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Study of The In Vivo Anti-Inflammatory, Antipyretic and Analgesic Effect of The Ethanol Extract Indian Nettle Plant (*Acalypha indica Linn*)

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ABSTRACT: The body can experience fever and pain, when inflammation occurs. Indian nettle plant (*Acalypha Indica Linn*.) are known to contain flavonoid compounds which are thought to have anti-inflammatory, antipyretic, and analgesic effect. This study aims to test the anti-inflammatory, antipyretic, and analgesic effect of the ethanol extract of 70% Indian Nettle plant. Method **e**ach test used 5 groups, negative control all given Na CMC 0,5%. Antiinflammatory use rats with Winter method, aspirin 40,43 mg/200 g BW, the test group dose 70 mg/200 g BW (I), 140 mg/200 g BW (II), 280 mg/200 g BW (III), antipyretics use rats with Brewer's yeast method, aspirin 21,45 mg/200 g BW, the test group dose 70 mg/200 g BW (I), 140 mg /200 g BW (II), 280 mg/200 g BW (III). Analgesic use mice with the Siegmund method, aspirin 3,10 mg/20 g BW, the test group dose of 10 mg/20 g BW (I); 20 mg/20 g BW (II); 40 mg/20 g BW (III). Results Kruskall Wallis test, the anti-inflammatory effect significant difference to negative control, p value <0,05. Percentage of inhibition positive control edema and test dosage I, II, and III obtained 34,44%; 18,88%; 21,37%, and 29,61%. The Mann Whitney test, the antipretic and analgesic effects significant difference to the negative control, p value <0,05. The decreased percentage of fever in positive control and dosage I, II, and III test preparations was 3,74%; 2,60%; 2,55%; 3,01%. Inhibition percentage of stretching positive control and test dosage I, II, and III obtained 41,05%; 24,46%; 27,78%; and 33,31%. Conclussion**:** Wriggling inhibition (analgesic) had the highest presentation dose III 33,31% compare to anti-inflammatory and antipyretic activities, lower than aspirin as positive control 41,05% (p<0,05).

KEYWORDS: Indian nettle plant; anti-inflammatory; antipyretic; analgesic.

1. INTRODUCTION

Inflammation is the body's response to negative stimuli, such as exposure to toxins or bacteria [1]. Inflammatory conditions could lead to unpleasant sensations in the body, including fever and pain [2]. Inflammation can be treated with non-steroidal and steroidal anti-inflammatory drugs such as aspirin, mefenamic acid and diclofenac. Furthermore there are several types of plants that can be used as anti-inflammatory drugs in addition to synthetic drugs.

Indonesia is a tropical country wealthy in biodiversity that can be used for medicinal purposes. Indian nettle plant is easy to find in fields, yards and ruBWish tips. This plant grows once a year. In traditional medicine, such as in India, this plant is used as a laxative, antihelminthic, for scabies, rheumatoid arthritis, antiemetic, expectorant, for earache, chronic bronchitis, asthma, lung disease, to relieve snake-bite pain, headache and as a cathartic [3]. The roots are also used as a tonic, febrifuge and strong laxative. An alcoholic extract of the root bark is used externally as an emollient. The root is used for chest pain, joint pain, migraine, blood dysentery and root extract lowers blood sugar levels by 30% [4].

Indian nettle plant contains a variety of powerful phytochemicals, one of these is flavonoid [3]. Flavonoids are polar and soluble in solvents such as water and ethanol as they contain many -OH groups. Flavonoids reduce inflammation by inhibiting the expression of the isoforms of inducible nitric oxide synthase, cyclooxygenase and lipoxygenase that produce high levels of nitric oxide [5]. It has been shown that this plant is rich in flavonoid compounds, which are capable of inhibiting free radicals. On the evidence of the above description, this study is to evaluate the anti-inflammatory, antipyretic and analgesic activity of Indian nettle plant in the form of 70% ethanolic extract.

