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The Analgesic Effect of Aqueous Extract of Sisik Naga Leaves (*Pyrrosia piloselloides* (L.) M.G. Price) on White Female Mice (*Mus musculus*)

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Research Article

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ABSTRACT

The use of the plant as one ingredient a treatment in indonesia has known since long time ago and until now there are still mostly done by the wider community. Sisik naga leaves are one of the plants that have been used traditionally by community as analgesic. This study aims to determine the analgesic effect of aqueous extract of Sisik naga leaves using three variations of dosage. The Complete Random Design (CRD) with 5 treatment groups was used in this study. The groups were: Group I, treated with tramadol 50 mg/kg BW, 0,5 ml/20 g BW as a positive control; Group II treated with distilled water 0,5 ml as a negative control; Group III-V treated with aqueous extract of Sisik naga leaves with 3 variations in dose 3.5 mg/20 g BW, 0,5 ml; 7 mg/20 g BW, 0,5 ml; and 14 mg/20 g BW, 0,5 ml. Thermal pain induction was done by placing the mice on hot plate with constant temperature of 55°C. The mice gave a respond in the way of lick its back foot or even jumping. The data was collected was analyzed using one direction ANOVA model and it was continued with LSD test in order to find out the difference every treatment group. The results showed that dosage of 14 mg/20 g BW had an analgesic function.

INTRODUCTION

Pain is a symptom of various diseases and experienced by almost every creature. Pain is subjective and there is no laboratory test that can measure the level of pain. Pain can only be felt and described by the patient [1,2]. A drug used to relieve pain and eliminate the so-called analgesic.

In Indonesia, in addition to public health services, treatment with traditional way is still widely practiced and maintained by the community. This traditional treatment by Indonesian society is a legacy of the nation's culture and principles back to nature makes treatment in this way becoming more popular [2]. A more healthy life with natural ingredients and the advancement of medical science makes people turn to traditional medicine. The development of traditional medicine is also increased along with the increasing number of problems encountered with the new-issue due to the use of chemical drugs, especially in terms of side effects [3].

Indonesia has about 30,000 species of plants and 940 species of which are medicinal plants. This natural wealth makes Indonesia as a source of medicinal plants in the world and the tropical climate of Indonesia is very possible that various plants can live well [2].

One of Indonesia's natural wealth which can be used as medicine is the leaves of Sisik naga. Empirical data shows that the water decoction of leaves of Sisik naga is trusted by communities to reduce and eliminate pain in the body [4]. Plants dragon scales (*Drymoglossum piloselloides*) is one of the plants that grow and spread wildly throughout tropical Asia region and has many names depending on the region where growth [5]. This plant is also an epiphytic plant because it can make your own meals. This plant has leaves shaped like dragon scales dark green, creeping stems and roots are strongly attached to the plant carrying [6]. Chemical constituents' present form Sisik naga leaves such as flavonoids and tannins have various effects one of which is

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the analgesic effect. Analgesic drugs a group of drugs that have activity reduces pain without losing consciousness. To test the analgesic activity, can usually be done in two ways: Chemically induced and induced pain thermik. The pain shown by test animals using chemical induction is the frequency of the movement of the test animals at specific times. While pain after induced by thermal shown to lick the back foot or jump when placed on a hot plate [7].

Based on the above, the researchers are interested in examining the analgesic effect of the aqueous extract of Sisik naga leaves on mice to obtain scientific data about potential analgesics on Sisik naga leaves.

EXPERIMENTAL

Materials and Devices of the Research

The main ingredient is Sisik naga leaves which is derived from Payakumbuh West Sumatra Province, Indonesia. Other materials are distilled water, analgesic drugs (tramadol) 50 mg, CMC 1% and ethanol 70%. While the equipment used is the analytical balance, oven, blender, fabric filter, Whatman filter paper no.1, stopwatch, test tubes, glass beaker, a petri dish, measuring cup, micropipette, syringe injection 1 ml, and water bath.

Grouping of Test Animals

Test animals were used in this study were 15 female white mice. Mice are grouped into 3 groups: Positive control group was given analgesic drug Tramadol, negative control group was given distilled water and the treatment group was given the aqueous extract of Sisik naga leaves with three variations of doses.

