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Research Article

Analgesic Effectiveness of ant Nest Ethanol Extract (Myrmecodia Pendens) on white Mice (Mus Musculus)

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ABSTRACT

Pain is an unpleasant sensation emotionally and subjectively, indicating damaged tissue such as inflammation or a more significant condition. Myalgia, or often known as muscle pain. In Indonesia, the percentage of Myalgia sufferers is around 45-59%. This study applies an experimental method with a pretest-posttest group design. In this study, 25 mice were used which were divided into 5 groups, namely negative control (Na CMC 1%), positive control (mefenamic acid) and 3 groups given a dose of ant nest extract (17.5 mg/kgBW, 35 mg/ kgBW, 70 mg/kgBW). The analgesic effectiveness test used was the hot plate method with the writhing response parameter measured for 3 hours with a measurement interval of every 30 minutes. The results showed that the ethanol extract of ant nests showed positive tannin, alkaloid and flavonoid compounds. Data analysis on the comparison of dose groups showed that the doses of 17.5 mg and 35 mg had a significant value of <0.05 with a dose of 70 mg, in the positive control group had a significant value of >0.05 compared to the 70 mg ant nest extract dose group, which means there is no significant difference between the two. On the contrary, the negative control group showed a significant value of <0.05 compared to all treatment groups, which indicates a significant difference. The conclusion of this study is that the dose of ethanol extract of ant nest 70 mg has good analgesic effectiveness, approaching the effectiveness of the positive control of mefenamic acid.

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INTRODUCTION

Pain is an emotional sensation that causes discomfort associated with actual or potential tissue damage (1). Thus, pain is often considered a warning indicator to protect the body from significant tissue damage. Pain often causes an unpleasant sensation such as being stabbed, electrocuted, or burned which can affect the living conditions of patients or individuals who experience it (2).

Pain is indicated to be several types, one of which is Myalgia. Based on data from the World Health Organization (WHO) in 2018, the percentage of people experiencing muscle pain disorders (Myalgia) ranged from 50% to 62% of the world's population. In Indonesia, the percentage of Myalgia sufferers is around 45-59% (3). Myalgia, or what is often referred to as muscle pain, is caused by excessive muscle use (4). Muscle pain can occur in any part of the body, including the back, neck, arms, thighs, cramps, calves, or soles of the feet (5).

Drugs that are usually used to relieve pain are included in the Non-Steroidal Anti-Inflammatory (NSAID) group (6). Analgesics are drugs that specifically reduce pain by affecting the central nervous system or the way pain works in the peripheral area, without affecting consciousness (7). The most common impacts that arise from the use of NSAID drugs are gastrointestinal (GI) problems, in the form of irritation or pain in the stomach, nausea, and abdominal pain. This occurs due to inhibition of the COX-1 enzyme, which is

responsible for the synthesis of prostaglandins from arachidonic acid. Prostaglandins function to protect the lining of the stomach mucosa (8).

Therefore, other options are needed such as using traditional medicine. In general, traditional medicine is considered safer when compared to modern medicines, because traditional medicine usually causes milder side effects than modern medicines (9). Indonesia has long known and utilized medicinal plants to overcome health problems. Medicinal plants are plants that have the potential to become medicinal ingredients, often used for healing and preventing disease. The chemical compounds contained in these plants have the ability as bioactivity (10).

For years, Papuans have used ant nests (*Myrmecodia pendens*) as part of their traditional healing practices. Papuans utilize ant nests (*Myrmecodia pendens*) by brewing them like making tea. Empirically, they use them to cure various diseases such as inflammation, strengthen the immune system, help in the treatment of tumors and cancer, and relieve muscle pain (11).

Based on the results of phytochemical screening tests and antioxidant activity on ant nest extracts (*Myrmecodia pendens*), the active compound components in ant nests consist of flavonoids and tannins (12). Flavonoid compounds found in ant nests are thought to have analgesic properties. The way flavonoid compounds work involves inhibiting the activity of the cyclooxygenase enzyme, which reduces prostaglandin production and results in reduced or eliminated pain (13).

Based on the description above, the ant nest plant (*Myrmecodia pendens*) contains flavonoids. One of the benefits of flavonoids is that they can inhibit the activity of the cyclooxygenase enzyme, especially COX-2, which functions in prostaglandin synthesis. However, there has been no research explaining the analgesic effectiveness of ant nest extract (*Myrmecodia pendens*). Thus, this study is intended to determine the analgesic effectiveness of ant nest extract (*Myrmecodia pendens*) in mice using the hot plate method.

