Analgesic Activity of Bitter Melon Leaves Ethanol Extract in Mice

(Mus musculus)

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Abstract

Pain is an unpleasant sensation and can be felt as sore sensation. This can be caused by mechanical, chemical or physical stimulation that could cause damage to the tissue and trigger the release of certain substances called pain mediators, such as histamine, bradykinin, leukotrienes and prostaglandins. Pain overcome by giving analgesic drugs. This study aims to determine the effectivity and effective duration of bitter melon (Momordica charantia L.) leaf extract as analgesic in mice (Mus musculus). The test method used in this study is the Hot plate method. This study was divided into 5

Kata Kunci: daun pare, analgesik, nyeri
treatment groups animals with 5 experimental animals in each group. Animal treatment of this study consist of a negative control group using CMC-Na (0.5%), a positive control group using Paracetamol, a suspension group of pare leaf ethanol extract with a dose of 0.212mg / 20gBW, the ethanol extract suspension group of bitter melon leaves with a dose of 0.423mg / 20gBW, the suspension group of ethanol extract of pare leaves with a dose of 0.635mg / 20gBW. The results of this study indicate that the ethanol extract of 96% bitter melon leaves has activity as an analgesic in mice with the best dose of 0.635mg / 20gBW and the highest duration of analgesic activity was in 40 minutes, these activity was the closest result to positive control of paracetamol in mice.

**Keywords:** bitter sweet leaves, analgesic, pain

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1 Introduction

Pain is an event related to tissue damage such as injury, inflammation or cancer [9]. It can be caused by a mechanical, chemical or physical stimulation and damage the tissue, which could cause the release of certain substances called pain mediators, such as histamine, bradykinin, leukotrienes and prostaglandins. Pain overcome by giving analgesic drugs [12].

Analgesics are substances that function to reduce pain without losing consciousness [14]. Analgesic drugs that are often used are paracetamol. This drug is a non-steroidal anti-inflammatory drug that has antipyretic and analgesic effects. The analgesic effect of the paracetamol drug which plays a role in inhibiting the cyclooxygenase enzyme both centrally and peripherally [5]. In analgesic treatment, apart from using chemical drugs, pain can be relieved using traditional medicine. The use of bitter melon leaves as an analgesic treatment is expected to minimize the hepatotoxic effect of using chemical drugs.

One of the plants that can be used as a traditional medicine for analgesics is bitter sweet leaves. These leaves contain vitamin A, vitamin B, vitamin C, saponins, flavonoids, steroids/triterpenoids, phenolic acids, alkaloids and carotenoids [11]. The flavonoids content in the form of flavones and flavonols in bitter melon leaves is 8.30% w/w [1].

Flavonoids can inhibit the cyclooxygenase enzyme, resulting in the inhibitory activity of cyclooxygenase, which plays a role in prostaglandin synthesis as a mediator of pain stimulation, which is the first step on the pathway to eicosanoids such as prostaglandins and thromboxane [10]. 70% ethanol extract of bitter melon leaves at a dose of 3.024 mg / 200 g BW of rats with a positive control drug paracetamol is effective as an antipyretic [4]. This study aims to determine the analgesic effectiveness of the 96% ethanol extract of bitter melon leaves. The levels of flavonoids are higher using 96% ethanol solvent than using 70% ethanol [6]. It is expected that the analgesic potential of 96% ethanol extract of bitter melon leaves could be informed into the society and considered as an alternative treatment to overcome pain.

2 Experimental section

2.1 Material

40 mesh sieve, stirring rod, brown glass bottle, grinder, Hot plate Analgesics (Intralab), mouse cage, batis cloth, parchment paper, oven (Memmert), water bath, Vacuum Dryer, stopwatch, syringe, oven tenure (Ney VULCAN), analytical weigh, waterbath.