Extraction of Sisik Naga Leaves

Sisik naga leaves that have been washed chopped and weighed as much as 60 g. Boiled with 600 ml of distilled water until the remaining 200 ml. Decoction is then filtered and evaporated to obtain a dry extract. This dry extract used in the study.

Preparation of Test Animals

Before treatment, female white mice adapted to the environment and food for 1 week and before administration of the test substances orally, female white mice were fasted for 11 h with fixed given to drink.

Preparation of Extract Solutions

Aqueous extract of Sisik naga leaves solution made experiments with varying doses at 3.5 mg/20 g BW; 7 mg/20 g BW and 14 mg/20 g BW were suspended in a solution of 1% CMC 0.5 ml/20 g BW.

Testing of Analgesic Effect

Mice induced thermic by way of placing it on the heater with temperature fixed at 55ºC as a painful stimulus. Mice will provide a response in the form of a lick of the hind legs or jumping. The time interval between administrations of pain stimuli with the response called the reaction time. This reaction time can be extended by administering analgesic drugs. Extension of time this reaction can then be used as a measure for evaluating analgesic activity. In this study, observations were made at min 30, 60, 90 and 120 after administration of the test substance and the reference solution with long observations 1 min.

Data Analysis

Data were analyzed using Varian Analysis (ANOVA) and if there is a significant difference between treatments followed by LSD (Least Square Difference) at significant level of 1%.

RESULTS AND DISCUSSION

In this study testing the analgesic effect of aquesous extract of sisik naga leaves as a test group with dose variation 3.5 mg/20 g BW, 0,5 ml; 7 mg/20 g BW, 0,5 ml and 14 mg/20 g BW, 0,5 ml. as a positive control used analgesic drug Tramadol and distilled water as negative control. Test animals that had been prepared, prior to administration of test substances, testing to painful stimuli in the form of securities and lick or jump. This observation aims to compare the response of test animals before and after administration of the test substance.

The observation of the response to jump or lick the feet of the test animals when were given painful stimuli in the form of heat for 1 min at a temperature of 55°C is presented in **Table 1**.

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Table 1. Response of mice observations negative control group (distilled water).

	Number of response														
	Before treatment			After treatment											
				30'			60'			90'			120'		
	L	J	T	L	J	T	L	J	T	L	J	T	L	J	T
I	-	7	7	8	3	11	8	2	10	11	1	12	8	1	9
II	1	9	10	11	2	13	8	0	8	13	0	13	15	0	15
III	3	7	10	3	1	4	10	2	12	9	1	10	7	2	9
Amount	27			28			30			35			33		
Average	9			9*			10			12*			11		

Note: L=Jump J=lick T=Total

*Rounding decimals

In **Table 1** above shows that prior administration of distilled water and the average number of responses in mice 9 times. At the 30th min after administration of distilled water, the average number of responses in mice remained unchanged. At the 60th min and the 90th min increased the amount of each are respectively 10 and 12 times. But the min-120 decreased the number became 11 times. The average response in **Table 1** can be depicted in the graph in **Figure 1**.

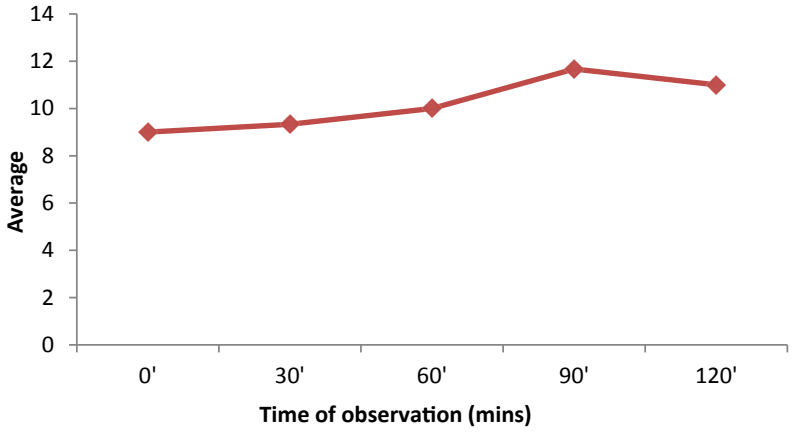

Figure 1. Graphs the average number of responses negative control (distilled water).

