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Evaluation of Analgesic Activity of Extract from Moringa Leaves (*Moringa Oleifera* Lamk) in Mice *(Mus Musculus)*

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ABSTRACT

Every human being experiences pain such as during chronic pain, infection, surgery or other medical interventions. In overcoming pain in patients, it is necessary to administer drugs in the form of analgesics. One of the herbal plants that can be used is Moringa leaves (*Moringa oleifera* Lamk) which contains metabolite compounds such as flavonoids, alkaloids, steroids and tannins that have analgesic activity. The aim of this research is to assess the analgesic activity of Moringa leaf extract (*Moringa oleifera* Lamk) in mice (*Mus musculus*) using 96% ethanol solvent and *writhing reflex* method. The experimental animals were divided into 3 groups: negative control group (Na-CMC 0.5%), positive control group (aspirin) and treatment group (Moringa leaf extract). The treatment material is given orally. Animals were treated with 0.6% acetic acid injection intraperitoneally to give the effect of pain in mice. Measurement of the analgesic effect with the *writhing reflex* method is seen from the number of writhing. Based on the results of the study it can be concluded that the administration of Moringa leaf extract (*Moringa oleifera* Lamk) with the *writhing reflex* method has analgesic activity, which can reduce the pain response characterized by a decrease in the number of writhing in mice (*Mus musculus*).

KEYWORDS: *Analgesic, Pain, Moringa Leaves, Writhing Reflex.*

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INTRODUCTION

Every human being experiences pain such as during chronic pain, infection, surgery or other medical interventions. Basically, pain is caused by mechanical, chemical or physical stimuli (heat, electricity) that cause tissue damage due to the stimulation of pain mediators such as prostaglandins, leukotrienes and others [4]. Dental pain has been ranked second (17.6%) compared to head, muscle and joint pain. The high prevalence of oral pain problems in Indonesia increased from 2007 (23.3%) to 2013 (25.9%). 31.1% of the community received treatment with the help of health workers and 68.9% of the community did not receive treatment with the help of health workers [5].

Tooth extraction is one of the actions in the field of dentistry that can cause pain due to trauma to the socket of the extracted tooth. Pain during treatment cannot be denied even though the correct procedure has been carried out. Preparations made to overcome pain in patients in the act of tooth extraction, one of which is by administering analgesic drugs [9]. Analgesic drugs are a group of drugs that have the activity of reducing pain without removing consciousness. Analgesics are used to relieve symptoms such as headache, toothache, menstrual pain, muscle pain, abdominal pain, fatigue and so on [8].

Analgesics are divided into 2 major groups, namely non-narcotic analgesics (non-opioid) and opioid analgesics. The most commonly used non-narcotic analgesics are mefenamic acid, aspirin and paracetamol [3]. These drugs provide faster effects compared to herbal medicines, but if consumed for a long time cause side effects such as stomach and intestinal disorders, blood damage, liver, kidneys and

allergic reactions. Aspirin is an analgesic drug of the NSAID (*non-steroidal anti-inflammatory drug*) class that can treat pain with mild to moderate intensity [14]. Side effects that can be caused are gastric ulcers. Therefore, other alternatives are needed by utilizing herbal plants that have relatively smaller side effects.

One of the herbal plants that can be used for analgesic drugs is Moringa leaves (*Moringa oleifera* Lamk). Moringa leaves (*Moringa oleifera* Lamk) are often known as miracle plants or the tree of life [12]. The active substances contained in moringa leaves are flavonoids, alkaloids, steroids and tannins [2]. Flavonoids, alkaloids, steroids and tannins are efficacious as analgesics whose mechanism of action inhibits the work of phospholipase, cyclooxygenase and lipoxygenase enzymes, which will reduce the production of prostaglandins, thromboxanes, prostacyclin and leukotriene (pain mediators) so as to reduce pain [13].

Moringa leaves at doses of 25 mg/kg BW, 50 mg/kg BW and 75 mg/kg BW given have an analgesic effect in terms of reducing the pain response [1]. The preliminary results showed that the dose of 75 mg/kg BW was the most effective dose. The author advises readers to conduct further tests using different solvents and methods. From the background that has been conveyed, researchers are interested in conducting research to test and find out how the analgesic activity of moringa leaf extract (*Moringa oleifera* Lamk) in mice (*Mus musculus*) using 96% ethanol solvent and different methods from preliminary tests conducted previously with the writhing reflex method. From the content of Moringa leaf extract (*Moringa oleifera* Lamk), it is hoped that researchers will be able to make this herbal plant another alternative for analgesic drugs.

