Analgesic Effectiveness Of Noni Leaf Infusion (*Morinda citrifolia* L.) In Mice By Writhing Method

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Abstract. *Pain is a sensory and emotional experience that occurs due to tissue damage. Pain can be eliminated by analgesics, which are drugs or medicinal materials used to reduce or suppress pain without removing awareness and working as general anesthesia. Noni leaf (Morinda citrifolia L.) can be used as an analgesic because it has flavonoid compounds, tannins,saponins, and alkaloids. This research is an experimental study with a Post-test-only Control Group Design. The test subjects used white male mice (Mus musculus) which amounted to 25 mice and were divided into 5 treatment groups namely negative control, positive control, and noni infusion with concentrations of 10%, 20%, and 30%. Before treatment, the mice were fasted and induced with 1% acetic acid. Observations were made by observing the effectiveness of analgesics ie the amount of writhing of mice at intervals of 5 minutes for 1 hour. The numerical data were then processed using SPSS with the One way ANOVA test. The results showed that the administration of noni leaf infusion concentrations of 10%, 20%, and 30% had an analgesic effect on mice with an effective concentration of 30%.*

Keywords: [*Noni leaf (Morinda citrifolia L.), Analgesic Effect, Writhing Method*]

INTRODUCTION

Pain is a sensory and emotional experience that occurs due to tissue damage. Pain often affects people and one of the most common reasons patients come to seek medical help is because it interferes with the effectiveness of a person's life. Pain is a comorbidity factor in many diseases (Amalia, Rantuwene, and Kembuan, 2016). In 2002, the Indonesian Association of Neurologists, which conducted research in teaching hospitals throughout Indonesia, reported that the number of women suffering from pain was 2,256 and 2,200 men. The most common cases suffered were myofascial pain, joints, shoulder joints, headaches, and lower back pain. , neuropathic pain, etc. Pain can be influenced by several factors such as age, occupation, education level, gender, culture, and habits or lifestyle (Amalia, Rantuwene, and Kembuan, 2016).

Pain mechanisms are divided into nociceptive (damage to somatic tissue or viscera), neuropathic pain (primary dysfunction of the nervous system), and mixed pain (between nociceptive and neuropathic pain mechanisms) (Sinda et al, 2018). Pain can be relieved with analgesics, namely drugs or medicinal substances used to reduce or suppress pain without losing consciousness, and work as general anesthesia. Analgesics consist of two groups, namely opioids and non-opioids. Opioid analgesics are a group of drugs that contain opium and are used for moderate to severe pain (Pandey, Bodhi, and Yudhistira, 2013).

Synthetic or chemical drugs have a higher risk of side effects than traditional medicines, although not all plants can be used as traditional medicines. One of the traditional plants that can be used as an analgesic is noni leaves (Morinda citrifolia L.) which belongs to the rabiciae family. Noni leaves contain active substances such as terpenoids, ascorbic acid, beta-carotene, i-arginine, xeronine, and proxeronine (Suwarni, Chayaningsih, and Yuda, 2016).

Compounds that have analgesic properties in noni leaves are flavonoids, tannins, saponins, and alkaloids. According to (Wemay and Wehantouw, 2013) Flavonoids and alkaloids as analgesics work by inhibiting the catalyst of the oxygen-binding enzyme cyclooxygenase, which is the substrate for cyclooxygenase in the formation of prostaglandins (Goenarwo, Chodidjah and Susanto, 2011). Meanwhile, tannin compounds are known to work by inhibiting COX-1 so they can reduce the amount of writhing in mice (Na'imah, 2017). Saponin compounds can work by inhibiting the prostaglandin dehydrogenase pathway which will inhibit the activation of prostaglandins, but saponins do not affect prostaglandin synthesis itself (Frediana, 2012).

According to research that has been conducted (Suwarni, Chayaningsih, and Yuda, 2016) noni leaves can be used in traditional medicine as an analgesic, one of which is by making an infusion.

According to the Indonesian Pharmacopoeia Edition III (1979), infusion is a liquid preparation made by extracting vegetable simplicia with water at a temperature of 90˚C for 15 minutes. Based on the above background, researchers are interested in researching the analgesic test of noni leaf infusion (Morinda citrifolia L.) in male mice using the writhing method.

