TEST ANALGESIC ACTIVITY OF 70% ETHANOL EXTRACT OF STARFRUIT LEAVES (*Averrhoa bilimbi* L.) ON MALE MICE WITH WRITHING TEST METHOD

Manar Syathotho[1*], Gumiarti[2], Wima Anggitasari[3]
[1,2,3] Department of Pharmaceutical, Faculty of Health Sciences , University of dr. Soebandi
Email: manar.syathotho@gmail.com

ABSTRACT

Introduction: Analgesics are drugs commonly used to reduce or eliminate pain without losing consciousness. Several chemical compounds such as alkaloids, tannins, flavonoids and saponins contained in starfruit leaves have a therapeutic effect in reducing pain (analgesics by inhibiting the cyclooxygenase enzyme in the process of forming prostaglandins. The aim of this research is to determine the analgesic activity of the 70% ethanol extract of starfruit leaves wuluh (averrhoa bilimbi L.) in male mice using the writhing test method. Method: The research was experimental with the writhing test method using experimental mice by observing the writhing of the mice after giving acetic acid induction. Divided into 5 treatment groups, namely positive control paracetamol 65 mg /kgBW, negative control CMC Na 0.5%, group of 70% ethanol extract of starfruit leaves, doses of 100, 300 and 500 mg/kgBW. Results: The results of the study showed that the 70% ethanol extract of starfruit leaves contains chemical compounds, flavonoids, alkaloids, tannins and saponins. The average number of writhing results for CMC Na was 123.6 ± 16,920, paracetamol was 46.6 ± 13,631, and 70% ethanol extract of starfruit leaves at a dose of 100 mg/kgBW was 85.8 ± 18,240, 300 mg /kgBW is 67.2 ± 16,574, and 500 mg/kgBW is 52.6 ± 16,742. Conclusion: Analysis of differences in the number of writhes using the LSD post hoc test, namely doses of 300 mg/kg BW and 500 mg/kg BW, has a significant difference with the CMC Na negative control group and does not have a significant difference with the paracetamol positive control group which shows that this dose is comparable to paracetamol of 65 mg/kgBB as an analgesic.

Keywords: Ethanol Extract 70% Starfruit Leaves (averrhoa bilimbi L.), Analgesic, Mice

INTRODUCTION

Analgesics are drugs commonly used to relieve and treat pain(Studenski et al., 2008). Analgesics or pain-blocking drugs are drugs that relieve pain without losing consciousness(Sariana, 2011). Pain is an unpleasant sensory and emotional experience, associated with actual or potential tissue damage(Kumar & Elavarasi, 2016). Pain arises due to mechanical or chemical stimulation which can cause damage to tissue and release certain substances which are also known as pain mediators such as bradykinin, histamine, serotonin and prostaglandins.(Afrianti et al., 2015).

The total prevalence of pain in Indonesia as a whole has never been researched. It is estimated that according to research, the prevalence of cancer pain in Indonesia is around 5% of the 12.5 million population. The rate of rheumatic pain is around 23.6-31.3% and low back pain is around 40%(Tanjung., 2016). Meanwhile, around 13% of women and 10% of men over the age of 60 experience symptoms of osteoarthritis. In people over 70 years old, the prevalence increases to 40 percent. The prevalence of osteoarthritis is also lower in men than women(Jang et al., 2021). According to

RISKESDAS data, the prevalence of osteoarthritis in Indonesia is around 7.3%, with a prevalence of around 6.3% in those aged 35-44 years who are diagnosed with osteoarthritis(Riskesdas, 2018). Osteoarthritis in general is common knee pain that appears gradually and worsens with activity(Khan et al., 2018).

Analgesics that are currently frequently used are Non-Steroidal Anti-Inflammatory Drugs (NSAIDs), steroids, opioids and antidepressants. NSAIDs are drugs that are often used in therapy because they have analgesic and anti-inflammatory effects at the same time(Singh et al., 2015). NSAIDs work by inhibiting the enzymes cyclooxygenase-1 and 2 (COX-1 and COX-2) thereby reducing the production of prostaglandin (PGE2) and prostacyclin (PGI2) which are inflammatory mediators, resulting in vasoconstriction. Apart from causing vasoconstriction, inhibition of prostaglandin production has the effect of increasing sodium retention(Lovell & Ernst, 2017). The analgesic group is divided into two, namely opioid (narcotic) and peripheral (non-narcotic) analgesics. Examples of central analgesics are morphine, codeine, tramadol HCl. Examples of peripheral analgesics are acetic acid, paracetamol, aspirin and ibuprofen. These drugs are also effective as pain relievers, fever reducers and anti-inflammatories (Tjay & Rahardja, 2007). NSAIDs often have mild side effects (usually in the form of allergic reactions) but can cause serious side effects. in form (diseases of the system, gastrointestinal tract: nausea, vomiting gastric bleeding). Long-term use certainly increases the risk of side effects of this drug (Aronson, 2010).

