ORIGINAL ARTICLE

EFFECT OF ASIATIC MANGROVE (*Rhizophora mucronata*) LEAVES EXTRACT AS ANALGESIC IN MALE ALBINO DDW MICE (*Mus musculus L.*) INDUCED BY 0,7% ACETIC ACID

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ABSTRACT

Background : Pain is an unpleasant emotional experience that illustrates ongoing tissue damage. Excessive use of non-steroidal anti-inflammatory drugs can cause peptic ulcer to gastric mucosal damage and perforation. Indonesia contains the largest area of mangrove forest in the world. There are 45 species of mangrove found and one of them is Asiatic Mangrove (*Rhizophora mucronata*). This type is easy to find and rich of alkaloids and flavonoids which can be used as analgesics.

Method: This study used *post-test only control group design*. The number of mice that used was 25 mice, divided into 5 groups. Which were given different therapies aquadest 10mL/KgBW, acetosal 150 mg/KgBW, extract of *Rhizophora mucronata* 250 mg/KgBW, 500 mg/KgBW, and 1000 mg/kg bw. The pain was induced by 0,7% glacial acetic acid at a dose of 10 mL/KgBW. The writhes of the mice was being calculated with an interval of 10 minutes in 30 minutes.

Result: The results of the analysis showed the decrease in writhes of mice in acetosal group dose 150 mg/kg bw, *Rhizophora mucronata* leaves extract dose 250 mg/kg bw, 500 mg/kg bw, and 1000 mg/kg bw. There was a significant difference in the results of the *Mann-Whitney U* test with $p<0,05$ in the aquadest group and the acetosal group with the *Rhizophora mucronata* leaves extract group dose 500 mg/kg bw, the difference between the acetosal group and the *Rhizophora mucronata* leaves extract group dose 250 mg/kg bw, and the difference between the *Rhizophora mucronata* leaves extract group dose 500 mg/kg bw and the *Rhizophora mucronata* leaves extract group dose 1000 mg/kg bw. **Conclusion:** *Rhizophora mucronata* leaves extract dose 500 mg/kg bw can provide analgesic effect and can reduce the writhing frequency in mice much better than acetosal group.

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Introduction

Pain is an unpleasant sensory and emotional experience associated with, or resembling that associated with, actual or potential tissue damage [1]. Almost all disease are accompanied by pain, pain arises because of a disturbance in a tissue so that is used by the body as a sign that an infection is occuring.

Estimated between long-term use of analgesics and anti-inflammatory drugs: 15-40% will experience upper gastrointestinal complaints; 10-25% suffer from gastric ulcers, especially peptic ulcers; and 1-4% will experiance life-threatening ulcer complications such as gastric bleeding and perforation [2]. In many countries including Indonesia, non-steroidal anti-inflammatory drugs (NSAIDs) are used for symptoms associated with arthritis and other symptomatic indications including myofascial pain, gout, fever, dysmenorrhea, migraine, perioperative pain, stroke prophylaxis and myocardial infarction [3].

Indonesia is an archipelagic country consisting of 17.500 islands with a coastline of about 95.181 km. The total area of Indonesia is about 9 million km^2 (2 million km^2 land and 7 million km^2 sea). Indonesia's area is only about 1,3% of the earth's area, but has a very high biodiversity. For plants, Indonesia is estimated to have 25% of flowering plant species in the world or the seventh largest country with a number of species reaching 20.000 species, 40% of which are endemic or native to Indonesia [4].

Mangroves are ecosystems located in intertidal areas, where there is a strong interaction between marine, brackish, river and land waters. This interaction makes the mangrove ecosystem has a high diversity of flora and fauna. Mangroves lives in the tropics and subtropics, especially at latitudes of life 25° North Latitude and 25° South Latitude [5]. Indonesia has the largest mangrove forest in the world, with an area of 42.550 km^2 and about 45 species of mangroves live in Indonesia [6].

Rhizophora mucronata or asiatic mangrove is a type of species that is often found and generally this tree has a height of up to 27 m, and rarely exceeds 30 m. stems up to 70 cm in diameter with dark to black bark and horizontal fissures. This plant grows in groups, near or on the bottom of tidal rivers and in river mouths, rarely growing in areas far from the tides. Optimal growth occurs in heavily flooded areas, as well as in humus-rich soil. The wood of this plant can be used for staining, and are sometimes used medicinally in cases of hematuria (bleeding in the urine). It is sometimes planted along ponds to protect the ambankments [7]. The phytochemical components of asiatic mangrove include: flavonoids, alcaloids, phenolics, terpenoids and glycoside [8], proteins, saponins, and tannins [9]. Based on the background, the study was designed to determine the analgesic effect of the leaves extract of asiatic mangrove (*Rhizophora mucronata*) in male albino ddw mice (*Mus musculus L.*) induced by 0,7% acetic acid.

Methods

Place and time of study

This research was conducted in the Biochemistry Laboratory of the Faculty of Medicine, Hang Tuah University, Surabaya for 7 days with the ethical clearance number 724/HRECC.FODM/X/2019.

