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Analgesic Activity Test of Leunca Leaves *(Solanum nigrum* L.*)* Ethanol Extract with Randall Selitto Method

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Abstract: Pain is a sensory and emotional experience related to tissue damage. This study uses the Randall Selitto method to determine the analgesic effect of launch leaf ethanol extract and the dose with the highest analgesic activity. Randall Selitto's method has been widely used to develop non-steroidal anti-inflammatory drugs. The maceration method extracted Leunca leaf powder with 96% ethanol solvent. A total of 25 Wistar male white rats were divided into five groups, namely positive control mefenamic acid 9 mg / 200 g BW, negative control CMC Na 1%, ethanol extract of leunca leaves doses of 5 mg / 200 g BW, 10 mg / 200 g BW and 20 mg / 200 g BW. The data obtained were analyzed using the ANOVA test, and then the LSD test was used to determine the differences between groups. The results showed that the extract doses of 5 mg / 200 g BW, 10 mg / 200 g BW, 20 mg / 200 g BW and positive control significantly differed from the negative control group. The extract dose of 20 mg / 200 g BB is comparable to the positive control, indicating that the extract dose of 20 mg / 200 g BB has the highest analgesic activity. Steroid and flavonoid compounds contained in leunca leaves are thought to have an effect as analgesics.

Keywords: Analgesic; Leunca Leaf; Randall Selitto.

Introduction

Pain is a condition with a function that provides a danger sign and protects against the presence of disorders in the body, such as rheumatism or inflammation, germ infections and muscle spasms caused by chemical and mechanical stimuli causing damage to tissues that release certain substances called pain mediators. Stimuli are transmitted through sensory nerves to the CNS through the spinal cord to the thalamus, then to the pain center in the cerebrum, where the stimulus is felt as pain [1] . The incidence of pain in the world is quite high. The latest research results from [2] The pain population in Indonesia reached 23.6% to 31.3%, with an average of joint pain (osteoarthritis). Overall, in 2007, the proportion of pain cases was 17.34%, which increased to 29.35% in 2008, and from 2009 to 2010, it increased from 39.47% to 48.32%. This figure shows that pain is enough to interfere with people's activities in Indonesia. Analgesic is a substance that reduces or alleviates pain without removing consciousness. The analgesic group is divided into two, namely central (narcotic) and peripheral (non-narcotic) analgesics. Examples of some central analgesic drugs are morphine, codeine, and tramadol HCl, while examples of peripheral analgesics are mefenamic acid, paracetamol, aspirin, and ibuprofen. Drugs with analgesic, antipyretic and anti-inflammatory properties at higher doses are widely used to relieve symptoms of rheumatic diseases such as rheumatoid arthritis, arthrosis, spondylosis [3]. Pain management can use analgesic drugs, but in the long term, analgesic drugs often give mild side effects (in the form of allergic reactions) or severe side effects (gastrointestinal system disorders, dyspepsia, nausea, vomiting, gastric bleeding). Treatment using traditional medicine is currently more favoured by the community because, besides being easy to obtain, it also has relatively mild side effects [4]. Traditional medicine is no less effective in treating various diseases, even though there has been a lot of more advanced and modern science, especially in the health sector. However, the lack of knowledge and information about various types of plants that can be used as traditional medicines and how to make them is a problem and difficulty for people interested in traditional medicine [5].

One of the plants that can be efficacious as an analgesic drug is leunca leaves (*Solanum nigrum* L). Leunca is a type of vegetable that is classified as eggplant. Sundanese people consider leunca plants as multi-purpose plants, both as a source of food, vegetable medicines and animal feed. Research on ethanol extracts of leunca leaves is still rarely done, seeing the increasingly high population of pain sufferers in Indonesia, so this study will be conducted on ethanol extracts of leunca leaves regarding analgesic activity, which will be tested on male white Wistar rats using the Randall Selitto method (mechanical stimulation), the principle of the Randall Selitto method is that the soles of the rat's feet are clamped and given the pressure of a certain weight with an increase in pressure stopped. The pressure is read in grams as the pain response threshold [6]. The extraction method used is maceration because maceration is a simple extraction method that involves soaking the simplicia powder in the liquid and is suitable for initial extraction [7]. The extraction method used is maceration because maceration is a simple way of extracting by immersing the simplicia powder in the liquid and is suitable for initial extraction [8]. The distiller used in this extraction process is 96% ethanol because 96% ethanol is stable, does

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not affect the efficacious substances, is not volatile, the heat required is low, and the heat used is high.