Treatment using herbal plants has existed and been known to Indonesian people since ancient times. Many medicinal plants have been reported to have therapeutic effects for several diseases, but knowledge about the efficacy and safety of these natural medicines is mostly only empirical and has not been scientifically tested.(Visser et al., 2014). The use of traditional medicine is generally considered safer than modern medicine, this is because traditional medicine has relatively few side effects than modern medicine.(Susianto et al., 2016).

One of the plants that is efficacious as a traditional medicine is starfruit leaves (Parikesit, 2011). Starfruit leaves have the property of relieving coughs, fever, diabetes, itching (external medicine), and rheumatism (external medicine) (Khomsan, 2009). The results of the phytochemical screening of 70% ethanol extract of starfruit leaves contain alkaloids, tannins, saponins, phenolics, triterpenoid flavonoids and glycosides. Several bioactive compounds such as alkaloids, tannins, flavonoids and saponins contained in starfruit leaves have a therapeutic effect in reducing pain (analgesic).(Panjaitan, 2017).

The chemical compounds contain alkaloids, tannins, flavonoids and saponins which can inhibit the cyclooxygenase enzyme in the process of forming prostaglandins(Mikaili et al., 2012). Flavonoids are compounds that can protect lipid membranes from damage and inhibit the cyclooxygenase enzyme which is the first pathway for the synthesis of pain mediators such as prostaglandins. This will reduce the production of prostaglandins by arachidonic acid thereby reducing pain (Gunawan et al., 2014).

Research that has been carried out regarding starfruit leaves states that a dose of 500 mg/kg BW of 96% ethanol extract of starfruit leaves has an analgesic effect on mice with a writhing inhibitor percentage of 68.49% and is close to the positive control paracetamol with a dose of 65mg/kg BW of 80.36%.(Putu et al., 2020). Several other studies conducted showed that the ethanol extract of Moringa leaves and starfruit leaves combined showed results that were able to overcome pain. Where the dose that must be given to mice is a 2:2 dose with a dose of 800 mg of Moringa leaves and 1000 mg of starfruit leaves.(Amalila et al., 2021).

Because of the risk of side effects associated with the use of this analgesic drug, research is needed to find alternative treatments that are able to provide analgesic therapeutic effects but with milder side effects. Therefore, the author is interested in carrying out tests and obtaining scientific data about the potential of ethanol extract of star fruit leaves (Averrhoa bilimbi L.) as an analgesic in mice induced by acetic acid using the writhing test method. This research is still in the testing stage on experimental animals and it is hoped that in the future it can be used on humans.

MATERIALS AND METHODS

This research was carried out experimentally. The tools used are maceration bottles, a set of rotary evaporators, analytical scales, animal scales, animal cages, syringes for injection, oral sondes, dropper pipettes, filter paper, funnels, stirring rods, knives, cloth filters, stopwatches, mortar, stamp, beaker glass, measuring cup, and stopwatch.

The materials used were starfruit leaves (Averrhoa bilimbi L.), male mice, mouse food, 70% ethanol, distilled water, 1% acetic acid solution, Paracetamol and Anhydrous Acetic Acid.

Plant Determination

Wuluh starfruit leaves (Averrhoa bilimbi L.) plant identification to determine the type and ensure the correctness of Simplicia. Testing was carried out at the Jember State Polytechnic Plant Laboratory

Making Simplisia and simplicia powder

The leaves of starfruit (Averrhoa bilimbi L.) are wet sorted by washing them thoroughly, then cutting them into small pieces and then drying them in the sun by covering them with a black cloth so that the active compounds are not lost. Once dry, blend and sift.

Making Ethanol Extract of Starfruit Leaves

Starfruit leaf powder was extracted using the maceration method, weighing 500 grams, adding 2 liters of 70% ethanol solvent so that the ratio was 1:4. Then let it sit for 3 days, after which it is filtered to obtain starfruit leaf extract. The extract of starfruit leaves is then rotated through the evaporator to obtain a thick extract of starfruit leaves.

