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ANALGETIC ACTIVITY TEST OF ETHANOL EXTRACT SUNGKAI LEAF (Peronema canescens Jack) IN WHITE MALE MOUSE (Mus musculus) INDUCED WITH ACETIC ACID

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ABSTRACT

Traditional medicine, which is generally considered safer than modern medicine, makes people choose alternative treatments using medicinal plants, one of which is to treat pain. One of the medicinal plants used to treat pain is a plant that contains flavonoids, one of which is the Sungkai plant (Penorema canescens Jack). The research design used was The Static Group Comparison, where the test animals used were divided into 5 groups, each group consisting of 5 mice. Group 1 was given 1 percent of CMC. Group II was given ethanol extract of Sungkai leaves at a dose of 300 mg/Kg BW. Group III was given EEDS at a dose of 600 mg/kg BW. Group IV was given EEDS at a dose of 900 mg/Kg BW. Group V was given paracetamol suspension. The results showed that there was an analgesic activity in the EEDS group, dose I was 50 percentage, dose II was 55,798 percentage, and dose III was 69,564 percentage. The study concluded that statisti-cally there was no significant difference in analgesic activity in white male mice between the EEDS doses I, II, and III groups and the paracetamol group.

Keywords: Extract; Sungkai Leaf; Analgesic

1. INTRODUCTION

Pain or pain is something that indicates a problem in the body, and includes a sign, whose function is to provide protection and indicate an unsafe signal regarding the presence of disturbances in the body, for example, inflammation (rheumatism, gout), contamination of germs, or muscle tension. The pain appears due to mechanical or chemical impulses, which can cause tissue damage and the release of pain-mediated substances or mediators such as braiding, histamine, serotonin, and prostaglandins (R. Afrianti et al., 2015). To reduce pain/pain can use medical or non-medical drugs such as the use of traditional medicine (Pasita, 2018; Mirzaie dkk., 2020).

The use of medicinal plants as a way of treatment already exists and is known to the people of Indonesia since ancient times. Many medicinal plants are known to have therapeutic properties for several indications, but knowledge about the benefits and whether or not these herbal medicines are safe is only hereditary and there is no scientific testing (Harniawati, D & Widya, T. Y., 2014).

Treatment using medicinal plants is generally considered safer than modern medicine. This is because the side effects of traditional medicine are relatively minimal compared to modern medicine (L. O. R. K. Sari, 2006). Indonesia has 7,000 types of plants whose benefits are known to more than 300,000 species of plants and only 300 types of plants are used for medicine or raw materials in the pharmaceutical industry (Saifudin, A, et al., 2011; Goyal, M. et al., 2021)

One of the many natural medicines that are often used for traditional medicine is Sungkai leaves (*Peronema Canescens*, Jack) (Islamudin, 2018)**.** Sungkai leaves contain alkaloids, saponins, flavonoids, tannins, glycosides, phenolics, and triterpenoids (Latief, M, 2021). According to R. Afrianti et al. (2015) Flavonoids are compounds that can protect against damage to the fatty layer and inhibit the formation of the cyclooxygenase 1 enzyme, which is the first pathway for the synthesis of pain intermediaries such as prostaglandins (Afrianti 2015; Kartikawati, E & Deswati, D. A, 2021).

By R. K. Sari (2021) for the treatment of bruises in the Serawai tribe, the leaves of *Peronema canescens*, Jack (Sungkai Leaf) are crushed into pieces. A district in Jambi Province, namely Merangin, uses a decoction of sungkai leaves as one of the traditional plants given to patients suffering from Covid-19. They believe that consuming a decoction of Sungkai leaves can cure confirmed Covid-19 patients more quickly (Mirzaie et al., 2020).

Previous studies have discussed the analgesic properties of traditional medicines containing secondary metabolites of flavonoids. Research by R. Afrianti et al. (2015) using extract doses of 100, 300, and 600 mg/BW concluded that the pain-reducing efficacy of papaya leaf ethanol extract in mice has a similar ability to paracetamol. Research by (Auliah et al., 2019). The research results obtained from the ethanol extract of jackfruit leaves (Artocarpus heterophyllus Lam.) have the greatest pain-reducing properties at a dose of 600 mg/kg BW which has the greatest percent protection.