2.2 Substance

Aquadest, ammonia, Bouchardat, bitter melon leaves, ethanol 96%, FeCl₃, gelatin 10%, male mice (Mus musculus.), magnesium, methanol, NaCl, CMC-Na 0.5%, Paracetamol 500 mg, HCl, H₂SO₄ , pellet BR-512, Dragendroff
Reagent, Mayer Reagent, 3% Iron (III) solution, Zn powder.

2.3 Simplicia Preparation Process

Pare leaves were collected as much as 7150 g, then sorted the ripe leaves, washed, put in the oven at a temperature of approximately 50-55 °C until dry, re-sorted, then grinded into simplicia powder [3].

2.4 Extract Preparation Process

The ethanol extract of bitter melon leaves was made using the maceration method. Bitter melon leaf powder was weighed as much as 312 g, soaked bitter melon leaves using ethanol 96% as much as 3000 mL. Soaking was carried out for the first 6 hours, stirring occasionally, then let stand for 18 hours. Filtered and separated the filtrate with residue. The residue resulting from the first maceration then macerated again (remaceration), then the obtained filtrate is evaporated using a vacuum dryer until a thick extract is formed [3].

2.5 Preparation of Bitter Melon Leaf Extract Suspension

Pare leaf extract suspension was prepared by weighing the bitter melon leaf extract according to the calculation then suspended with 0.5% CMC-Na (as a carrier) and stirring until homogeneous.

2.6 Preparation of Test Animals

A total of 25 healthy male mice (Mus musculus) were weighed and calculated the coefficient variant (CV) then grouped randomly according to the number of treatment groups. The experimental mice were acclimatized for approximately 7 days to adapt the experimental animals to their new environment and produce a body weight of 20-30 g. Before being given the treatment, the mice were weighed again and recalculated the coefficient variant (CV) on the condition that it met CV <15% [8]. When CV results have met the requirements, treatment could be given in mice.

2.7 Treatment of Experimental Animals

25 mice were fasted for 18 hours without being fed, but given water. Then the body weight of each mouse was calculated, then the initial analgesic test was carried out using the hot plate method with a temperature of 50-55°C. The parameter observed was the time that mice raised their legs. The time interval between giving a pain stimulus and the response is called reaction time [7]. Then the mice were randomly divided into 5 treatment groups. Before the mice were treated, they were rested for 30 minutes. Each group was given orally as follows: CMC-Na (0.5%), Paracetamol (1.82 g/20g BW), bitter melon leaf extract suspension 0.212 mg, 0.423, and 0.635/20 g BW.

The mice were then observed again at 0 minutes. The hot plate was placed at a temperature of 50-55°C as a pain stimulus. After the temperature reached 50-55°C, mice in each group were put into the hot plate one by one and the response was observed, respond is counted as mice lifting the legs. Time observations were made at time intervals of 0, 10, 20, 30, 40, 50, and 60 minutes.

3 Result and Discussion

After the acclimatization process, followed by analgesic activity test using the Hot Plate method. The treatment was divided into several treatment groups; CMC-Na (0.5%) as negative control, paracetamol 1.82mg/20g BW as positive control and bitter melon leaves extract dose 0.212, 0.423, and 0.635 mg/20 g BW. Later, the experimental animals are rested for 30 minutes to provide a time span for the drug to be properly absorbed.

The next step is the initial analgesic test with the hot plate method with a temperature of 50-55°C. The objective of the initial analgesic is to identify and differentiate thickness of mice feet. Test animals will respond within 3-6 seconds after being placed on the hot plate. If it does not respond later than that time, the mice cannot be used. The parameters observed were lifting the legs after the animal was put into the hot plate. The time interval between giving a pain stimulus and the response is called reaction time [7]. The parameter observed in the response is in the form of a leg lifting movement. Then the Stopwatch is turned on, time observations are made at time intervals of 0, 10, 20, 30, 40, 50, and 60 minutes.

The results of the observation of the analgesic effectiveness test of the ethanol extract of bitter melon leaves in mice can be seen in the following table.
extract of bitter melon against mice given pain stimulation by the (hot plate) method can be seen in Table 1. Data on the results of the analgesic effectiveness test were analyzed using a completely randomized design with a factorial pattern, using the ANOVA (Analysis of Variant) method and Duncan continued test.