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2. MATERIALS AND METHODS

2.1. Materials

Indian nettle plant was collected from Lembang, Bandung, and then plant identification was carried out. Plant determination is carried out to ensure the correctness of the simplisia of the plant to be used in research. The results from the Indonesian Institute of Sciences (LIPI) number 2025/IPH.1.01/If.07/XI/2019 show Indian nettle plant (*Acaliypha indica* Linn) of the family Euphorbiaceae. Another material used is aspirin® tablets for control positif.

2.2. Methods

2.2.1 Preparation of ethanol extract of Indian nettle plant

Indian nettle plant were washed with clean water, mashed with a blender, then macerated with 70% ethanol for 6 hours, extracted 5 times. After that, it evaporated with a rotary evaporator.

2.2.2 Phytochemical Screening [6]

Prepare 1 mg of sample extract in a test tube, add 10 drops of methanol, stirred with a spatula to dissolve. Then add 6 pieces of Mg tape and 4 drops of HCL to the mixture. The result is positive flavonoid when the mixture changes color to yellow or red.

2.2.3 Preparation of Experimental Animals

This study used mice and rats and had obtained ethical review approval from the University of Indonesia with number KET-131/UN2.F1/ETIK/PPM.00.02/2019. The experimental animals used were 25 male Sprague-Dawley white rats weighing 150-250 g and 2-3 months old for anti-inflammatory and antipyretic tests. For analgesic test, 25 male DDY mice aged 2-3 months and weighing 20-30 g were used. All test animals were acclimated for one week for environmental adjustment.

2.2.4 Anti-inflammatory activity test [7]

The anti-inflammatory activity test was performed using the Winter method on the soles of the feet of rats. There were five groups of five rats each: group I negative control with Na CMC 0.5%, group II dose 70 mg/20 g BW, group III dose 140 mg/200 g BW, group IV dose 280 mg/200 g BW and group V positive control with aspirin 18 mg/200 g BW. The rats were initially fasted for ± 16 h while still drinking. On the day of the test, the rats were weighed, marked on the tail and the initial volume of the rat's paw was measured by dipping the rat's paw into the plethysmometer. For each anti-inflammatory test group, the rats were given the test substance preparation orally according to the treatment dose of each group. Thirty minutes later, the rat's paw was induced with 1% carrageenan up to 0.2 mL intraplantarly and the volume of the rat's paw edema was measured every 1 hour for 5 hours.

2.2.5 Antipyretic activity testing [7]

Mice were made fever with Brewer's yeast pyrogen induction, consisting of 5 groups of 5 rats each, negative control group I was given Na CMC 0.5%, group II dose 70 mg/20 g BW, group III dose 140 mg/200 g BW, group IV dose 280 mg/200 g BW and group V positive control was given aspirin 9 mg/200 g BW. Rectal temperature measurement of mice with a digital thermometer. Rectal temperature was measured at the 60, 90 and 120 minutes after administration of the test preparation. The analgesic effectiveness of the test preparation is seen by a decrease in temperature and BNT (Least Real Difference) test if the data is normally distributed and homogeneous.

2.2.6 Analgesic activity testing [7]

The experiment to determine analgesic activity was carried out using the Siegmund method, the experimental animals consisted of 5 groups of 5 mice each, group I negative control was given 0.5% Na CMC, group II dose of 10 mg/20 g BW, group III dose of 20 mg/20 g BW, group IV dose of 40 mg/20 g BW and group V positive control was given aspirin 1.3 mg/20 g BW. Mice were first fed for ± 16 hours while still given a drink. Mice were given the test substance preparation orally according to the treatment dose of each group. Thirty minutes later the mice were induced with 3% acetic acid as much as 0.2 mL/20 g BW intraperitoneally. mice will give a writhing response and the writhing is counted.