After tracing previous research regarding the benefits of sungkai leaves, it was found that sungkai leaves have antipyretic activity (Brata, A. & Wasih, E.A., 2021), and as an anti-inflammatory (Rahman, U., 2021). However, no research has been found on Sungkai leaves as an analgesic. To the description, it is appropriate to conduct a study entitled "Test of Analgesic Activity of Sungkai Leaf Ethanol Extract (Peronema canescens Jack) in Male White Mice (Mus Musculus) Induced by Acetic Acid".

2. METHOD

The research was carried out using the pre-experimental method which was carried out using two types of objects, namely control and treatment. The control subjects used positive control (paracetamol) and negative control (CMC NA) with the Static Group Comparison design which was carried out at the Phytochemical and Pharmacology Laboratory of the Department of Pharmacy, Poltekkes Kemenkes Jambi.

2.1. Sample

From calculations according to Federer using the formulation (n-1) (t-1) ≥15, this study was divided into 5 experimental groups, with 2 for control and 3 experiments/intervention with each group using 5 mice.

2.2. Research design

2.2.1. Tools

Digital scale, stir bar, beaker glass, Erlenmeyer, measuring cup, mok, 1cc oral sonde, 1cc syringe, mortar and stamper, rotary evaporator tool, hot plate.

2.2.2. Ingredients

Sungkai leaf ethanol extract dose of 300 mg/kg, 600 mg/kg, 900 mg/kg, 500 mg paracetamol, 1% acetic acid, 1% Na-CMC.

2.3. Research Stages

At the simplicia manufacturing stage, sungkai leaves that are not too old are selected, sorted, cut, dried, and then sorted again. After the simplicia dried, it was made into dry powder. The dry powder was extracted using 70% ethanol and evaporated in a rotary evaporator until a thick extract was obtained. The extract was suspended with concentrated Na-CMC. Continue to

produce 1% Na-CMS, 1% paracetamol suspension, and 1% acetic acid, provided that the usual dose for humans is 500 mg. Conversion from a human weighing 70 kg to a 20-gram mouse produces 1.3 mg/kg BW.

2.4. Procedure

2.4.1. Qualitative identification

Qualitative identification of the ethanol extract of Sungkai leaves must be carried out before the analgesic test to determine whether or not the active substance is present

2.4.2. Stages of labor

Formula = 100 - (Number of stretches in the treatment divided by the number of stretches of the negative control multiplied by 100 percentage

a. Thirty rats fasted from eating while still being given water for 8 hours, and acclimatized and homogeneous temperature, humidity, and lighting for about 7 days in the laboratory while still being given food and drink before testing.
b. Then 25 mice were divided into 5 groups, which were divided into negative control (CMC), treatment (Sungkai leaf extract at a dose of 300 mg/kg, 600 mg/kg, 900 mg/kg, and positive control (Paracetamol).
c. Weighing and random grouping were carried out for each mouse, each group was given the test solution orally, namely, the negative control was in the form of 1% Na-CMC solution; II positive control in the form of paracetamol with a dose of 1.3 mg/20 g BW; III in the form of sungkai leaf extract at a dose of 300 mg/kg BW; IV in the form of Sungkai leaf extract at a dose of 600 mg/kg body weight; V in the form of Sungkai leaf extract at a dose of 900 mg/kg body weight.
d. After 30 minutes, 0.1 mL/20g BW of 1% acetic acid solution was given by intraperitoneal injection.
e. Then pay attention and count the total stretching every 5 minutes for 60 minutes.
f. The existence of wriggling is indicated by the presence of deflation of the stomach with the body of the mouse looking elongated due to the pulling of the legs toward the back.
g. The processing of statistical test data for One-Way ANOVA analysis was then connected using the Post Hoc Scheffe test
h. Make observations in each group and then tabulate the total data, which will then calculate the comparison between controls and tests (Islamudin, 2018).

2.5. Ethical Clearence

Ethical Clearance is carried out at the Health Office Poltekkes Ethics Committee, Jambi Ministry of Health No. LB. 06/02/29/2022.