Phytochemical Screening of Starfruit Leaves

1) Alkaloid Compound Test
   A total of 1 gram of sample extract was put into a test tube which was then dripped with 3 mL of HCl. After that, 1 mL of Dragendrof reagent was added. positive for alkaloids if a reddish brown precipitate is formed (Mutmainah, 2019).
2) Flavonoid Compound Test
   A total of 1 gram of sample extract was put into the hole of the test tube and then magnesium powder and concentrated HCl were added. After that, heat it for 15 minutes in a water bath. Positive for containing flavonoids if the color is red or yellow (Mutmainah, 2019).
3) Tannin Compound Test
   A total of 1 gram of sample extract was put into a test tube and 10 mL of hot water was added and boiled for 5 minutes. After that, 3-4 drops of FeCl3 were added. Positive for containing tannin if the color shown is blackish blue or brownish green (Mutmainah, 2019).

4) Saponin Compound Test
   A total of 1 gram of sample extract was put into a test tube and 10 mL of hot water was added, cooled. After that, shake vigorously for approximately 10 seconds. If there is 1-10 cm of foam in no less than 10 minutes and if 1 drop of HCl is added, the foam is still there (Mutmainah, 2019).

Making 1% Acetic Acid Solution

Pipette 1 ml of 100% glacial acetic acid solution then dilute to 100 ml with distilled water for injection.

Preparation of 0.5% CMC Na Solution

A total of 0.5 grams of CMC Na was sprinkled into a mortar containing 10 ml of hot water. CMC Na was allowed to stand until homogeneous, then poured into a 100 mL measuring flask and adjusted with distilled water.

Making Paracetamol Solution

Weigh 10 paracetamol tablets (each tablet contains 500 mg paracetamol), then calculate the average weight and grind them evenly. Paracetamol was weighed by mice at 1.3 mg-20 g, then suspended gradually in 0.5% CMC Na solution while grinding in a mortar until homogeneous, then put into a measuring flask and 100 mL was added to the volume. The volume given is 0.13 ml/20 gBB orally.

Paracetamol dosage

The type of test animal used is mice, based on the dose calculation conversion table for various types of test animals from various species and humans, the conversion dose for humans weighing 70 kg to mice weighing 20 grams is 0.0026.

Paracetamol dose = 500 mg / tab

Dosage conversion = 500 mg x 0.0026

=1.3 mg /20 kg BW

The dose used was given to mice

$$x = \frac{1.3\ mg}{20\ g} x \frac{x}{100\ mg}\ .$$

x = 65 mg/kg BW

Preparation of Test Animals

1) Mice were adapted for 1 week and fasted for ± 18 hours first and given water
2) Mice were grouped randomly into 5 groups, each consisting of 5 mice. Mice were marked on their tails using a marker to make it easier to observe.
3) Mice were weighed one by one and their weights were then recorded

Experimental animals were divided into 5 groups

1) Group 1: a group of mice were given 0.5% CMC Na suspension orally
2) Group 2: group of mice that were given Paracetamol suspension at a dose of 65 mg/kgBW orally
3) Group 3: administered orally with a solution of starfruit leaf extract at a dose of 100mg/KgBW
4) Group 4: administered a solution of starfruit leaf extract at a dose of 300mg/KgBW orally
5) Group 5: administered a solution of starfruit leaf extract at a dose of 500mg/KgBW orally

Testing Analgesic Activity Using the Writhing Test Method

A total of 25 mice were divided into 5 groups with 5 mice in each group and given the test treatment, namely: Positive control paracetamol 65/kgBW, negative control CMC Na 0.5%, ethanol extract of star fruit leaves at a dose of 100 mg/kgBW. , Ethanol extract of starfruit leaves, dose of 300 mg/kgBW, Ethanol extract of starfruit leaves, dose of 500 mg/kgBW. Given oral treatment to all groups. After 30 minutes, 0.1ml/kgBW of 1% acetic acid was induced intraperitoneally. Observe stretching every 5 minutes for 60 minutes. Writhing was counted when the mouse began to feel pain, which was indicated by the mouse's body stretching, followed by a crushing of the stomach on the floor.