Research Methods

Preparation of ethanol extract of Leunca Leaf

Ethanol extract of leunca leaves was made using the maceration method. Leunca leaf powder was weighed as much as 1000 grams. The powder was placed in a maceration bottle, and 7500 ml of 96% ethanol was added. The maceration bottle was kept at room temperature, prevented from direct sunlight and constantly stirred every 3 times a day. After 5 days, the marinade results were filtered using flannel cloth and filter paper. The liquid extract was concentrated with a rotary evaporator at 40°C until a thick extract was obtained.

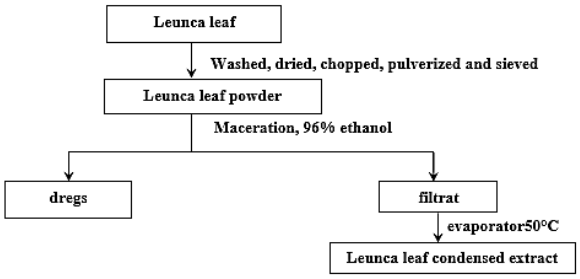

Figure 3. Scheme of Preparation of ethanol extract of leunca leaves

Testing the analgesic effect

Procedure for testing the analgesic effect of Randall Selitto method. Twenty-five rats were randomly divided into five groups for each method and fasted for 18 hours while drinking. Rats were adapted to the device for 2-3 minutes, tested Randall Selitto first before being treated, and recorded time as T0. Group I CMC Na (negative control), Group II mefenamic acid (positive control) dose 9 mg/200 g BW, Group III ethanol extract of leunca leaves at a dose of 5 mg / 200 g BW, Group IV ethanol extract of leunca leaves at a dose of 10 mg / 200 g BB, Group V ethanol extract of leunca leaves at a dose of 20 mg / 200 g BB.

After being given a single dose treatment orally, 30 minutes later, the rats were given pain stimuli as pressure with a basic analgesia meter with a certain load pressure. Holding the rat must be correct, and make sure the rat is calm enough to adapt to the test equipment. The left foot of the rat is placed between the support base and the load pressure. Then, the load is run and stopped if the rat responds by withdrawing the foot. The weight of the load was recorded in grams. This test was carried out for 4 hours with a recorded time range, namely 30 minutes, 60 minutes, 120 minutes, 180 minutes and 240 minutes.

Results and Discussion

Preparation of ethanol extract of leunca leaves

The leunca leaf powder was extracted with 96% solvent because it is selective, non-toxic, does not affect the efficacious substances, and requires less heat for the concentration. The method used was maceration. The macerate was concentrated with a rotary evaporator at 400°C until a concentrated extract was obtained. The results of strong leaf extract obtained from the maceration process using 96% ethanol have a yield of 16.16% can be seen in Table 1.

Table 1. Leunca leaf ethanol extract yield

Leunca leaf powder (g)	Condensed extract (g)	Yield (%
500	80.66	16.13

Randal Selitto method analgesic activity testing

This test was conducted to determine the analgesic activity of ethanol extract from leunca leaves by measuring the ability of the test compound to overcome the pain sensation given. The purpose of the Randall Selitto method is to assess the presence of analgesic effects measured at the threshold of pain response to mechanical pressure stimulation that many researchers have used to evaluate the presence of pain activity [7]. This method is also called the mechanical stimulation method, where the pain threshold is measured on the rat's paw, which is pressed by giving a certain weight to cause pain sensation [8]. The pain sensation arises if a stimulus exceeds a certain threshold value that can cause tissue damage, the stimulus is transmitted to the brain through the spinal cord until it reaches the thalamus impulses and is felt as pain [9]. This method has been used to evaluate the type of peripheral analgesics (Randall and Selitto 1957) referenced in [9]. Research conducted by [10], this method can also be used to evaluate the effectiveness of the therapeutic potential of central analgesics. According to [11], his research developed and modified this method to evaluate the ability of the combination of The analgesic effect can be shown by the response of increasing pain threshold with increasing load endurance values. The analgesic effect can be shown by the response of increasing the pain threshold with increasing values of load resistance given [12]. In this study, the extract test was orally administered at 5 mg / 200 g BW, 10 mg / 200 g BW, and 20 mg / 200 g BW. The dose variation was obtained based on the results of the previous orientation. The positive control was mefenamic acid at 9 mg / 200 g BW. Mefenamic acid is used because it has been proven to have analgesic effects, and the negative control used is CMC Na 1%. Observations in this method were carried out for 4 hours, with measured times of 30 minutes, 60 minutes, 120 minutes, 180 minutes, and 240 minutes. The pain response was recorded in the form of the rat's paw withdrawal by the given load, and the weight of the load was recorded in grams. The results obtained in the observation are in the form of a certain weight value, which will then be used in the calculation of AUC to determine the percent value of the increase in pain threshold. The results of the average increase in pain threshold by a load in the treatment group at each time are shown in Table 2 and Figure 1.