Plant collection and preparation of the extract

Rhizophora mucronata leaves were collected from Wonorejo Mangrove Forest, Surabaya. Fresh *Rhizophora mucronata* leaves were cleaned, shade dried and crushed to powder by grinder. They were extracted used maceration technique. 1000 grams of dried *Rhizophora mucronata* leaves were macerated with 96% ethanol solvent for 3x24 hours. Then after producing the extract, the resulting extract is concentrated with a water bath to produce a thick blackish green extract. Furthermore, evaporation is carried out and then evaporated at room temperature to produce a thick extract of ethanol.

Animals

25 male albino ddw mice (*Mus musculus L.*) aged 7-9 weeks with an average weight of 20-25 grams were used for these studies. The animals were obtained from the Pusat Veteriner Farma Surabaya (Pusvetma Surabaya). The animals were placed in boxes and given husks. Each box contains 5 mice.

Analgesic activity study

The design of this study was a laboratory experimental study using *post-test only control group design.* Analgesic test of this leaves extract was carried out by induction of 0,7% acetic acid in mice 0,7% acetic acid was injected intraperitoneally for one time to cause pain in mice. Mice were divided into 5 groups with 5 mice in each groups. Group A as the negative control group was given 10 mL/KgBW of aquadest orally, group B as a positive control group was given acetosal at a dose of 150 mg/KgBW orally, group C as the first treatment group was given *Rhizophora maucronata* leaves extract at a dose of 250 mg/KgBW orally, group D as the second treatment group was given *Rhizophora mucronata* leaves extract at a dose of 500 mg/KgBW orally, group E as the third treatment group was given *Rhizophora mucronata* leaves extract at a dose of 1000 mg/KgBW orally.

After 30 minutes of oral administration, 0,7% acetic acid was injected intraperitoneally in each mice. After 5 minutes, the number of writhing performed by the mice was counted for 30 minutes. Calculations were carried out at 10 minutes intervals. Then determine the percentage of inhibition (%) of analgesic activity with the formula [10]:

$$\% \text{ inhibition} = \frac{(\text{Control} - \text{Sample})}{\text{Control}} \times 100\%$$

Results

In acetic acid induction, *Rhizophora mucronata* extract gave significant analgesic results at a dose of 500 mg/KgBW with percentage of inhibition of 75,15% and a dose of 250 mg/KgBW with a percentage of inhibition of 52,87% compared to the control. And the results of the study in Table 1, shows that there is an analgesic effect asiatic mangrove (*Rhizophora mucronata)* leaves extract in male mice (*Mus musculus L.*) with 0,7% acetic acid induction (Table 1).

Table 1 Writhling Frequency Observation Results

Group	Mean ± S.D	% inhibition
A	31,4 ± 27,18	0%
B	29,4 ± 14,6	6,39%
C	14,8 ± 11,56	52,87%
D	7,8 ± 4,65	75,15%
E	20,4 ± 4,39	35,03%

S.D=standard deviation

The results of the writhing rate per period showed that the leaves extract of the *Rhizophora mucronata* had a significant analgesic effect. At 10 minutes, the highest mean was found in the acetosal group or group B with the value of 12, while the lowest mean was found in the group with *Rhizophora mucronata* leaves extract dose 500 mg/KgBW or group D with the value of 2,4. At 20 minutes, the highest mean was found in the aquadest group or group A with the value of 11,6, while the lowest mean was found in the group with *Rhizophora mucronata* leaves extract dose 500 mg/KgBW or group D with the value of 3,8. At 30 minutes, the highest mean was found in the aquadest group or group A with the value of 9,6, while the lowest mean was found in the group with *Rhizophora mucronata* leaves extract dose 500 mg/KgBW or group D with the value of 1,6 (Table 2).

Table 2 Averange Number of Writhing

Group	Averange number of writhing (minutes)		
	10mins ± S.D	20mins ± S.D	30mins ± S.D
A	10,2 ± 9,41	11,6 ± 12,30	9,6 ± 6,94
B	12 ± 4,06	13,2 ± 8,58	4,2 ± 3,11
C	4,2 ± 3,76	6,4 ± 4,77	4,2 ± 3,89
D	2,4 ± 1,67	3,8 ± 2,48	1,6 ± 1,81
E	7,2 ± 3,83	7,2 ± 3,27	6 ± 3,24

S.D=standard deviation

Furthermore, the normality test was carried out using the *Saphiro-Wilks* test. The results of the *Saphiro-Wilks* test, it was found that all groups had significance $p > \alpha$ ($\alpha = 0.05$), except for the 10 minutes of group E and the 30 minutes of group A the significance value was $p < \alpha$ ($\alpha = 0.05$), meaning that the data not normally distributed (Table 3).