Data analysis

Data analysis was carried out using parametric statistical tests. The data obtained was first tested for normality using Shapiro Wilk. Next, a homogeneity test was carried out. Data is said to have a normal and homogeneous distribution if the P-value is>¤0.05. The aim of the normality test and homogeneity test is to be used as a requirement to be able to use the one way ANOVA test. After carrying out the normality and homogeneity test, it is then analyzed using the one way ANOVA test with a confidence level of 95 % to see the significance of each group. If a p value <0.05 is obtained, a further test is carried out, namely a post hoc test with LSD (least significant differences) to see significant differences between groups, indicated by a p value <0.05.

Research Ethics

The ethics of using experimental animals is that experimental animals used in this research will experience suffering such as discomfort, pain, and even death. Therefore, animals sacrificed in research whose results can be used by humans need to be cared for properly, and efforts must be made to adapt their lifestyle to the same pattern as in nature. Researchers who will use experimental animals in health research must assess the feasibility and reasons for using animals by considering the suffering that will be experienced by experimental animals and the benefits that will be obtained for humans.

Experimental research ethics are the rules or principles that must be implemented in carrying out experiments. The ethical test for this research will be carried out through the ethics committee at Dr. University. Soebandi Jember. No. 125/KEPK/UDS/IV/2023.

RESULTS AND DISCUSSION

Plant Determination Results

Plant determination was carried out at the Jember State Polytechnic. Based on the letter of determination issued by the UPA. Jember State Polytechnic Integrated Agricultural Development with No: 96/PL17.8/PG/2023, the results can be stated that the specimens below (attached) are: Kingdom: Plantae; Devisio; Spermatophyta; Sub Division; Magnoliophyta; Class: Magnoliopsida; Sub Class: Rosidae; Order: Geraniales; Family: Oxalidaceae; Genus: Averrhoa; Species: Averrhoa bilimbi, L

Results of 70% ethanol extraction of Wuluh Starfruit Leaves

The weight of starfruit leaf simplicia powder was weighed as much as 500 grams and 2L of 70% ethanol as a solvent for the maceration process. Next, thickening is carried out using a rotary evaporator at a temperature of 50°C. The result of the thick extract obtained was 57.19 grams, so the yield value obtained from the extraction (maceration) was 11.43% w/w.

Results of Identification of Chemical Compounds of Starfruit Leaves in 70% Ethanol Extract

The results of phytochemical screening show that the 70% ethanol extract of starfruit leaves contains chemical compounds which can be seen in table 2.

Based on the table above, it is found that the 70% ethanol extract of starfruit leaves contains flavonoids, alkaloids, tannins and saponins. The results of the phytochemical screening showed that the 70% ethanol extract of starfruit leaves contained chemical compounds. Based on the results, it was found that the 70% ethanol extract of starfruit leaves contains flavonoids, alkaloids, tannins and saponins.

This is in line with research (Susi Yanti and Yulia Vera 2019) that 70% ethanol extract of starfruit leaves contains flavonoids, alkaloids, tannins and saponins. Several other studies conducted showed that the results of phytochemical screening of 70% ethanol extract of starfruit leaves contain chemical compounds that have analgesic effects, namely flavonoids, alkaloids, tannins, saponins (Aryantini et al., 2018).

Based on this, the 70% ethanol used in starfruit leaf extract can attract compounds that are polar in nature and have an analgesic effect such as flavonoids, alkaloids, tannins and more saponins because 70% ethanol is a more polar solvent compared to organic solvents. other. Where the 70% ethanol solvent has higher levels of flavonoid, alkaloid, tannin and saponin chemical compounds than the 50% and 96% solvents. The test results show that the 70% ethanol extract of starfruit leaves contains positive chemical compounds such as flavonoids, alkaloids, tannins and saponins.

Results of Identification of the Number of Writhing Mice in the Negative Control (CMC Na), Positive Control (Paracetamol) Group, 70% Ethanol Extract of Starfruit Leaves Dose 100 mg/kg BW, 70% Ethanol Extract of Wuluh Starfruit Leaves Dose 300 mg/kg BW, Ethanol Extract 70% Starfruit Leaves Dosage 500 mg/kg BW

The test was carried out on 5 test groups and the number of movements of each mouse was observed every 5 minutes for 60 minutes. The results of the number of wriggles for each group were then calculated as the average writhing of mice every 5 minutes for 60 minutes in each test group after being given acetic acid. After conducting research, it can be seen that the average cumulative amount of writhing for CMC Na is 123.6 ± 16,920, Paracetamol is 46.6 ± 13,631, and 70% ethanol extract of starfruit leaves at a dose of 100 mg/kgBW is 85.8 ± 18,240. the 70% ethanol extract of starfruit leaves at a dose of 300 mg/kgBW was 67.2 ± 16,574, and the 70% ethanol extract of starfruit leaves at a dose of 500 mg/kgBW was 52.6 ± 16,742. The highest average number of writhes was the CMC Na negative control group, which was 123.6 ± 16,920, while the lowest average number of writhes was the paracetamol positive group, which was 46.6 ± 13,631. Can be seen in table 3.