Observations on the negative control group which were given distilled water showed that the average response of test animals to pain stimuli before and after administration of relatively stable distilled water as negative control did not provide an analgesic effect, so the decline and increase the average response of test animals was not significant.

Table 2 shows that before treatment, the average number of responses in mice was as much as 9 times and up to the 30th min after drug administration, the average number of responses of mice still did not decline. In the 60th min a new decline in the response of mice to 8 times and continued to decline until the 90th min to 6 times and stable up to 120 min. The average response in **Table 2** can be illustrated in the graph in **Figure 2**.

Table 2. Observations Response Mice positive control group (Tramadol).

	Number of response														
	Before treatment			After treatment											
				30'			60'			90'			120'		
	L	J	T	L	J	T	L	J	T	L	J	T	L	J	T
I	2	8	10	9	1	10	7	0	7	0	1	1	5	0	5
II	0	7	7	1	6	7	6	3	9	3	7	10	7	0	7
III	5	4	9	8	1	9	7	0	7	6	1	7	7	0	7
Amount	26			26			23			18			19		
Average	9*			9*			8*			6			6*		

Note: L=Lick J=Jump T=Total. *Rounding decimals

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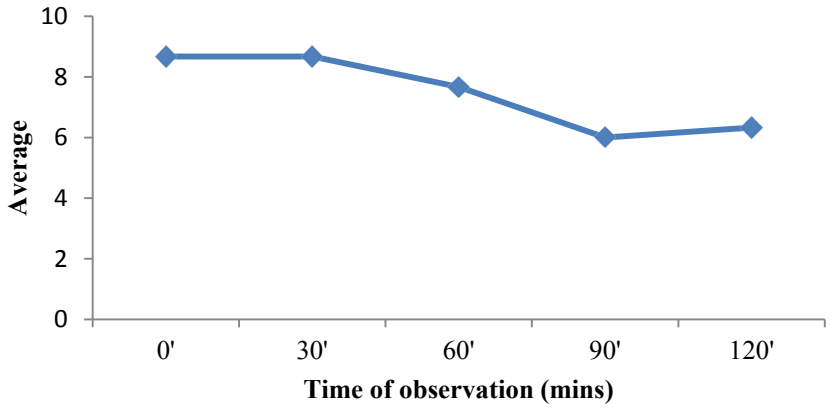

Figure 2. Graph the average number of responses in the positive control.

A decrease in the average number of responses of test animals mice to stimulation of pain in the positive control group were given the analgesic tramadol drug had begun in the 30th min after drug administration. Average decline in the 30th min was from 9 times to 6 times the response and continued to decline until min 120. Tramadol analgesic effects began to be seen at min 60-120 min with a peak analgesic effect of tramadol in the 90th min. Observations response to stimuli pain test animals in each group which were given doses of the aqueous extract of Sisik naga leaves are presented in **Tables 3-5**.

Table 3. Observations response aqueous extract of Sisik naga leaves dosage 3.5 mg/0.5 ml.

MICE		Number of response														
		Before treatment			After treatment											
					30'			60'			90'			120'		
		L	J	T	L	J	T	L	J	T	L	J	T	L	J	T
3,5 mg	I	6	6	**12**	7	2	**9**	12	0	**12**	12	0	**12**	8	0	**8**
0,5 ml	II	4	4	**8**	6	1	**7**	11	0	**11**	6	0	**6**	12	0	**12**
	III	4	4	**8**	12	0	**12**	5	0	**5**	8	1	**9**	8	0	**8**
	Amount	**28**			**28**			**28**			**27**			**28**		
	Average	**9***			**9***			**9***			**9**			**9***		

Note: L=Jump J=lick T=Total. *Rounding decimals

Table 4. Observations response aqueous extract of Sisik naga leaves dosage 7 mg/0.5 ml.