METHODS

Material

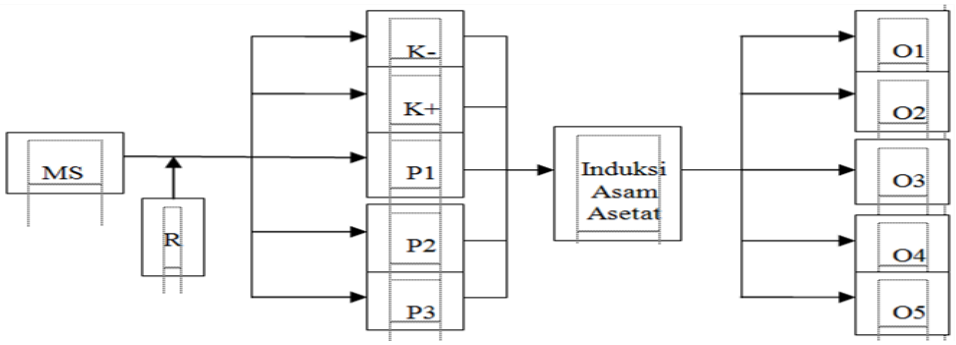
Noni leaf, 1% acetic acid, distilled water, ibuprofen, CMC-Na powder, Nacl, Mg powder, Fecl3, Dragendrof, Mayer, and concentrated HCl.

Tool

Analytical balance, infusion pan, Erlenmayer, water bath, spatula, magnetic stirrer, filter paper, funnel, stopwatch, beaker glass, measuring glass, measuring flask, pipette, 1cc injection syringe, marker, test animal cage, mice probe, flannel cloth.

Research design

The research design used was Post-test Only Control Group Design. Because variable examination cannot be done at the beginning or before treatment but after treatment (Post-test). The research has received ethical clearance/approval from the Medical/Health Research Bioethics Commission, Faculty of Medicine, Sultan Agung Islamic University, Semarang. Ethical Clearance No. 119/IV/2020/Bioethics Commission.



Picture 1. Research design

Information:
MS = Healthy Mice
R = Random
K- = Negative control group with CMC-Na K+ = Positive control group with Ibuprofen
P1 = Noni leaf infusion group with 10% concentration
P2 = Noni leaf infusion group with 20% concentration
P3 = Noni leaf infusion group with 30% concentration
O1-O5 = Observation of the number of mice writhing

Making noni leaf infusion

Noni leaves were obtained from Karang Rowo village, Undaan District, Kudus Regency. sorted to separate them from other plants. Next, the noni leaves are washed thoroughly with running water, drained, and air-dried. Noni leaves are dried by heating them in the sun and covered with a black cloth. After the simplicia is dry, the simplicia is then blended and sieved with a 40 mesh sieve.

Then 100 mL of noni leaf infusion was made 10%, 20%, and 30% by weighing 10 grams, 20 grams, and 30 grams of noni leaf simplicia respectively. Then put each simplicia into the infusion pot and add 100 mL of water (+ 2x the weight of the simplicia). Each infusion pot is heated over a water bath for 15 minutes starting from the solvent temperature reaching 90˚C, then filtered using a flannel cloth until the infusion water reaches 100 mL (Indonesian Pharmacopoeia Edition III, 1979).

Phytochemical Screening

The phytochemical examination carried out was the examination of Flavonoids, Tannins, Saponins, and Alkaloids.

a. Flavonoid phytochemical test was done by adding 4 grams of noni leaf samples to hot water and boiling for 15 minutes at 90˚C and filtering, then after the filtrate was filtered, a little Mg powder and 1 mL of concentrated HCl were added to the filtrate and then shaken. Positive results are indicated by the formation of red, yellow, or orange (Septiadi, Pringgenies, and Radjasa, 2013).
b. Tannin phytochemical test by dripping noni leaf infusion with 3 drops of 1% FeCl3. If a blackish-green color forms, it is positive for containing tannin (Sugiarti & Fitrianingsih, 2018).
c. Saponin phytochemical test by infusion of noni leaves plus HCl then shaking. If stable foam is formed, it is positive for containing saponin (Sugiarti & Fitrianingsih, 2018).
d. Alkaloid phytochemical test by infusion of leaves dissolved in 5 mL of 2N HCl. The solution obtained was then divided into 3 test tubes. The first tube was used as a blank, 3 drops of Dragendroff's reagent were added to the second tube, and 3 drops of Mayer's reagent were added to the third tube. The formation of an orange precipitate in the second tube and a white-to-yellowish precipitate in the third tube indicates the presence of alkaloids (Simaremare, 2014).