Figure 1 shows that overall, in the treatment groups, there was an increase in pain threshold. The negative control group produced the lowest average load endurance value compared to other control groups. This is in line with the research of [13], which showed that CMC Na as a control group could not increase the pain threshold and other controls. This is because CMC Na does not contain active substances that can inhibit pain, so it cannot withstand longer and heavier loads. The positive control group given

mefenamic acid was seen at the 30th minute to produce a greater increase in load endurance compared to the negative control group at the same minute after treatment. Still, the administration of mefenamic acid has not been able to provide an analgesic effect at the 30th minute because, based on the results of the LSD statistical test, there is no significant difference between the positive control group and the negative control group (table 3), which means that there is no inhibition of pain by mefenamic acid at the 30th minute. At the 60th minute, the average load resistance produced by mefenamic acid administration decreased; this also means no pain inhibition at the 60th minute. The analgesic effect on the positive control was seen at minutes 120 and 180. The results of this test are in accordance with research conducted by [14], which found that the analgesic effect of mefenamic acid peaks at 120 minutes because the peak concentration reaches 2-4 hours after oral administration. The species differences between humans and test animals may cause differences in metabolic processes. Still, the analgesic effect of mefenamic acid in this method is in accordance with the theory, which states that the analgesic effect of mefenamic acid reaches a peak within 2-4 hours. The mechanism of action of this drug is by [15]. Inhibiting the synthesis of prostaglandins by inhibiting the work of cyclooxygenase enzymes (COX-1 and COX-2) so that it can provide a good analgesic effect [16].

Table 2. Average load endurance response of Randall Selitto method

Group	Average load endurance response (gram) minute- X ± SD					
	0	30	60	120	180	240
Negative control (CMC Na 1%)	0±0	8.8±5.26	11.8±6.26	10.4±3.64[b]	12.8±2.16[b]	11±4.18
Positive control (Mefenamic acid 9 mg/ 200 g BW)	0±0	17±7.21	15.4±13.79	24±3.80[a]	22.6±8.08[a]	12.4±8.44
Extract dose 5 mg / 200 g BW	0±0	13±9.46	16.2±11.16	19.6±4.39[a]	18±2.44	10.2±5.89
Extract a dose of 10 mg / 200 g BW	0±0	21±6.36[a]	18.8±9.83	16.4±6.18[b]	16.6±9.91	13.2±8.52
Extract dose 20 mg / 200 g BW	0±0	18.4±4.82[a]	20.4±1.67	22.2±5.49[a]	20±9.72	8.8±7.12

Notes:
a = Significantly different from negative control with LSD test
b = Significantly different from positive control with LSD test