RESEARCH METHOD

Type and Design of Research

This research uses an experimental method with a pretest-posttest group design.

Location and Time of Research

This research was conducted at the Natural Materials Laboratory and Pharmacology Laboratory, Faculty of Applied Science, Muhammadiyah University of Education Sorong. The research was conducted in March - May 2024.

Research Tools and Materials

The equipment used in this study were blender, sieve, jar, analytical balance, injection syringe, cage, water bath, glassware, filter paper, sonde needle, bottle, gloves, 10 ml glass bottle, mask, thermogun, stopwatch, and hot plate. While the materials used in this study were ant nest tubers, 70% ethanol, mefenamic acid, mice, Na CMC, and distilled water.

Preparation of ant nest samples

The sample used was 2 kg of white ant nest tubers and wet sorting was carried out to remove any remaining dirt, the cleaned tubers were then chopped to obtain a uniform size and facilitate the drying process. then washed thoroughly using running water, the washed ant nest tubers were then dried using an oven at a temperature of 50°C-55°C until they reached the standard quality of the simplicia. After drying, the simplicia was ground using a blender and sieved.

Making Ant Nest Extract

The extraction process is carried out with a ratio of powder to solvent of 1:6, namely 85 g of ant nest simplicia is put into a maceration container and 510 ml of 70% ethanol is added. After that, it is tightly closed and stored in a place that is not exposed to sunlight. Maceration is carried out for 3 x 24 hours, followed by re-maceration for 2 x 24 hours with stirring every 1 x 24 hours. The liquid extract obtained from the extraction process is then evaporated using a water bath at a temperature of 40 ° C-50 ° C to produce a thick extract.

Skrining Fitokimia

Flavonoid Test

Flavonoid test by diluting the thick extract using 70% ethanol, the mixture is added with 4-5 drops of Pb2 acetate, positive results will be seen with a color change to orange yellow to dark red (magenta).

Alkaloid Test

Alkaloid test is done by mixing the thick extract that has been diluted with 70% ethanol with 3-4 drops of Dragendorff's reagent. Positive results of the alkaloid test are indicated by the appearance of a brick red precipitate.

Tannin Test

The tannin test is carried out by mixing the thick extract of ant nest that has been diluted using 70% ethanol with a few drops of $FeCl_3$ solution. Positive results from the tannin test indicate a change in the color of the solution to blackish green or blackish blue.

Making 1% Na CMC Suspension

Weigh 1 gram of Na CMC, then gradually add it into 50 mL of distilled water heated to 70°C while stirring until a colloidal solution is formed. Then, add distilled water until the solution volume reaches 100 mL.

Determination of Dosage and Preparation of Mefenamic Acid Suspension

Mefenamic acid is the only fenamic acid that shows both central and peripheral effects. The mechanism is by inhibiting the action of the cyclooxygenase enzyme (Sumonda et al., 2021). The difference factor between humans and mice is 0.0026. Therefore, the dose of mefenamic acid that can be given to mice is:

Mouse dose = human dose x human to mouse conversion factor

= 500 mg x 0,0026

= 1,3 mg.

Mefenamic acid suspension is made by weighing 1.3 mg/kg BW of mefenamic acid powder, then suspending it in 10 ml of 1% Na CMC.

Determination of Ant Nest Extract Dosage

The doses of ant nest extract used were 17.5 mg/kgBW, 35 mg/kgBW, and 70 mg/kgBW. To make the suspension, the ant nest extract was weighed according to the specified dose and suspended in 10 ml of 1% Na CMC.

Preparation of Test Animals

This study used 25 mice as test subjects. Before the test was carried out, the researcher obtained ethical clearance from the Makassar Pharmacy College of Health Sciences. The mice were separated into 5 groups, each group containing 5 mice. The negative control group (K-) was given 1% Na CMC, the positive control group (K+) was given mefenamic acid suspension at a dose of 1.3 mg/30 g BW, while the dose groups (F1, F2, and F3) were given ant nest extract suspension at doses of 17.5 mg/KgBW, 35 mg/KgBW, and 70 mg/KgBW, respectively.