Grouping and Treatment of Test Animals

The test animals used in this research were white Swiss mice aged 2-3 months with a body weight of 20-30 grams. All mice are maintained including food, water, cage, and bedding. Before being treated, the mice were first fasted for 18 hours while still being given water, this aimed to reduce the influence of food on the test results. The mice used were 25 mice which were randomly assigned to 5 groups.
1. Group 1 is the negative control (1 mL of CMC-Na 0.1% was given to each mouse)
2. Group 2 is the positive control (ibuprofen dose 41 mg/kg BW given 1 mL to each mouse)
3. Group 3 is the noni leaf infusion treatment group with a concentration of 10%
4. Group 4 is the noni leaf infusion treatment group with a concentration of 20%
5. Group 5 is the noni leaf infusion treatment group with a concentration of 30%.

Each was given 1 mL/gram BW to test animals orally. After 15 minutes, mice were given an acid induction chemical stimulus of 0.5 mL of 1% acetate was then observed for the writhing response and recorded at intervals of 5 minutes for 1 hour (Safitri, 2013).

Data Analysis

The research results were analyzed using one-way ANOVA. If there are significant differences, the LSD (Least Significance Difference) test is used to determine whether there are differences between groups.

RESULTS AND DISCUSSION

Research on the analgesic test of noni leaf infusion (Morinda citrifolia L.) using the stretching method using male mice as test animals. Swiss strain male mice were chosen as test animals because they have several advantages such as having basic physiological characteristics like humans, their small size, more economical, and male mice do not experience an estrus cycle so the sample is homogeneous, the results are expected to be more accurate and easy to control (Safitri, 2013). This study used induction with acetic acid, this is because induction with acetic acid intra-peritoneally in mice can work by releasing free arachidonic acid from phospholipid tissue via the prostaglandin and cyclooxygenase biosynthesis pathway, or acetic acid induces writing which is associated with an increase in the amount of PGF2α and PGE2 intraperitoneally. This results in an increase in the amount of prostaglandin in the intraperitoneal cavity which results in an increase in inflammatory pain due to an increase in capillary permeability resulting in a stretching effect (Zulfiker et al., 2010).

The results of phytochemical screening show that noni leaves contain flavonoids, tannins, saponins, and alkaloids as analgesics. Flavonoids and alkaloids as analgesics work by inhibiting the catalyst of the oxygen-binding enzyme cyclooxygenase, which is the substrate for cyclooxygenase in the formation of prostaglandins (Goenarwo, Chodidjah and Susanto, 2011). Meanwhile, tannin compounds are known to work by inhibiting COX-1 so they can reduce the amount of writhing in mice (Na'imah, 2017). Saponin compounds can work by inhibiting the prostaglandin dehydrogenase pathway which will inhibit the activation of prostaglandins, but saponins do not affect prostaglandin

synthesis itself (Frediana, 2012). The results of phytochemical screening can be seen in Table 1.

Table 1. Results of Identification of Noni Leaf Infusion Content

Golongan Senyawa	Reaksi	Hasil	Warna
Flavonoid	Mg+ HCl	+	Merah Jingga
Tanin	Fecl3	+	Biru Kehitaman
Saponin	HCl2N	+	Terbentuk Busa
Alkaloid	HCl2N+ Mayer	+	Orange
	HCl2N+ Dragendroft	+	Biru Kehitaman

Number of writing

Testing the analgesic effect of noni leaf infusion was carried out by looking at the number of mice writhing. Noni leaf infusion was tested with 3 concentrations, namely concentrations of 10%, 20%, and 30%. On average, the least amount was found at a concentration of 30% with a result of 42.2 writing times. This data can be seen in Table 2.

Table 2. The average number of writhing mice in each group for one hour

	Treatment Group				
	Control(-)	Control (+)	Infusion 10%	Infusion 20%	Infusion 30%
Mice 1	151	29	92	85	25
Mice 2	180	31	109	89	56
Mice 3	172	109	121	56	62
Mice 4	122	89	98	79	47
Mice 5	66	56	115	65	21
Avarage± SD	138.2 ± 41.30#	40.6 ± 31.68*	107.0 ± 10.67*	74.8 ± 12.43*	42.2 ± 16.43*

Information:
(*): Indicates a significant difference with the negative control group (P<0.05)
(#): Indicates a significant difference from the positive control group (P<0.05)