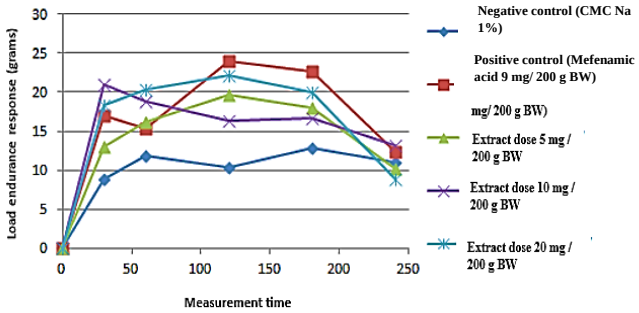

Figure 1: Graph of average measured load data

In the treatment control group given the test preparation of ethanol extract of leunca leaves, the average increase in load resistance began to appear at the 30th minute, but when compared to the negative control according to the results of the LSD statistical test, the extract dose of 5 mg / 200 g BW was not able to inhibit pain at the 30th minute. Still, the extract dose of 10 mg / 200 g BW and 20 mg / 200 g BW had an analgesic effect at the 30th minute, meaning both doses could inhibit pain at that minute. The extract dose of 5 mg / 200 g BW has an analgesic effect at the 120th minute because it increases the pain threshold to be able to withstand greater load power, this is due to the inhibition of pain stimuli at the 120th minute and then the decrease in pain threshold occurs at the 180th minute until the final 240th minute. In contrast to the extract dose of 10 mg / 200 g, which has a rapid analgesic onset time and visible analgesic effect at the 30th minute but has a short duration as seen from the decrease in pain threshold that occurs from the 60th minute to the 120th minute with the average results of load resistance produced smaller than the average results of load resistance produced in the extract dose of 5 mg / 200 g BW and 20 mg / 200 g BW at the same minute. This may be due to the body's natural response when experiencing pain because the body has a natural analgesic, namely endorphin, so the body will adapt to the pain stimulus that will cause pain in withstanding pain [17]. The treatment group at a dose of 20 mg / 200 g BW extract also experienced an increase in pain threshold from the 30th minute to the 120th minute, but the analgesic effect raised by the extract dose of 20 mg / 200 g BW was seen at two times, namely the 30th and 120th minutes. The increase in pain threshold indicates pain inhibition so that rats can withstand heavier loads. The load resistance increases and each extract dose's apparent analgesic effect differs. This states that there are also different analgesic effects. The overall response data of increasing pain threshold in the form of increasing load endurance is used to calculate AUC, and the percentage of increasing pain threshold as analgesic power can be seen in Table 3.

Giving ethanol extract from leunca leaves increases the average load endurance in response to an increased pain threshold. Statistical results with the ANOVA test (appendix 18) show that the percentage increase in pain threshold is normally distributed (P>0.05) and homogeneous with a value of P = 0.107. One-way ANOVA test with the result of P = 0.000, which shows the percentage increase in pain threshold is significantly different. The results of the research test of the treatment group were significantly different from the negative control group. Still, the extract dose of 20 mg / 200 g BW was comparable to the positive control. The resulting AUC can be calculated as the

percentage of pain threshold increase value as analgesic power. The greater the AUC value, the greater the percentage increase in pain threshold. The percentage of increase in pain threshold is the magnitude of the ability of the test compound to overcome pain due to suppression by the load given. The greater the dose, the greater the load power the test animals can withstand.

Table 3. AUC data and percentage increase in pain threshold in the treatment group

Test Group	AUC data (mean±SD)	Percentage increase in pain threshold (%) (mean±SD)
(-) (CMC Na 1%)	8.39±1.09[b]	-
(+) (Mefenamic acid)	14.22±2.21[a]	68.96±6.32
Extract dose 5 mg / 200 g BW	12.27±1.95[ab]	45.85±6.68
Extract a dose of 10 mg / 200 g BW	12.84±2.12[ab]	52.41±6.34
Extract dose 20 mg / 200 g BW	14.57±1.93[a]	73.74±5.51

Notes:
a = Significantly different from negative control with LSD test
b = Significantly different from positive control with LSD test

Analgesic activity of test preparation is indicated by the analgesic power produced greater than or equal to 50% of the negative control group, then considered effective as an analgesic [18-21]. The percentage of pain threshold increase produced by the extract dose of 5 mg / 200 g BW is 45.85%. This indicates that the extract dose of 5 mg / 200 g BW has a weak analgesic activity because the percentage value <50%. The third variation of the extract dose with the highest percentage increase in pain threshold is the extract dose of 20 mg / 200 g BW, which is 68.96% below the percentage increase in pain threshold produced by mefenamic acid. It can be assumed that the extract dose of 20 mg / 200 g BW has more active compound content with the amount that is absorbed more so that it can provide a better analgesic effect.

Conclusion

Ethanol extract of leunca leaves at doses of 5 mg / 200 g BW, 10 mg/200 g BW and 20 mg/200 g BW has analgesic activity by the Randall Selitto method, and 20 mg/ 200 g BW extract had the highest analgesic activity and was comparable to the positive control with two compound contents for analgesic test, steroid compounds and flavonoids contained in Leunca leaves are thought to have an effect as an analgesic, the mechanism of action of flavonoids as analgesics is by inhibiting the action of the cyclooxygenase enzyme which will reduce the production of prostaglandins to reduce pain. Steroids work by inhibiting phospholipases, preventing the release of arachidonic acid, and blocking the cyclooxygenase and lipooxygenase pathways to inhibit the formation of prostaglandins and leukotrienes to reduce pain.

Author's Contributions

Mia Ariasti: Analysis data and dose determinations in research trials and article compiler. Sri Winarni Sofya: data collection related to research, article compiler.

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