METHOD

This type of research is *experimental laboratories* with *post test only control group design*. This research was conducted at the Integrated Agricultural Development UPT of Jember State Polytechnic for the identification of Moringa leaves (*Moringa oleifera* Lamk), the Pharmacy Technology Laboratory of the Jember Academy of Pharmacy for the process of making Moringa leaf extract (*Moringa oleifera* Lamk), and the Biomedical Animal Laboratory of the Faculty of Dentistry, University of Jember for the maintenance and treatment of experimental animals in this study.

RESEARCH SUBJECT

The experimental animals were divided into 3 groups: negative control group (Na-CMC 0.5%), positive control group (aspirin) and treatment group (Moringa leaf extract) was given Moringa leaf extract at a dose of 75 mg/kg BW. Moringa leaf extract (*Moringa oleifera* Lamk) was obtained using maceration and remaceration techniques with 96% ethanol as a solvent.

RESEARCH PROCEDURE

All materials are given orally using a gastric sonde according to the predetermined dose. 30 minutes later all experimental animals will be injected with 0.6% acetic acid as pain induction intraperitoneally for each mice, because 30 minutes is estimated that the drug has reached each receptor. The pain effect is characterized by the number of writhing, namely abdominal retraction, appearing in mice stretching their legs and their abdomen touching the floor. After administering 0.6% acetic acid, the mice waited for 5 minutes. Then the number of writhing can be observed and recorded every 5 minutes for 30 minutes.

DATA ANALYSIS

Data analysis using the *Shapiro-Wilk* Test was used to test the normality of the data obtained, and the *Levene* Test was used to determine homogeneity. Data showed normal distribution but not homogeneous. So proceed with non-parametric tests using the *Kruskal-Wallis* test to determine whether there are significant differences in all sample groups with a significance value ($p < 0.05$). Then to test whether there is a significant difference between each group, the data was tested using the *Mann-Whitney U* test with a significance value ($p < 0.05$).

RESULTS

The results of the analgesic effect test in the group of mice with the writhing reflex method given Na-CMC 0.5% (K-), the group of mice given aspirin at a dose of 0.065 mg/kg BW (K+), the group of mice given moringa leaf extract at a dose of 75 mg/kg BW (KP) in the form of averages that have been presented in the form of Table 1 and Figure 1.

Table 1. Average number of writhing mice with the Writhing Reflex method.

Time (Minutes)	Mean number of mice writhing (mean ± standard deviation)		
	Na-CMC (K-)	Aspirin (K+)	Moringa Leaf Extract (KP)
5’	21.25 ± 7.588	18 ± 3.559	9.5 ± 1.290
10’	28 ± 11.888	15 ± 1.825	8 ± 1.414
15’	26.5 ± 4.358	15.75 ± 0.957	7 ± 0.816
20’	22 ± 7.958	12.75 ± 1.258	6 ± 1.414
25’	14.25 ± 5.123	10.25 ± 1.707	5.25 ± 0.957
30’	12.25 ± 4.924	8 ± 1.414	4 ± 0.816

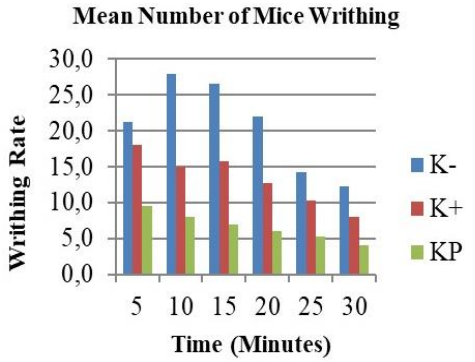

Figure 1. Graph of the average number of writhing mice through the writhing reflex method.

The graph (Figure 1) shows the average number of writhing mice in each treatment. The average number of mice writhing decreased. The mean number of writhing of the treatment group (KP) is smaller than the positive control group (K+), the mean number of writhing of the positive control group (K+) is smaller than the negative control group (K-) and the mean number of writhing of the negative control group (K-) is greater than the treatment group (KP). The mean number of writhing mice from the smallest in a row, namely the Moringa leaf extract group, the positive control group (aspirin) and finally the negative control group (Na-CMC 0.5%) can be written as KP < K+ < K-.