The average number of writhing results for CMC Na was 123.6 ± 16,920, Paracetamol was 46.6 ± 13,631, and 70% ethanol extract of starfruit leaves at a dose of 100 mg/kgBW was 85.8 ± 18,240, 70% ethanol extract of starfruit leaves wuluh dose of 300 mg/kgBW was 67.2 ± 16,574, and in 70% ethanol extract of star fruit leaves wuluh dose of 500 mg/kgBW was 52.6 ± 16,742.

This is in line with research (Putu et al 2020) that ethanol extract of starfruit leaves resulted in a decrease in the average number of mice writhing, indicating that the average number of mice writhing in the positive control group and extract group was reduced compared to the negative control group. This shows that the group given the extract and the positive control can reduce the average number of writhing in mice. The lower the average number of mice writhing, the better the analgesic effect of the test material.

The test was carried out on 5 test groups and the number of movements of each mouse was observed every 5 minutes for 60 minutes. The results of the number of wriggles for each group were then calculated as the average writhing of mice every 5 minutes for 60 minutes in each test group after being given acetic acid. After conducting the research, it can be seen that the highest average number of writhes was the CMC Na negative control group, which was 123.6 ± 16,920, while the lowest average number of writhes was the paracetamol positive group, which was 46.6 ± 13,631. This is because the negative control group CMC Na given to mice showed an increase in writhing from the first 5 minutes because CMC Na does not have analgesic activity and automatically does not have the effect of reducing writhing because the function of CMC Na is only as a solvent. Meanwhile, in the positive group, paracetamol given to mice showed a decrease in writhing from the first 5 minutes because paracetamol can inhibit prostaglandin synthesis, especially in the central nervous system (CNS), thereby providing an analgesic effect in reducing writhing in mice and paracetamol can also reduce and eliminate mild pain. to medium. In the 70% ethanol extract group of starfruit leaves, the doses were 100 mg/kgBW, 300 mg/kgBW and 500 mg/kgBW based on the results. The average cumulative number of mice writhing decreased with increasing dose, where the chemical compound content obtained was higher. This is due to the chemical compound content in the 70% ethanol extract of starfruit leaves which can protect lipid membranes from damage and inhibit the cyclooxygenase enzyme which is the first pathway for the synthesis of pain mediators such as prostaglandins. This will reduce the production of prostaglandins by arachidonic acid thereby reducing pain. It can be shown that increasing the dose of 70% ethanol extract of starfruit leaves (*Averrhoa bilimbi L*.) reduces the amount of writhing in mice.

Results of Analysis of Differences in the Number of Mice Writhing in the Negative Control Group (CMC Na), Positive Control (Paracetamol), 70% Ethanol Extract of Starfruit Leaves Dose 100 mg/kg BW, 70% Ethanol Extract of Wuluh Starfruit Leaves Dose 300 mg/kg BW, Extract Ethanol 70% Starfruit Leaves Dosage 500 mg/kg BW

The normality test with Shapiro Wilk showed that the data were distributed normally, it can be seen that the value obtained was P-value>$^{\alpha}$0.05, namely the positive control for paracetamol was 0.687 and the negative control for CMC Na was 0.962, while the 70% ethanol extract of star fruit leaves at a dose of 100 mg/ kgBW was 0.816, 70% ethanol extract of starfruit leaves at a dose of 300 mg/kgBW was 0.910, and 70% ethanol extract of starfruit leaves at a dose of 500 mg/kgBW was 0.986. The data homogeneity test results obtained were 0.978 and were homogeneous because they had a significant value showing P-value>$^{\alpha}$0.05. The results of the normality and homogeneity tests can be seen in table 4.

After the data was normally and homogeneously distributed, it was continued with the one way ANOVA test to find the significance of the relationship between the five groups. Then the result obtained was 0.00, which means the P-value <$^{\alpha}$0.05 indicates there is a significant difference between groups. Because there were significant differences between groups, a post hoc test with LSD (least significant differences) was continued to see the differences between groups. LSD results are shown in table 5.