Table 3 *Saphiro-Wilks* Test Results

Group	Significance		
	10’	20’	30’
A	0,169	0,330	0,015
B	0,627	0,639	0,670
C	0,194	0,899	0,093
D	0,314	0,384	0,254
E	0,006	0,914	0,111

Furtheremore,a non-parametric test is carried out using the *Kruskal-Wallis* test. The results of the *Kruskal-Wallis* test there is a significant value of $p < \alpha$ ($\alpha = 0.05$), which means that there is a difference in the analgesic effect of *Rhizophora mucronata* leaves extract in male mice (*Mus musculus L.*) with 0,7% acetic acid induction bertween groups (Table 4).

Tabel 4 Kruskal Wallis Test Results

	10 Minutes	**20 Minutes**	**30 Minutes**
Asymp. Sig. (2-tailed)	0,019	0,388	0,169

To determine whether there was a difference in the mean of the study groups, a post-hoc *Mann-Whitney U* test was performed. There was a significant difference in the results of the *Mann-Whitney U* test with p<0,05 in the aquadest group and the acetosal group with the *Rhizophora mucronata* leaves extract group at a dose of 500 mg/KgBW, the difference between the acetosal group and the *Rhizophora mucronata* leafves extract group at a dose of 250 mg/KgBW and the *Rhizophora mucronata* leaves extract group at a dose of 1000 mg/KgBW (Table 5).

Table 5 *Mann Whitney U* Results in the first 10 minute observation period

10 Minutes					
Sig	**Group A**	**Group B**	**Group C**	**Group D**	**Group E**
Group A		0,401	0,140	0,044	0,743
Group B			0,027	0,009	0,084
Group C				0,454	0,129
Group D					0,041
Group E					

Discussion

In this study, 25 mice were used which were divided into 5 groups. Each groups consisted of 5 white mice (*Mus musculus L.*) which was induced by pain with 0,7% acetic acid injection. The injection was carried out 30 minutes after being given therapy, such as aquadest, acetosal, and *Rhizophora mucronata* leaves extract.

Group A as a negative control was given 10 mL/KgBW of aquadest, group B as a positive control was given 150 mg/KgBW of acetosal, group C as the first treatment group was given *Rhizophora mucronata* leaves extract at dose of 250 mg/KgBW, group D as the second treatment group was given *Rhizophora mucronata* leaves extract ast dose of 500 mg/KgBW, group E as the third treatment group was given *Rhizophora mucronata* leaves extract ast dose of 1000 mg/KgBW.

After being given the extract, wait about 30 minutes first, then 0,7% acetic acid injected with a dose of 10 mL/KgBW intraperitoneally and wait for 5 minutes and do the calculations at intervals of every 10 minutes for 30 minutes.

Based on the data of this study, it was found that the *Rhizophora mucronata* leaves extract at a dose of 250 mg/KgBW (group C), a dose of 500 mg/KgBW (group D), a dose of 1000 mg/KgBW (group E) had a significant analgesic effect, as evidenced by the mean of writhing which is smaller than the negative control group (group A). The percentage of inhibition from group C was 52,87%, group D was 75,2%, and group E was 35,03%, this indicates that the three doses of *Rhizophora mucrnata* can inhibit pain process. *Rhizophora mucronata* containing flavonoids, alkaloids, phenolics, terpenoids and glycosides [9] which some substances can help in inhibiting the pain process [11].

Acetic acid induces an inflammatory response in the abdominal cavity with subsequent activation of nociceptors. When animals are injected intraperitoneally with acetic acid, acute pain and an inflammatory reaction appear in the peritoneal area. Constriction caused by acetic acid is considered a nonselective antinociceptive model, because acetic acid acts indirectly by stimulating endogenous mediators that increase nociceptive neurons sensitive to sensitive to nonsteroidal anti-inflammatory drugs, and other active drugs [12]. *Rhizophora mucronata* leaves extract contains many flavonoids and alkaloid that are effective in delaying the response time of mice to stimulation, and the researchers identified that this compound is luteolin which interacts strongly with cyclooxygenase and forms a number of specific hydrogen bonds and they identified that the central and peripheral anti-nociceptive activity of the extract *Rhizophora mucronata* leaves invole opioid receptors [13].

In the group with high doses of *Rhizophora mucronata* leaves extract

produced more writhing, this can be associated with the administration of too many flavonoids, the higher dose, the more flavonoids are given and can cause opioid receptor binding. If opioid receptors are activated frequently, it can lead to an opioid-induced hyperalgesia respons [14]. However, luteolin in *Rhizophora mucronata* leaves extract can reduce the intensity of neuropathic pain and can relieve hyperalgesia and nociception and can relieve acute and chronic pain [15].

Conclusion

Asiatic mangrove (*Rhizophora mucronata*) leaves extract at a doses of 250 mg/KgBW, and 1000 mg/KgBW was able to give the effect of decreasing the amount of writhing with 0,7% acetic acid induction method.

Asiatic mangrove (*Rhizophora mucronata*) leaves extract at a dose of 500 mg/KgBW can provide a better analgesic effect than aspirin in reducing the amount of acetic acid by 0,7% acetic acid induction method.

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