Analgesic Effectiveness Test

The analgesic effectiveness test uses the heat stimulation method, or often called the hot plate method. The procedure for testing the analgesic effect on test animals is as follows: the beaker glass is placed on a hot plate and heated to a temperature of 50°C-55°C, then the mice are put into the beaker glass. Mice are considered to have a pain response if they show jumping and licking their feet, which is observed for 15 seconds. After being observed for 15 seconds, the mice are given a suspension based on each treatment group, the mice are rested to be observed in the next observation. Observations are carried out every 30 minutes for 3 hours. This duration was chosen because the half-life of mefenamic acid is 2-4 hours.

Data Analysis

The analgesic observation data were tested for normality and homogeneity. If the significance value $p > 0.05$, further testing was carried out using the paired samples t-test and independent samples t-test methods. Statistical analysis was carried out using the SPSS 25 program with a significance level of 95%.

RESULTS AND DISCUSSION

This study used white ant nest plants taken from Folley Village, East Misool District, Raja Ampat Regency, Southwest Papua. The part used was the white ant nest tuber.

Table 1. Phytochemical screening of ant nest extract

Type of test	Reactor	Results	Information
Flavanoid	Pb2 asetat	Orange yellow	+
Alkaloid	Dragendorf	Brick red sediment	+
Tanin	FeCl3	Blackish green	+

The results of phytochemical screening of ant nest extract in table 1 showed that ant nest extract has active compounds such as flavonoids, alkaloids, and tannins. Flavonoid and tannin compounds are able to provide analgesic effects, because the way they work is by inhibiting the activity of the cyclooxygenase enzyme. By inhibiting this enzyme, prostaglandin production can be reduced, which ultimately reduces the sensation of pain (14).

The results of the flavonoid test on ant nest extract gave red or orange results after a few drops of Pb2 acetate solution were added (15). The addition of Pb2 acetate solution aims to detect the presence of flavonoid compounds in ant nest extract, which will produce a red or orange color.

The results of the tannin test were carried out by adding 2-3 drops of FeCl3 to the extract, which produces a blackish green color. Tannin compounds, which are polar because they have an OH group, will change color to blackish green when FeCl3 is added. This color change occurs because tannin reacts with FeCl3, forming a blackish green complex due to hydrolysis (15).

Positive results of alkaloid compounds in the Dragendorff reagent are indicated by the presence of brick red precipitates. If a compound contains alkaloids, then in testing with the Dragendorff reagent it will form brown-orange or orange precipitates, because the alkaloid compound will interact with tetraiodobismuta ions (15). Observations on the analgesic effect of ant nest extract were carried out for 3 hours with 30-minute intervals. The average results of writhing can be seen in Table 2 and Figure 1.

Table 2. Average results of observations of the effectiveness test of ant nest extract

Treatment group	Average Observation of movement							
	Pre test	Post test						Average
		30th minute	60th minute	90th minute	120th minute	150th minute	180th minute	
K-	8	9	11,2	13,2	14	16,2	17	12,7
K+	9	7	6	5	4	3,8	3,8	5,5
F1	8	9,6	10,6	10	9,4	8,2	7,6	9
F2	8,4	9	9,8	9,2	8,4	7,8	7	8,5
F3	9	8,2	7,2	6	5	4	3,8	6

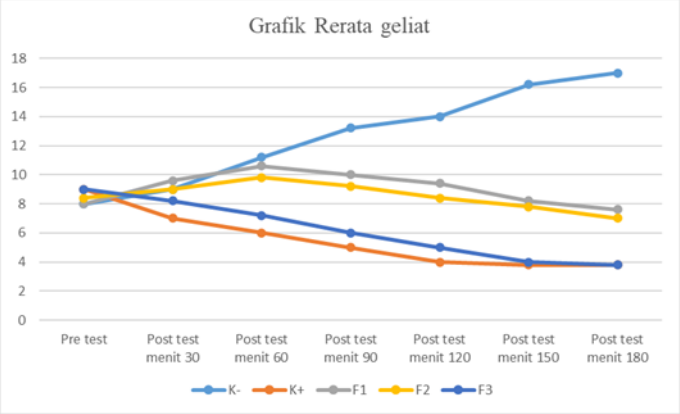

Figure 1. Average graph of observation results of the effectiveness test of ant nest extract

The test results that have been obtained are then analyzed for normality and homogeneity data. The results of the normal distribution test show a sig value> 0.05, which means that the results of the analgesic effectiveness test of ant nest extract are normally distributed, and the results of the homogeneity test show a sig value> 0.05, which means that the results of the analgesic effectiveness test of the nest extract are homogeneous overall.