The results of the analysis showed that the CMC Na negative control group showed results that had significant differences with the paracetamol positive control group, the 70% ethanol extract group of starfruit leaves at doses of 100 mg/kgBW, 300 mg/kgBW and 500 mg/kgBW with a P-value < $^{\alpha}$0.05. This shows that CMC Na as a negative control failed to inhibit pain. Meanwhile, the positive control group of paracetamol showed results that were not significantly different from the 70% ethanol extract group of starfruit leaves at doses of 300 mg/kgBW and 500 mg/kgBW with a P-value>$^{\alpha}$0.05 and significantly

different from the 70% ethanol extract of leaves. starfruit at a dose of 100 mg/kgBW with a P-value <ᵅ0.05. This shows that a dose of 100 mg/kg of 70% ethanol extract of starfruit leaves is not equivalent to a dose of 65 mg/kgBW. The 70% ethanol extract group of starfruit leaves at a dose of 100 mg/kgBW showed results that were not significantly different from the group of 70% ethanol extract of starfruit leaves at a dose of 300 mg/kgBW with a P-value>ᵅ0.05 and significantly different from the extract. ethanol from starfruit leaves at a dose of 500 mg/kgBW with a P-value <ᵅ0.05. Meanwhile, the 70% ethanol extract group of wuluh starfruit leaves at a dose of 300 mg/kgBW showed results that were not significantly different from the 70% ethanol extract group of wuluh starfruit leaves at a dose of 500 mg/kgBW with a P-value>ᵅ0.05.

The data obtained is in line with research (Putu et al 2020) that starfruit leaf extract shows activity as an analgesic due to the chemical compound content in starfruit leaf extract which can inhibit the cyclooxygenase enzyme which is the first pathway for the synthesis of pain mediators such as prostaglandins.

From this analysis it can be seen that CMC Na as a negative control has a significant difference from the positive control group of paracetamol, the 70% ethanol extract group of star fruit leaves at a dose of 100 mg/kgBW, 300 mg/kgBW and 500 mg/kgBW. This is because CMC Na does not contain active substances as an analgesic. CMC Na is commonly used as a solvent or additive in the food and pharmaceutical industries, which is a derivative of cellulose and has no effect on reducing pain in mice. Meanwhile, in the positive control group paracetamol, paracetamol at a dose of 65 mg/kgBW showed results that were not significantly different from the 70% ethanol extract group of starfruit leaves at doses of 300 mg/kgBW and 500 mg/kgBW and were significantly different from the 70% ethanol extract of starfruit leaves at dose 100 mg/kgBB. This is due to the content of chemical compounds flavonoids, alkaloids, tannins, saponins. Flavonoids are efficacious in inhibiting the cyclooxygenase enzyme which affects the formation of prostaglandins so that pain is reduced. Alkaloids play a role in blocking an important stage of prostaglandin formation in the cyclooxygenase enzyme in the arachidonic acid pathway. Tannin has the function of stimulating the release of the lipomodulin enzyme which causes the phospholipase enzyme to be inhibited so that the cyclooxygenase and lipooxygenase pathways are interrupted and no metabolites are formed. Meanwhile, tannin can reduce pain because it is effective in inhibiting COX-1. Meanwhile, saponins inhibit nitric oxidase by inhibiting central mechanisms involving opiates, dopaminergic, noradrenergic, or peripherally involving prostaglandins, leukotrienes and other endogenous substances in reducing pain. At a dose of 100 mg/kg, the 70% ethanol extract of star fruit leaves contains low levels of flavonoid, alkaloid, tannin and saponin chemical compounds. Even though it can provide an analgesic effect, the analgesic effect obtained is low and not equivalent to a paracetamol dose of 65 mg/kgBW so the effect given is less effective in reducing pain in mice. The 70% ethanol extract of starfruit leaves at a dose of 300 mg/kgBW and 500 mg/kgBW, both doses are able to provide an analgesic effect comparable to paracetamol at a dose of 65 mg/kgBW which contains high levels of flavonoid, alkaloid, tannin and saponin chemical compounds as analgesic so it can be said to be able to reduce pain at that dose. At doses of 300 mg/kgBW and 500 mg/kgBW, these two doses have different decreases in the average number of writhes.

