of inflammation (Figure 3). At the 4th hour, the percent of inflammation inhibition in the positive control group was 82.09%, while in the treatment group 1st dose (0.3 mg/gBW), 2nd dose (0.6 mg/gBW), and 3rd dose (0.9 mg/gBW) were 28.87%, 35.98%, and 55.97%, respectively.

Table 4. Post-hoc LSD percent inflammation at 4th hour

<table>
<thead>
<tr>
<th>Comparison</th>
<th>Significance at 4th hour</th>
</tr>
</thead>
<tbody>
<tr>
<td>NC vs PC</td>
<td>0.000</td>
</tr>
<tr>
<td>NC vs 1st Dose</td>
<td>0.022*</td>
</tr>
<tr>
<td>NC vs 2nd Dose</td>
<td>0.011*</td>
</tr>
<tr>
<td>NC vs 3rd Dose</td>
<td>0.000*</td>
</tr>
<tr>
<td>PC vs 1st Dose</td>
<td>0.001*</td>
</tr>
<tr>
<td>PC vs 2nd Dose</td>
<td>0.002*</td>
</tr>
<tr>
<td>PC vs 3rd Dose</td>
<td>0.071</td>
</tr>
<tr>
<td>1st Dose vs 2nd Dose</td>
<td>0.756</td>
</tr>
<tr>
<td>1st Dose vs 3rd Dose</td>
<td>0.054</td>
</tr>
<tr>
<td>2nd Dose vs 3rd Dose</td>
<td>0.099</td>
</tr>
</tbody>
</table>

DISCUSSION

The results of the phytochemical test of purple eggplant skin extract in this study were shown to contain flavonoids, tannins, and steroids/triterpenoids. This is in accordance with the results of research by Nisha (2009) which also states that purple eggplant contains flavonoids, alkaloids, tannins, and also saponins. The content of nutrients and active substances in eggplant can be influenced by environmental factors where the plant is cultivated. Heating techniques and the process of making extracts can also determine the quality of chemical compounds obtained.

The main compound content in purple eggplant skin extract that plays a major role as an anti-inflammatory is flavonoids of the anthocyanin type, this is in accordance with previous studies by Toppino et al. in 2016. Flavonoids cause anti-inflammatory activity through the arachidonic acid metabolic pathway by preventing the biosynthesis of prostaglandins, thromboxanes, and leukotrienes through inhibition of PLA2, COX, or LOX enzymes.

In this study, edema began to form early and reached a peak at the 4th hour. This is in line with previous studies which stated that edema formation by 1% carrageenan began to form in the first hour and reached its peak at the 4th hour. The use of carrageenan is often the main choice because it does not cause tissue damage like other irritant compounds, does not cause marks, and is more sensitive to

Figure 2. 4th hour percent inflammation graph

Figure 3. Percent of inflammation inhibition at 4th hour
anti-inflammatory drugs. The formation of edema in rat feet occurs because carrageenan induces the release of mediators that initiate the inflammatory process, then supported by PGE1 and PGE2 through decreased vascular permeability. Proinflammatory mediators such as histamine, prostaglandins, bradykinin, tachykinins, complement and reactive oxygen, and nitrogen species also play a role in the inflammatory process. Inflammatory signs such as edema, erythema and hyperalgesia develop rapidly and can be observed immediately after subcutaneous injection. Edema usually becomes maximal within 3 to 5 hours post-injection. It may last for 6 hours and slowly decrease within 24 hours.21,22

The positive control group and several treatment groups in this study showed a decrease in the volume of rat feet starting at the 2nd hour. This is in line with research conducted by Hartati (2016), with the same research method and using extracts with anthocyanin compound content showed a decrease in edema in the positive control and several treatment groups starting at the 2nd hour.23 Other research by Lawarence & Murugan (2019), showed slightly different results where the decrease in edema volume in the positive control group and the treatment group began at the 3rd hour observation.24

CONCLUSIONS AND SUGGESTIONS

The results of this study prove the draft hypothesis that has been made, which is to accept Ha and reject H0, where there is anti-inflammatory activity from purple eggplant skin extract with the best effectiveness at a dose of 0.9 mg/gBW which provides inflammatory inhibition at the 4th hour of 55.97%.

This study still has several shortcomings and confounding factors, including the use of traditional plethysmometer tools that still use mercury so that the reading results can differ from one to another and are subjective from the researcher's point of view, therefore it can be recommended to use a digital plethysmometer to get more accurate results. It is also recommended that the body weight of the rats be made more uniform to make it easier to compare between research groups. In addition, other studies can also be done to add and update literature related to purple eggplant, such as observations over a longer period of time, testing the percentage of active substance content in the extract, and making extracts using different types of solvents.

REFERENCES