Data from the analgesic activity test observations on *Balb*-C mice before being statistically analyzed, first the normality test was carried out using the *Shapiro-Wilk* test and the data homogeneity test with the *Levene* test. In statistical tests that have been carried out, the data is normally distributed ($p > 0.05$) but the data is not homogeneous ($p < 0.05$), so statistical testing is carried out with the *Kruskal-Wallis* and *Mann-Whitney U* non-parametric tests to see any differences in the number of writhing mice in each group.

Table 2. *Kruskal-Wallis* test results with a significance value of $p < 0.05$

Subject	*p value*
Group and observation	0.00*
(*) = There is a difference	

Based on the results of the *Kruskal-Wallis* statistical analysis test in (Table 2) between the negative control group (Na-CMC), the positive control group (aspirin) and the treatment group with moringa leaf extract there is a significant difference with a p value of 0.00 which means ($p < 0.05$). This shows the ability of Na-CMC, aspirin and moringa extract in the analgesic activity test in pain response characterized by the frequency of the number of different writhes. So that further *Mann-Whitney U* test can be done to determine the significant differences in each research group at each observation time interval.

Table 3. Mann-Whitney U test results for each observation time interval

						p value
Group	**5'**	**10'**	**15'**	**20'**	**25'**	**30'**
Na-CMC (K-) x Aspirin (K+)	0.663	0.021*	0,019*	0.189	0.309	0.189
Aspirin (K+) x Moringa Leaf Extract (KP)	0.021*	0.020*	0.019*	0.019*	0.020*	0.019*
Moringa Leaf Extract (KP) x Na-CMC (K-)	0.021*	0.020*	0.019*	0.020*	0.020*	0.020*
(*) = There is a difference						

Based on the results of the *Mann-Whitney U* test statistical analysis of the number of writhes (Table 3) between the moringa leaf extract treatment group (KP) and the Na-CMC negative control group (K-), there was a significant difference ($p < 0.05$) at each observation time interval. This significant difference means that the moringa leaf extract treatment group (KP) in the analgesic activity test is characterized by a decrease in the frequency of the number of writhing which is lower than the negative control group Na-CMC (K-). This indicates the administration of moringa leaf extract has analgesic activity compared to Na-CMC. The results of the *Mann-Whitney U* statistical analysis test of the number of writhing (Table 3) between the positive control group of aspirin (K+) and the treatment group with moringa leaf extract (KP) found a significant difference with a value ($p < 0.05$) at each time interval of observation, where the moringa leaf extract treatment group (KP) has analgesic activity in terms of the mean characterized by a decrease in the frequency of the number of writhing lower than the positive control group with aspirin (K+). This shows that the administration of moringa leaf extract has stronger analgesic activity compared to aspirin.

DISCUSSION

Ethanol 96% was chosen because it is selective, non-toxic, good absorption and high filtering ability so that it can attract non-polar, semi-polar and polar compounds. The 96% ethanol solvent is easier to penetrate into the cell wall of the

sample than the ethanol solvent with a lower concentration, resulting in a more concentrated extract. Ethanol solvents can dissolve almost all secondary metabolites contained in simplisia, not toxic like other solvents such as methanol which can cause blindness. Ethanol solvents have the properties to penetrate cell wall materials so that they can perform cell diffusion and attract bioactive compounds faster [15]. The Moringa leaf extraction process uses a maceration method carried out by researchers for one day which is then remacerated. Remaceration is the process of repeating the addition of solvent after filtering the first macerate and so on, for remaseration here is done twice until the desired sample/compound has been completely extracted.

For this study, we will use the writhing reflex method, where the results of this study are the frequency of the number of writhing mice observed and counted after 5 minutes of 0.6% acetic acid induction intraperitoneally observed for 30 minutes with an observation interval of every 5 minutes. In analgesic testing this time using the writhing reflex method because this method is quite sensitive in assessing the pain stimuli given. Acetic acid is an irritant that can damage tissues locally, which can cause a pain response in the abdominal cavity with intraperitoneal administration [11]. Acetic acid 0.6% provides a fairly good pain stimulus to test animals by triggering a local inflammatory response resulting from the release of free arachidonic acid from phospholipid tissue through cyclooxygenase (COX) and prostaglandin biosynthesis. The response of mice that can be assessed in the form of abdominal contractions until they touch the bottom of the floor/stand and the mice's legs are pulled back and forth which can be seen in Figure 2.