Further data analysis involves statistical tests of paired samples t-test and independent samples t-test. The paired samples t-test is used to evaluate the effect of administering ant nest extract before and after treatment.

Table 3. Effect of giving ant nest extract between pre-test and post-test treatments.

Group	N (25)	Pre-test	Post-test	Δ (pre-post difference)	P Value
K-	5	8,00 ± 0,707	13,60 ± 0,548	-5,600 ± 1,140	0,000
K+	5	9,00 ± 1,225	5,20 ± 0,837	3,800 ± 0,837	0,000
F1	5	8,00 ± 0,707	9,40 ± 1,140	-1,400 ± 0,894	0,000
F2	5	8,00 ± 0,707	8,60 ± 0,894	-0,600 ± 0,894	0,000
F3	5	9,00 ± 0,707	5,80 ± 0,447	3,200 ± 0,837	0,000

The results of the paired sample t-test, as shown in Table 3, show that all treatment groups have a p value (sig) <0.05, which indicates a significant effect of the administration of ant nest extract. The p value for each treatment group is as follows: negative control 0.000, positive control 0.000, dose of ant nest extract 17.5 mg 0.000, dose of ant nest extract 35 mg 0.000, and dose of ant nest extract 70 mg 0.000.

Further data analysis was carried out using a statistical test using the Independent Samples t-test method, which was used to compare the differences between each treatment group with a significance value <0.05.

Table 4. Comparison of differences in the administration of ant nest extract between treatment groups.

Group		Δ (pre-post difference)	P Value
K-	K+	-9,40 ± 0,894	0,000
	F1	-4,20 ± 0,837	0,000
	F2	-5,00 ± 1,225	0,000
	F3	-8,80 ± 1,483	0,000
K+	K-	-9,40 ± 0,894	0,000
	F1	5,20 ± 0,837	0,000
	F2	4,40 ± 1,140	0,000
	F3	0,60 ± 1,517	0,290
F1	K-	-4,20 ± 0,837	0,000
	K+	5,20 ± 0,837	0,000
	F2	-0,80 ± 1,483	0,195
	F3	-4,60 ± 1,517	0,000
F2	K-	-5,00 ± 1,225	0,000
	K+	4,40 ± 1,140	0,000
	F1	-0,80 ± 1,483	0,195
	F3	-3,80 ± 1,095	0,000
F3	K-	-8,80 ± 1,483	0,000
	K+	0,60 ± 1,517	0,290
	F1	-4,60 ± 1,517	0,000
	F2	-3,80 ± 1,095	0,000

The results of the independent samples t-test, as shown in Table 4, show that the comparison between the negative control and the positive control, as well as the doses of ant nest extract of 17.5 mg, 35 mg, and 70 mg, produced a p value (sig) of 0.000. This indicates a significant difference between the groups.

The results of the independent samples t-test show that the comparison between the positive control and the doses of ant nest extract of 17.5 mg and 35 mg produced a p value (sig) of 0.000, indicating a significant difference. However, the comparison between the positive control and the dose of ant nest extract of 70 mg produced a p value (sig) of 0.290, indicating that there was no significant difference between the positive control and the dose of ant nest extract of 70 mg, or in other words, the results were almost the same.

The results of the independent samples t-test show that there is no significant difference between the doses of ant nest extract of 17.5 mg and 35 mg, with a p value (sig) of 0.195. However, compared to the dose of 70 mg ant nest extract, there was a significant difference with a p value (sig) of 0.000. This shows that the dose of 70 mg ant nest extract is more effective than the doses of 17.5 mg and 35 mg.

CONCLUSION

Based on the results of the study, it can be concluded that the ethanol extract of ant nest (*Myrmecodia pendens*) contains several active compounds, namely tannins, alkaloids, and flavonoids. The ethanol extract of ant nest (*Myrmecodia pendens*) also showed analgesic effectiveness in mice (Mus musculus). The dose of ethanol extract of ant nest 70 mg showed better analgesic effectiveness compared to the doses of 17.5 mg and 35 mg. However, its effectiveness is still lacking compared to the positive control, namely mefenamic acid.

SUGGESTION

It is recommended for subsequent researchers to explore further research on the development of formulations or comparison of other analgesic testing methods for ethanol extracts of ant nests.

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