2.2.7 Statistical analysis

Data on paw volume, writhing frequency and changes in body temperature of the mice obtained from each treatment group were processed by statistical analysis using the Statistical Package for the Social Sciences (SPSS) version 22.0. The data obtained were tested for normality (Shapiro-Wilk test) and homogeneity (Levene test). If the data were normally distributed and homogeneous, parametric statistical tests were performed using the one-way analysis of variance (ANOVA) method. If one of the conditions for ANOVA was not met, non-parametric Kruskal-Wallis analysis was performed.

3. RESULTS

3.1 Anti-inflammatory test

The data in Table 1 below show the average volume of paw edema in rats induced with carrageenan. A decrease in paw volume was observed for all doses of the test preparation, especially at the 4th hour. This shows that the test compound has anti-inflammatory activity, as it can prevent the increase in inflammation characterized by an increase in the volume of the soles of the rats' feet caused by the inducer substance. The decrease in paw volume was observed from the 2nd hour after carrageenan induction.

Table 1. Average decrease in rats paw volume

Test groups	Average sole volume of the foot (mL)					
	Hour to-0	**Hour to -1**	**Hour to -2**	**Hour to -3**	**Hour to -4**	**Hour to -5**
Control (-)	1.16±0.41	1.61±0.10	1.97±0.09	2.1±0.11	1.91±0.07	1.80±0.11
Control (+)	0.99±0.06	1.14±0.05	1.33±0.07	1.53±0.07	1.42±0.05	1.28±0.05
Dose I	1.26±0.04	1.47±0.04	1.69±0.08	1.84±0.06	1.69±0.090.08	1.58±0.09
Dose II	1.067±0.05	1.49±0.04	1.66±0.05	1.79±0.05	1.64±0.01	1.50±0.03
Dose III	1.06±0.04	1.2±0.06	1.34±0.06	1.64±0.03	1.55±0.01	1.45±0.02

3.2 Antipyretic Test

Measurements were taken using a digital thermometer, with temperature changes measured via the rectum, as this was easier to do with a rat. Brewer's yeast is used as a pyrogen, which can cause fever. Table 2 provides data on average rectal temperature measurements in rats

Table 2. Average rectal temperature measurement of rats

Test group	Rectal temperature measurement of rats (^{0}C)				
	İnitial temperature	After induction (minute-0)	Minute to-60	Minute to -90	Minute to -120
Control (-)	36.4±0.11	37.32 ±0.25	37.46 ±0.22	37.32 ±0.21	37.32 ±0.37
Control (+)	35.52 ±0.58	36.48 ±0.36	36.10 ±0.33	35.74 ±0.51	35.40.63
Dose I	35.64 ±0.27	36.5±0.46	36.6±0.22	36.34 ±0.27	36.08 ±0.23
Dose II	36±0.21	36.94±0.20	36.46±0.24	36.22 ±0.22	36±0.25
Dose III	35.5±0.25	36.74 ±0.38	36.46 ±0.29	36±0.27	35.48 ±0.27

Table 3 showed the statistical results have no significant difference between the test groups with antipyretic effectiveness.

Table 3. Statistical Results of the Least Significant Difference Test for Antipyretics

Dependent Variable: Temperature

LSD

(I) Groups	(J) Groups	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Negative	Positive	-.32000*	.11806	.022	-.5831	-.0569
	Dose I	-.10000	.11806	.417	-.3631	.1631
	Dose II	-.27333*	.11806	.043	-.5364	-.0103
	Dose III	-.38000*	.11806	.009	-.6431	-.1169
Positive	Negative	.32000*	.11806	.022	.0569	.5831
	Dose I	.22000	.11806	.092	-.0431	.4831
	Dose II	.04667	.11806	.701	-.2164	.3097
	Dose III	-.06000	.11806	.622	-.3231	.2031
Dose I	Negative	.10000	.11806	.417	-.1631	.3631
	Positive	-.22000	.11806	.092	-.4831	.0431
	Dose II	-.17333	.11806	.173	-.4364	.0897
	Dose III	-.28000*	.11806	.039	-.5431	-.0169
Dose II	Negative	.27333*	.11806	.043	.0103	.5364
	Positive	-.04667	.11806	.701	-.3097	.2164
	Dose I	.17333	.11806	.173	-.0897	.4364
	Dose III	-.10667	.11806	.388	-.3697	.1564
Dose III	Negative	.38000*	.11806	.009	.1169	.6431
	Positive	.06000	.11806	.622	-.2031	.3231
	Dose I	.28000*	.11806	.039	.0169	.5431
	Dose II	.10667	.11806	.388	-.1564	.3697

*. The mean difference is significant at the 0.05 level.