3. RESULTS AND DISCUSSION

The ethanol extract of Sungkai leaves in this study was made by starting from collecting raw materials in the form of fresh Sungkai leaves. Then it is processed in several steps such as wet sorting, cutting, drying, dry sorting, and powdering. After refining the Simplicia, then proceed with the maceration extraction process. This method was chosen with several advantages, namely the method and use are easy, as well as the tools are simple. The solvent chosen in this maceration process is 70% ethanol, 70% ethanol is chosen because the material used is in the form of dry simplicia. Soaking was carried out for 5 days, then filtered with a flannel cloth. Then evaporated to separate the solvent using a rotary evaporator with a temperature setting at the boiling point of 70% ethanol (Inayati, A, 2010).

Qualitative identification of the ethanol extract of Sungkai leaves must be carried out before the analgesic test to determine whether or not the active substance is present. In the screening stage for the secondary metabolites of the ethanol extract of Sungkai leaves, it was found that the extract contained flavonoid compounds and it was found that the ethanol extract of Sungkai leaves

had analgesic activity in male white mice (Mus musculus) given 1% acetic acid and its effectiveness was close to that of paracetamol.

One of the most abundant groups of compound compounds found in plant tissues is flavonoids. Flavonoids include phenolic compounds whose chemical structure is C6-C3-C6. The flavonoid part consists of an aromatic ring A, an aromatic ring B, and a middle ring in the form of a heterocyclic form in which oxygen is contained. Flavonoids are compounds that can protect against damage to the fat membrane and inhibit the cyclooxygenase I enzyme, which is the first pathway for the synthesis of pain intermediaries such as prostaglandins (R. Afrianti et al., 2015).

25 male mice were used in this study and before the test was carried out, the mice were adapted for ± 7 days so that the mice were able to adjust to a new place and not be shocked. Next, mice were selected that met the inclusion criteria. Mice must also be fasted ± 8 hours before being given the action, to minimize the effect of food on the research results (Pasita, 2018).

Testing the efficacy and analgesic power of the ethanol extract of Sungkai leaves has the aim of knowing whether or not there is a decrease in pain in the ethanol extract of Sungkai leaves. This test was carried out using chemical stimulation. This method is done because it is simple, not difficult to implement, and is sensitive to testing compound components that have less strong pain-reducing power (Henny, 2010).

In this test, the negative control used 1% Na-CMC, the positive control was paracetamol at a dose of 1.3 mg/20gBW, the test group used ethanol extract of Sungkai leaves at a dose of 300 mg/kg BW, 600 mg/kg BW, and 900 mg/kg BW. This test was carried out using a chemical stimulation method, namely 1% acetic acid intraperitoneally. The presence of acetic acid can cause pain due to local tissue irritation, this occurs because H+ ions are released from acetic acid, therefore the level of acidity in the tissues decreases and tissue injury/irritation appears (Henny, 2010).

The existence of writhing in mice means that mice experience pain. Stretching is observed every 5 minutes for 60 minutes. The total stretching data was then changed to the form of stretching inhibition ability in the form of a percentage value according to the Hendersoth-Farsaith equation, then statistical analysis was carried out using the one-sample Kolmogrov-Smirnov test to find out whether the data was evenly distributed or not if the data was evenly distributed or $p>0.05$ then continued with One-Way ANOVA statistical test. If the difference is stated to be significant or the p-value <0.05, it is continued with Scheffe's posthoc test using a 95% confidence level to get clearer results on the meaning of the difference between each part of the test (Henny, 2010; Sentat & Pangestu, 2016; Lumintang et al., 2015).

From the research that has been carried out, it is obtained that the average number of mice stretches is as follow **Table 1**.

Table 1. Error the number of mice writhing

Group	The average number of stretches (n)	Standard Deviation
Negative Control (Na CMC) (Group I)	27.6	4,13094
Positive Control (Paracetamol) (Group II)	11	5,37304
Dosage 300 mg/kg BW (Group III)	13.8	6,47873
Dosage 600 mg/kg BW (Group IV)	12.2	6,06549
Dosage 900 mg/kg BW (Group V)	8.4	5,72847

The total writhing results of each treatment after administration of the test solution, then the average stretching of the mice was calculated. From the data in the **Table 1**, it can be seen that the three doses of Sungkai leaf ethanol extract and the positive control (paracetamol) showed that there was a difference in the mean total stretchiness compared to the positive control of 11; 13.8; 12.2; and 8.4 while with a negative control of 27.6. The less the average number of stretches obtained, the better the analgesic effect (Puspitasari et al., 2023; Arifin et al., 2018).