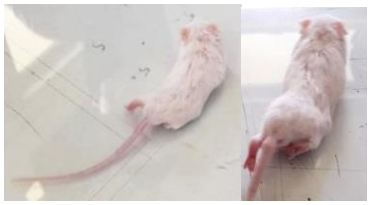

Figure 2: The mice feel pain visibly through writhing movements.

According to the theory that Na-CMC is a neutral material so that it has no effect in reducing pain, mice responded to pain through higher writhing movements compared to using aspirin and Moringa leaf extract. Then, the results seen in the study using aspirin showed that mice experienced a decrease in pain response. Aspirin is an analgesic drug proven to be able to reduce low to moderate intensity pain through inhibition of the cyclooxygenase process, because in that process the synthesis of prostaglandins and thromboxane if inhibited will reduce the pain [7]. This study shows that the administration of moringa leaf extract at a dose of 75 mg/kg BW in mice has a stronger analgesic activity as shown by mice experiencing a decrease in pain response characterized by a lower number of writhing. This is thought to be due to the content of compounds contained in moringa leaf extract, namely flavonoids, alkaloids, tannins and steroids (Figure 3). The mechanism of these compounds as analgesics is to inhibit phospholipase, cyclooxygenase and lipoxygenase enzymes so that they have more optimal analgesic activity. As for aspirin, it only blocks the cyclooxygenase pathway, not the lipoxygenase enzyme, so it does not inhibit the formation of leukotriene [7].

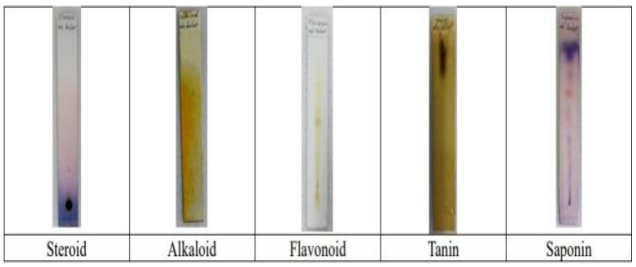

Figure 3: Phytochemical screening results of moringa leaf extract (Moringa oleifera Lamk).

Whereas in research that has been conducted that moringa leaf extract using 96% ethanol contains flavonoid, alkaloid, tannin and steroid compounds that have analgesic activity (Figure 3). Where flavonoids themselves have analgesic activity in inhibiting the formation of cyclooxygenase and lipoxygenase enzymes, so as to inhibit the synthesis of prostaglandins, thromboxanes, prostacyclin and leukotriene which are mediators of pain so as to reduce the pain [6]. Alkaloids have a function as inhibitors of an important phase in prostaglandin biosynthesis, namely in cyclooxygenase in the arachidonic acid metabolic pathway. The mechanism of action of tannins by inhibiting the enzymatic work of phospholipase, an enzyme responsible for the release of arachidonic acid and blocking the cyclooxygenase and lipoxygenase pathways so that its metabolites, namely prostaglandins, leukotrienes, prostacyclin and thromboxane, are not formed [10]. Steroids have activity as anti-inflammatory by inhibiting inflammatory factors so that the production of pain mediators decreases, causing pain perception to decrease. The mechanism of steroids as analgesics is that the inhibition of phospholipase enzymes will cause the cyclooxygenase and lipoxygenase pathways to be blocked so that the formation of pain mediators is inhibited [13].

The overall results in this study is moringa leaf extract has analgesic activity, as evidenced by the average results of each group, that in the treatment group using moringa leaf extract has the results of a lower number of twists. Moringa leaf extract contains flavonoids, alkaloids, tannins and steroids whose mechanism of action inhibits phospholipase, cyclooxygenase and lipoxygenase enzymes so that the formation of pain mediators is inhibited. From this research, moringa leaf extract has analgesic activity and can be an alternative analgesic herbal preparation indicated by a decrease in pain response.

CONCLUSION

The conclusion of this study is that Moringa leaf extract (*Moringa oleifera* Lamk) at a dose of 75 mg/kg BW has analgesic activity with the writhing reflex method, which can reduce the pain response characterized by a decrease in the number of writhing in mice (*Mus musculus*).

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