3.3 Analgesic test

The chemical compound used in this test was glacial acetic acid induced intraperitoneally in mice. To determine the presence of analgesic activity in the test preparation, it is calculated from the decrease in the number of mice writhing for 1 hour at 5-minute intervals, as shown in Figure 1. The pain felt is due to the administration of acetic acid, which causes contraction of the abdomen of the mice so that their legs are withdrawn.

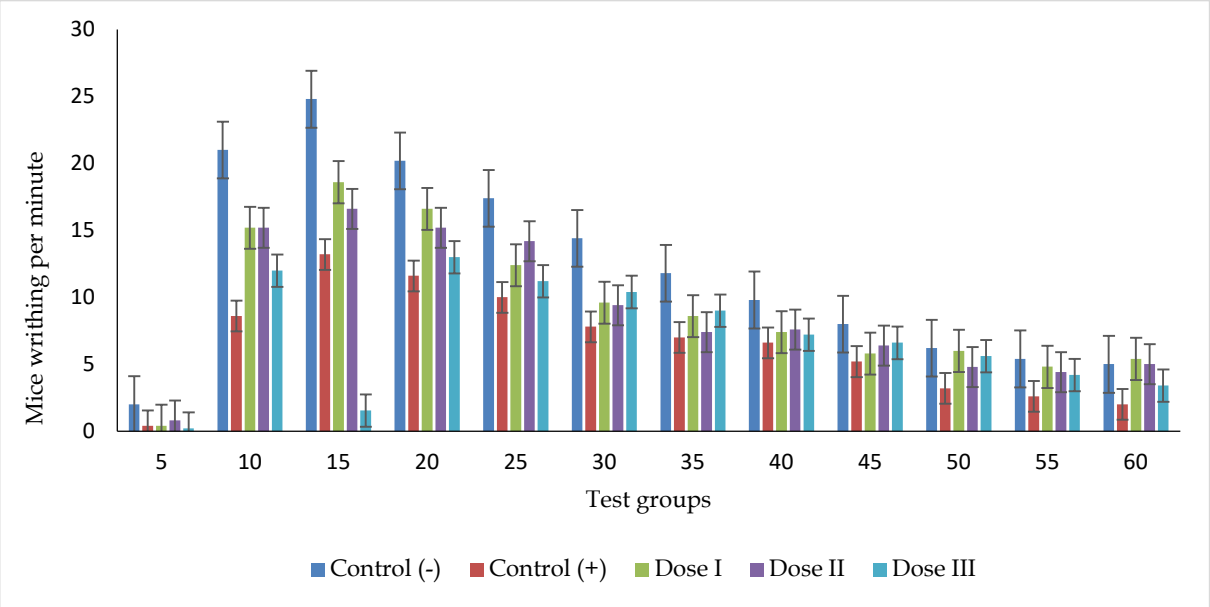

Figure 1. Average decrease in mice writhing per 5 minutes

The data in Figure 1 show that the decrease in the number of writhing mice occurred at the 20 minute after acetic acid induction and the peak of writhing mice occurred at the 15 minute. This means that both the positive control and the test substance are able to reduce the amount of writhing of the mice. It can be seen from Table 4 that the statistical results show significant differences between the test groups in terms of analgesic efficacy.

The percentage of inhibition can be calculated from the average of the DDK (area under the curve) data from the test group and the negative control. It can be clearly seen that Indian nettle plant extract has the lowest inhibition of fever at all doses compared to the positive control, doses I, II and III. The inhibition of writhing in mice with the best analgesic activity is at dose III which is 33.31% compared to doses I and II. However, this is still lower than the inhibition by aspirin.