After calculating the mean total stretching for each treatment, then the percentage of analgesic protection was calculated. The percentage of analgesic protection is whether or not the test material can minimize the mice's writhing response due to acetic acid administration. The percentage of analgesic protection was obtained from the comparison of the total stretch average of the test section to the negative control (Galani, V.J & Patel, B.G., 2011). The results in the **Table 1** show that the smallest percentage of analgesic protection is in the negative control. The negative control group, i.e., the mice that were put in 1% Na-CMC suspension, could not inhibit stretching. This happens because 1% Na-CMC has no effect on experimental animals and has no pain reliever and has a role as a comparison so that the value of the ability to inhibit stretching is known. Next is the positive control section, namely mice that received paracetamol suspension treatment at a dose of 1.3 mg/20gBW. Paracetamol is a non-narcotic painkiller that works by inhibiting the formation of prostaglandins mainly in the central nervous system (CNS) thereby reducing pain in mice given acetic acid.

The results of the percentage of analgesic protection sequentially from large to small, namely the ethanol extract of Sungkai leaves at a dose of 900 mg/kg bb, positive control (paracetamol), doses of 300 mg/kg bb and 600 mg/kg bb with a percentage of analgesic protection of 69.564 percentage; 60.144 percentage; 55,798 percentage and 50 percentage.

An inverse comparison between the mean number of stretches and the percentage of analgetic protection in the test section against the negative control means that the more total stretches the mean, the smaller the percentage of analgetic protection obtained and vice versa (Puspitasari et al., 2023).

Based on **Table 1**, the results of calculating the percentage of analgesic protection in male white mice:

Formula = 100 - (Number of stretches in the treatment divided by the number of stretches of the negative control multiplied by 100%)

a. Na-CMC stretch protection (negative control)
   Average percentage of protection = 100 - [27.6/27.6 x 100%] = 0 %
b. Paracetamol stretch protection (positive control)
   Average protection percentage = 100 - [11/27.6x 100%] = 60.144%
c. Protection dose of 300 mg/kg BW
   Average protection percentage = 100 - [13.8/27.6 x 100%] =50%
d. Protection dose of 600 mg/kg BW
   Average percentage of protection = 100 - [12.2/27.6x 100%] =55.798%
e. Protection dose of 900 mg/kg BW
   Average percentage of protection = 100 - [8.4/27.6x 100%] = 69.564%

A dose of 600 mg/kg BW has the best analgesic ability because it has results close to paracetamol, namely 55.798 percent ability.

After the test results were obtained, we continued to process the data with the Kolmogorv-Smirnov test, the results were obtained p> 0.05, which means that the data is spread evenly, and in the homogeneity test, we also get p> 0.05, which also means homogeneous variance (**Table 2**). After that, proceed with the One-Way ANOVA test. One-Way ANOVA analysis is an analysis of data processing that aims to determine whether or not there are differences in values in two or more sections (Riduwan, 2003). The One-Way ANOVA analysis serves to show whether or not there is a significant difference in total stretching data for each test section. The use of One-Way ANOVA must meet the requirements. Namely, the data must be spread evenly, and the variance of the parts must be homogeneous.

The normality test results can be seen in the Symp. Sign. (2-tailed) column, if p>0.05, then the data is normally distributed. P value in negative control = 0.941, Dose 1 = 0.921, Dose 2 = 0.930, Dose 3 = 0.987, and positive control = 1. From these data, it is known that all data is evenly

distributed so that it can be continued using the One-Way ANOVA test to be able to be forwarded to the Post Hoc Scheffe test.