CONCLUSIONS AND RECOMMENDATIONS

Conclusion

Based on the research results above, it can be concluded as follows:

1) The 70% ethanol extract of starfruit leaves (Averrhoa bilimbi L.) contains alkaloid, flavonoid, tannin and saponin chemical compounds.
2) The average number of writhes for CMC Na was 123.6 ± 16,920, Paracetamol was 46.6 ± 13,631, and 70% ethanol extract of starfruit leaves at a dose of 100 mg/kgBW was 85.8 ± 18,240, 70% ethanol extract of wuluh starfruit leaves a dose of 300 mg/kgBW was 67.2 ± 16,574, and in 70% ethanol extract of starfruit leaves a dose of 500 mg/kgBW was 52.6 ± 16,742
3) Based on the analysis of differences in the number of writhes through the LSD post hoc test, namely doses of 300 mg/kg BW and 500 mg/kg BW as an analgesic because these doses did not have a significant difference with the positive control group of paracetamol which showed that this dose was comparable to paracetamol of 65 mg/kgBW.

Suggestion

Based on the results of the research above, the researchers suggest several things as follows:

1) For Further Researchers
   In future researchers, it is hoped that they will be able to determine the levels of flavonoids and toxicity contained in starfruit leaves (Averrhoa bilimbi L.)
2) For Pharmaceutical Institutions
   Through this research, it is hoped that it can increase knowledge about testing the analgesic activity of extracts. The analgesic activity of 70% ethanol extract of starfruit leaves (Averrhoa bilimbi L.) in mice.

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TABLE AND FIGURE

Table 1 Calculation of % Yield Value

Simplicia Powder	Condensed Extract	%Maceration Yield
500 grams	57.19 grams	11.43%

Table 2 Chemical Compounds of Starfruit Leaves, 70% Ethanol Extract.

Identification	Reactor	Results	Conclusion
Flavonoids	Mg powder and concentrated HCl	Orange red color (orange)	Positive
Alkaloids	Dragendorf	The precipitate is reddish brown	Positive
Tannin	FeCI3	Blackish blue or brownish green	Positive
Saponins	Aquadest and HCl	Stable foam forms for more than 10 minutes	Positive

Table 3 Average Number of Writhes in Each Test Group

Group	Number of Mice Writhing 60 Minutes After Acetic Acid Induction					Mean writhing ± SD
	M1	M2	M3	M4	M5	
K- CMC Na	136	122	145	110	105	123.6 ± 16.920
K+ Paracetamol	53	66	40	30	44	46.6 ± 13.631
EEDBW 100 mg/kgBW	90	74	65	113	87	85.8±18,240
EEDBW 300 mg/kgBW	57	77	64	48	90	67.2 ± 16.574
EEDBW 500 mg/kgBW	50	62	31	75	45	52.6±16,742

Description =

EEDBW =Ethanol Extract 70% Starfruit Leaves

M= Mice

Table 4 Normality and Homogeneity Tests

Group	Normality test	Homogeneity test
K+ CMC Na	0.687	

K- Paracetamol	0.962	
EEDBW 100 mg/kgBW	0.816	0.978
EEDBW 300 mg/kgBW	0.910	
EEDBW 500 mg/kgBW	0.986	

Description =EEDBW =Ethanol Extract 70% Starfruit Leaves

Table 5 Analysis of Differences Between Groups

Group	Sig.	Information
CMC Na with Paracetamol	0,000	Have Meaningful Differences
CMC Na with EEDBW 100 mg/kgBB	0.002	Have Meaningful Differences
CMC Na with EEDBW 300 mg/kgBW	0,000	Have Meaningful Differences
CMC Na with EEDBW 500 mg/kgBW	0,000	Have Meaningful Differences
Paracetamol with EEDBW 100 mg/kgBB	0.001	Have Meaningful Differences
Paracetamol with EEDBW 300 mg/kgBB	0.062	Have No Meaningful Differences
Paracetamol with EEDBW 500 mg/kgBB	0.572	Have No Meaningful Differences
EEDBW 100 mg/kgBW with EEDBW 300 mg/kgBW	0.090	Have No Meaningful Differences
EEDBW 100 mg/kgBW with EEDBW 500 mg/kgBW	0.005	Have Meaningful Differences
EEDBW 300 mg/kgBB with EEDBW 500 mg/kgBB	0.177	Have No Meaningful Differences

Description =EEDBW =Ethanol Extract 70% Starfruit Leaves