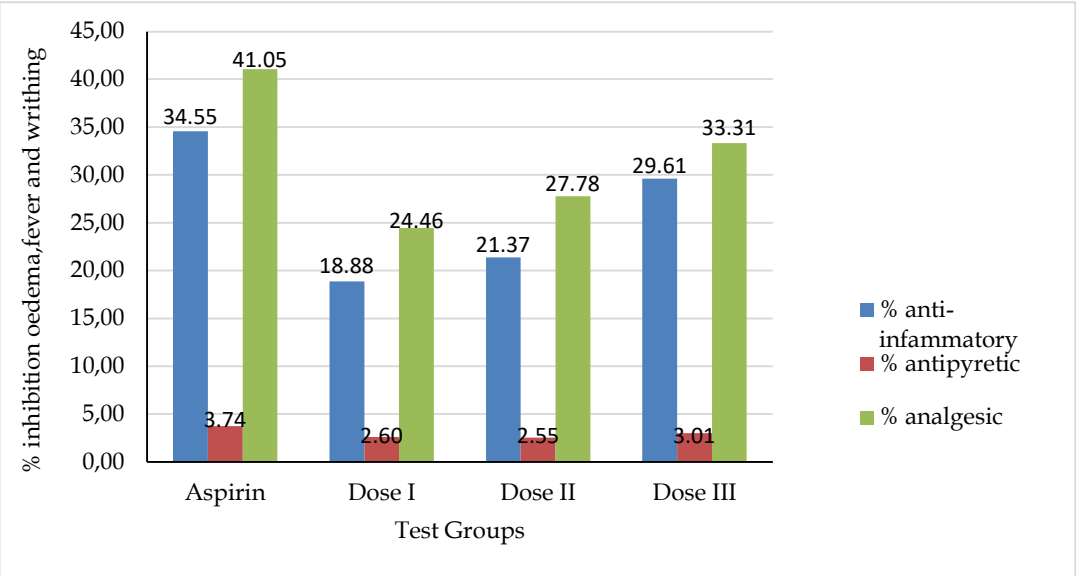

Figure 2. Percentage inhibition of Indian nettle plant extracts

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Table 4. Statistical Results of the Analgesic Least Significant Difference Test

Dependent Variable writhing

LSD

(I) Groups	(J) Groups	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Negative	Positive	297.00000*	31.69700	.000	230.8812	363.1188
	Dose I	177.00000*	31.69700	.000	110.8812	243.1188
	Dose II	201.00000*	31.69700	.000	134.8812	267.1188
	Dose III	241.00000*	31.69700	.000	174.8812	307.1188
Positive	Negative	-297.00000*	31.69700	.000	-363.1188	-230.8812
	Dose I	-120.00000*	31.69700	.001	-186.1188	-53.8812
	Dose II	-96.00000*	31.69700	.007	-162.1188	-29.8812
	Dose III	-56.00000	31.69700	.093	-122.1188	10.1188
Dose I	Negative	-177.00000*	31.69700	.000	-243.1188	-110.8812
	Positive	120.00000*	31.69700	.001	53.8812	186.1188
	Dose II	24.00000	31.69700	.458	-42.1188	90.1188
	Dose III	64.00000	31.69700	.057	-2.1188	130.1188
Dose II	Negative	-201.00000*	31.69700	.000	-267.1188	-134.8812
	Positive	96.00000*	31.69700	.007	29.8812	162.1188
	Dose I	-24.00000	31.69700	.458	-90.1188	42.1188
	Dose III	40.00000	31.69700	.221	-26.1188	106.1188
Dose III	Negative	-241.00000*	31.69700	.000	-307.1188	-174.8812
	Positive	56.00000	31.69700	.093	-10.1188	122.1188
	Dose I	-64.00000	31.69700	.057	-130.1188	2.1188
	Dose II	-40.00000	31.69700	.221	-106.1188	26.1188

*. The mean difference is significant at the 0.05 level.