Table 2. Percent Normality Test for Analgesic Protection (One Sample Komogorv-Smirnov Test)

	Negative Control	Dose 1	Dose 2	Dose 3	Positive Control
Number of animal test	5	5	5	5	5
Normal Parameters	0.0	50	55.798	69.564	60.144
Kolmogorv Smirnov Z	0.530	0.331	0.534	0.450	0.306
Async. Sig. (2-tailed)	0.941	0.921	0.930	0.987	1

Table 3. One-Way ANOVA Test of Analgesic Protection Percentage

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	14886.320	4	3721.580	118.135	0.000
Within Groups	630.054	31.503			
Total	15516.375	24			

Based on the **Table 3** a significance value of p = 0.000 was obtained. This value means that the total data on the movement of mice for each test group has a significant difference (p <0.05). The results of the analysis showed that the negative control was significantly different in the amount of writing (p<0.05) to the positive control, at a dose of 300 mg/kg BW, a dose of 600 mg/kg BW, and a dose of 900 mg/kg BW, from the amount of decreased writhing at three doses of the extract. It has been proven that it is good for reducing pain from the three doses of the extract. For a dose of 300 mg/kg BW, there was a significant difference with a dose of 900 mg/kg BW indicated by a p-value <0.05, namely p=0.01, and had no significant difference with a dose of 600 mg/kg BW and positive control with a p-value> 0.05. Meanwhile, the dose of 600 mg/kg BW was also significantly different from the dose of 900 mg/kg BW with a p<0.05, namely p=0.019, and not significantly different from the dose of 300 mg/kg BW and positive control. For the positive control, namely paracetamol, the difference was not significant at all experimental doses where the p-value was shown > 0.05. The analgesic effect is proven if the percentage of analgesic power is ≥50 percent (Depkes, 1993). So, it is known that the dose of 300 mg/kg and dose of 600 mg/kg has an analgesic effect that is close to paracetamol, and the dose of 900 mg/kg has greater pain-reducing activity than paracetamol but does not have a significant difference. The ethanol extract of Sungkai leaves at a dose of 900 mg/kg has a greater percentage of analgesic power than the positive control, but this dose cannot be stated to have the best analgesic activity, because this study was limited to testing the analgesic activity of the ethanol extract of Sungkai leaves in white rats. Induced by acetic acid and a positive control comparator, namely paracetamol.

Although the ethanol extract of sungkai leaves at a dose of 900 mg/kg BW had the most significant percentage of analgesic protection, after 2 hours of observing the movement, three dead mice were found. In previous studies regarding the toxicity test of the ethanol extract of Sungkai leaves by (Melisa et al., 2022) it was found that the ethanol extract at a dose of 175-5000 mg/kg bb did not result in death in the test animals, but resulted in damage to the kidneys which was observed from the creatinine and histological values. Research by (Nabila, 2021) also showed that the ethanol extract of Sungkai leaves at a dose of 175-5000 mg/kg BW did not cause death but did cause the liver to become abnormal after macroscopic observations.

The results using the ANOVA test can be seen from the significance value. If the p value> 0.05, then H0 is rejected, and the results of the ANOVA test are significant, which means that each group is significantly different.

Table 4. Scheffe Test of Mice

Group	N	Subset for alpha=0.05		
		1	2	3
KN	5	0.000		
Dose 1	5		50.0020	
Dose 2	5		55.7980	
KP	5		60.1440	60.1440
Dose 3	5			69.5640
sig		1	0.127	0.176

Based on **Table 4** showed that there was no difference between groups with high computational freedom or small sample size in this study.

The death of the treated animals at a dose of 900 mg/kg BW could occur due to damage to the liver and kidneys, which was possibly more severe than the lower experimental doses, namely doses of 300 mg/kg BW and 600 mg/kg BW.

4. CONCLUSION

After analyzing the observed data, it was concluded that there was an analgesic activity of the ethanol extraction of Sungkai leaves in male white mice given acetic acid induction. The ethanol extract of Sungkai leaves at a dose of 600 mg/kg BW was the most effective and the effect was close to paracetamol. Sungkai leaf ethanol extract has the potential as an analgesic, so it can be used as an alternative to reduce pain with this dose. However, to make it easier to use as an analgesic, research can be continued to make solid preparations or extracts that are more practical.

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