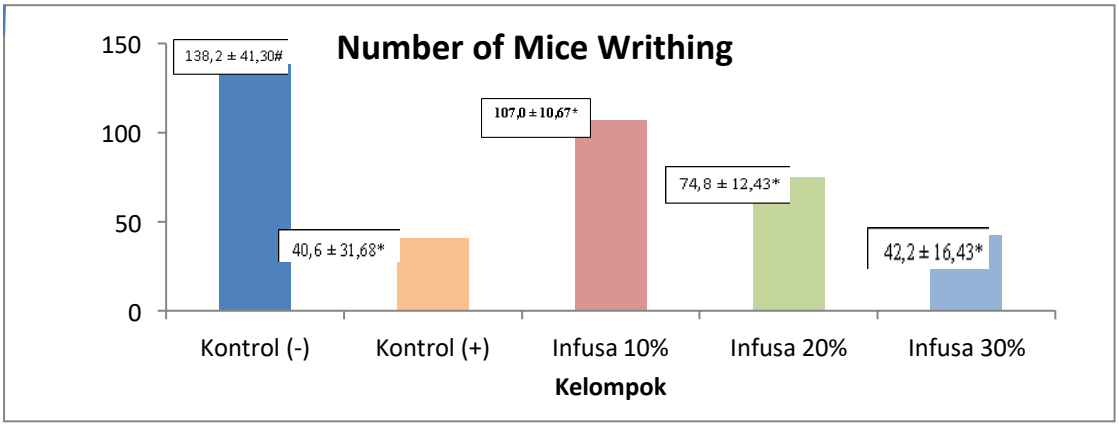


Figure 2. Graph of the Number of Mice Writhing

The results of the average number of mice writhing showed a decrease in the number of writhing in the positive control group and the noni leaf infusion group with concentrations of 10%, 20%, and 30% compared to the negative control group. The results of observing the number of mice stretching were then followed by a data normality test, homogeneity test, One Way Anava test, and LSD test using SPSS 16.0.

Protection percentage

The results of the percentage of protection for each group in the analgesic effect test of noni leaf infusion can be seen in Table 3.

Tabel 3. Protection Percentage

Kelompok Uji	Perlakuan	Persentse Proteksi
II	Positif control (Ibuprofen)	70,63%
III	Noni Leaf Infusion Concentration 10%	22,58%
IV	Noni Leaf Infusion Concentration 20%	45,88%
V	Noni Leaf Infusion Concentration 30%	69,47%

Based on the table and figure, the percentage of analgesic effectiveness at a concentration of 30% gives results close to the effectiveness of ibuprofen, namely 98.35%, so an infusion with a concentration of 30% can provide an analgesic effect almost equivalent to ibuprofen.

The normality test obtained in this study from the number of mice writhing in all treatments showed significant results greater than 0.05 ($P > 0.05$), which means the data was normally distributed. The homogeneity test resulted in <0.05, and it can be concluded that the data is not homogeneous. Continuing with the One-way ANOVA test on all treatments, it showed a significant value of $P<0.05$, which means there was a significant difference between the treatment groups. Post Hoc test using the Games Howell method (because the data was not homogeneous) and the results obtained for comparing the negative control with the positive control showed a significant difference ($P<0.05$). The writhing effect due to acetic acid induction can be reduced by administering ibuprofen. Ibuprofen as a positive control has an analgesic effect by inhibiting the cyclooxygenase enzyme in the formation of prostaglandins so that it can have the effect of reducing writhing in mice (Muhammad, Saeed & Khan, 2012). Meanwhile, CMC-Na as The negative control did not have an analgesic effect because CMC-Na only functioned as a solvent.

The comparison value between the positive control with an infusion of 10% concentration and 20% concentration shows a significant difference. Infusion concentrations of 10% and 20% were able to reduce the number of mice writhing but were not equivalent to the analgesic effect of ibuprofen. The comparison value of the positive control with the 30% concentration infusion showed that there were no significant differences. Noni leaf infusion with a concentration of 30% has a higher content of flavonoids, tannins, saponins, and alkaloids compared to noni leaf infusion with a concentration of 10% and 20%, and it can be said that the greater the concentration used, the greater the content of compounds that act as analgesics, namely flavonoids, tannins, saponins and alkaloids (Safitri, 2013). It can be concluded that noni leaf infusion with a concentration of 30% is an effective dose that is almost the same as ibuprofen. From these results, it can be concluded that noni leaf infusion with a concentration of 30% is an effective concentration because it has almost the same effect as ibuprofen.

CONCLUSION

Noni leaf infusion has analgesic effectiveness on male mice induced with 1% acetic acid, and noni leaf infusion with a concentration of 30% has an effect almost equivalent to ibuprofen.

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