4. DISCUSSION

4.1 Anti-inflammatory test

The method used for anti-inflammatory testing is the Winter method. This method was chosen because it is simple and widely used. The anti-inflammatory effects were studied using a mercury plethysmometer with measurements based on Archimedes' law, which states that an object immersed in a liquid exerts an upward force or pressure equal to the volume moved. Chemical induction of inflammation using a 1% carrageenan solution, up to 0.2 ml, was injected intra plantarly into the soles of the rats' feet [8].

The inflammatory response can be categorized into different aspects such as redness, heat, pain and oedema. The inflammatory response results in the release of various systemic mediators, cytokines and chemokines that control cellular infiltration, resulting in resolution of the inflammatory response and recovery of the tissue [9]. However, prolonged inflammatory stimulation can lead to chronic inflammation. Flavonoids are secondary metabolites with known anti-inflammatory activity. Flavonoids are secondary metabolites with known anti-inflammatory activity. Some flavonoid-containing plants are able to reduce the regulation of cyclooxygenase-2, which is thought to contribute to inflammation [10].

After statistical testing, the positive control and test preparations (all doses) showed significant differences in reducing the volume of oedema. This indicates that the positive control has a greater anti-inflammatory effect than the test compounds at different doses. The anti-inflammatory effect at the doses of 70 mg/200 g BW and 140 mg/200 g BW also showed significant differences for the reduction in volume of oedema, as did the doses of 70 mg/200 g BW and 280 mg/200 g BW, and 140 mg/200 g with 280 mg/200 g BW, all of which showed significant differences. This suggests that as the dose increases, so also the anti-inflammatory effect. Statistical tests showed that the data were normally distributed and homogeneous, and the Kruskal-Wallis statistical value showed a significant difference from each test group with a value of $p<0.05$

4.2 Antipyretic test

Antipyretic test, using the Brewer's yeast 20% method, induced ± 18 hours prior to the administration of test compounds and negative controls. Measurements are made using a digital thermometer and temperature changes are made in the rectal area as it is easier to measure temperature in mice. Brewer's yeast is used as a pyrogenic substance that can cause fever [11]. The reason for using Brewer's yeast is that of all pyrogens causing fever, Brewer's yeast is the cheapest and is still used in some in vivo antipyretic studies. In this antipyretic test it can be seen that there are significant differences between the negative control group and the positive control and test preparation groups. This indicates that both the positive control and the test preparation have antipyretic activity. This decrease was observed at the 60 minutes after administration of the positive control and test preparations. Then, when comparing the decrease in temperature by the positive control with the test preparation dose of 70 mg/200 g BW and 140 mg/200 g BW, there is a significant difference in the decrease in temperature. This indicates that the analgesic effect of the positive control is greater than that of the two dosed groups and that dose affects temperature reduction. In the test group, there was no significant difference in temperature reduction at the 280 mg/20 g BW dose with the positive control

4.3 Analgesic test

The Siegmund method is used in pain research. This is a chemical stimulation method and is the most commonly used method. It is tested by looking at the reduction in writhing or ability to relieve pain in mice following intraperitoneal administration of acetic acid. The symptoms of pain in mice as a result of acetic acid administration are characterized by contractions of the abdominal wall that cause the legs to be pulled back, stretched and the abdomen to touch the floor of the space it occupies, this symptom is called writhing [12]. In order to determine whether the test preparation has a protective effect against the pain caused by the inducer, the preparation is administered 30 minutes before the administration of acetic acid (the inducer) [13,14].

5. CONCLUSION

Extract ethanol 70% of Indian nettle plant has anti-inflammatory and analgesic effects as it can reduce the volume of oedema on the soles of rats' feet and writhing and no antipyretic effect. The best anti-inflammatory effect at dose III 33.31. Statistical test there is a significant difference in anti-inflammatory with a p-value 0.005.

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