Based on table 1, it can be seen that the difference in the length of time the rats can stay on the hot plate with the longest mean time of 9.9 seconds (treatment with paracetamol) followed by bitter melon leaf extract at a dose of 0.635 mg / 20g BW for 9.5 seconds. While the fastest average time was found in the group treated with CMC Na. From the results of the Duncan statistical test (table 3 column 9), it shows that the negative control was significantly different from all treatment groups of bitter melon leaf extract and paracetamol. CMC Na as a solvent has no effect as an analgesic, the method used is valid and the treatment of paracetamol and bitter melon leaf extract has an effect on the analgesic effect in experimental animals. Meanwhile, the dose 3 group had the closest mean time to the hot plate with the positive control group.

Table 1. Duration of Mice to Survive on Hotplate

<table>
<thead>
<tr>
<th>Treatment dose</th>
<th>Time (minute)</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>10</td>
</tr>
<tr>
<td>CMC Na (0.5%)</td>
<td>5.65±0.32</td>
<td>6.92±0.42</td>
</tr>
<tr>
<td>Paracetamol</td>
<td>4.38±0.62</td>
<td>5.12±0.45</td>
</tr>
<tr>
<td>1st dose of bitter melon leaves extract</td>
<td>5.71±0.63</td>
<td>6.46±0.53</td>
</tr>
<tr>
<td>2nd dose of bitter melon leaves extract</td>
<td>5.70±0.26a</td>
<td>6.90±0.65</td>
</tr>
<tr>
<td>3rd dose of bitter melon leaves extract</td>
<td>5.11±0.60a</td>
<td>6.01±0.24a</td>
</tr>
<tr>
<td>Mean</td>
<td>5.31±0.58b</td>
<td>6.2±0.73c</td>
</tr>
</tbody>
</table>

Note: The average value followed by the same superscript letter in the same row and row shows the same effect (p> 0.05)

Figure 1. Experimental Animal Response Graph on the Hotplate

Bitter melon leaf extract has effectiveness as an analgesic presumably because the content of flavonoids acts as an analgesic by inhibiting the action of the cyclooxygenase enzyme by reducing the production of prostaglandins by arachidonic acid, thereby reducing pain [2].

Based on Figure 1, it can be seen that the dose of 0.635mg / 20g BB bitter melon leaves extract, resulting in the analgesic effect closest to a positive control. At this dose, the experimental animals lifted their legs the longest at 40 minutes. The time to achieve the analgesic effect was quite varied, both positive
control and the third dose of bitter melon leaf extract, namely at 40 minutes and then gradually decreased thereafter. While the treatment groups at doses 1 and 2 experienced peak analgesic effect at 60 minutes and then showed a decrease. However, until the 60th minute the analgesic effect resulting from the treatment has not fully returned, which means that the duration of each treatment is more than 60 minutes.

In the observation, there is a decrease in analgesic effect which occurs on average from 50 to 60 minutes. This can be influenced by several factors, namely internal factors and external factors. Internal factors are influenced by the metabolism of the extract given to mice, genetics or heredity, differences in age, diet and disease. The external factor is the hot plate temperature that is not right at 55 °C (can be reduced or more than 55 °C) so that the response of mice can be faster or slower to receive the response than it should be.

4 Conclusion

There was analgesic effectiveness of 96% ethanol extract of bitter melon leaves in male mice with the dose closest to positive control, namely at a dose of 0.635 mg / 20 gBW. Both treatments experienced peak analgesic effect at 40 minutes. Until the 60th minute, all treatment groups had not shown a decreased analgesic response to normal values before being given treatment so it was estimated that the duration of bitter melon leaf extract was more than 60 minutes.

5 Ethical Clearance

Number 53/KEPHP-UNPAK/7-2019 from the ethics Committee for Handling Experimental Animals Universitas Pakuan Bogor.

6 References