Mice		Number of response														
		Before treatment			After treatment											
					30'			60'			90'			120'		
		L	J	T	L	J	T	L	J	T	L	J	T	L	J	T
7 mg	I	0	10	**10**	7	4	**11**	12	0	**12**	12	0	**12**	8	0	**8**
0,5 ml	II	2	6	**8**	3	1	**4**	8	0	**8**	8	0	**8**	10	0	**10**
	III	0	10	**10**	13	0	**13**	5	1	**6**	4	2	**6**	7	0	**7**
	Amount	**28**			**28**			**26**			**26**			**25**		
	Average	**9***			**9***			**9***			**9***			**8***		

Note: L=Jump J=lick T=Total. *Rounding decimals

Table 5. Observations response aqueous extract of Sisik naga leaves dosage 14 mg/0.5 ml.

Mencit		Number of response														
		Before treatment			After treatment											
					30'			60'			90'			120'		
		L	J	T	L	J	T	L	J	T	L	J	T	L	J	T
14 mg	I	1	9	**10**	9	0	**9**	9	0	**9**	8	0	**8**	14	0	**14**
0,5 ml	II	3	6	**9**	11	0	**11**	7	0	**7**	10	0	**10**	3	2	**5**
	III	3	6	**9**	5	2	**7**	9	1	**10**	7	0	**7**	5	0	**5**
	Amount	**28**			**27**			**26**			**25**			**24**		
	Average	**9***			**9**			**9***			**8***			**8**		

Note: L=Jump J=lick T=Total. *Rounding decimals

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Tables 3-5 shows that before the extract, the average number of responses in mice 9 times and survived for three variations of the dose to the 60th min after administration of the extract. In the 90th min doses of 3.5 mg/20 g BW and 7 mg/20 g BW has not been decline, while a dose of 14 mg/20 g BW decreased the response to 8 times. After 120 min of treatment, the dose of 7 mg and 14 mg decreased to 8 times, while a dose of 3.5 mg did not decrease.

Based on existing data in **Tables 3 and 4** and, it can be described by the graph of the average response to the control mice each group at each dose treatment **(Figure 3)**.

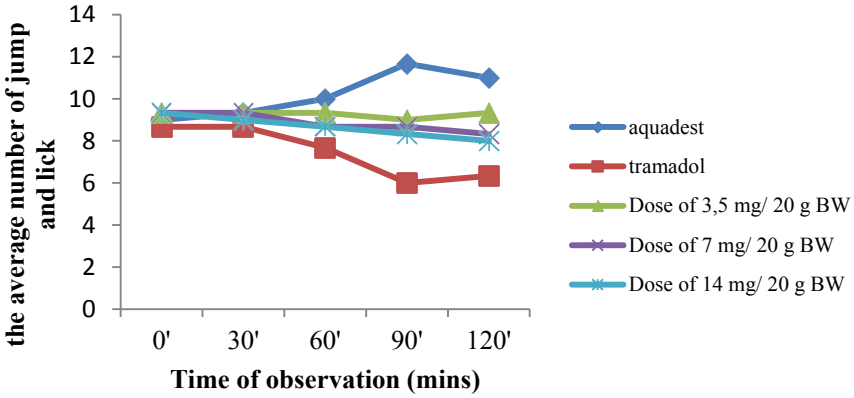

Figure 3. Graph comparison of the average dose of treatment to control.

In Figure above shows the average response of mice positive control group and the treatment group decreased after treatment compared to before treatment, while the average response of mice negative groups tend to be stable. The most powerful decline was seen in test animals that were given water extract 14 mg/20 g BW.

Testing test substance dose of 3.5 mg/20 g BW began to decline in the 90th min and went back up after 120 min. The test substance at a dose of 7 mg/20 g BW decreased at min 60-120 min. While at a dose of 14 mg of test substance in min 30-120 min continues to decline, it showed that at high doses began working test substance after 30 min and reached a peak at 120 min.

From the graph method Hot-Plate seen that new Tramadol decreased the effect of jumping and licking the feet on 60 min and reaching the peak of decline in the 90th min. According to the literature it is known that Tramadol start working after 1 h and the effect began to diminish in the minutes to 120. This is evidenced by the increasing number of jumping back and or licking of test animals. **Tables 3-5** show that the extract tend to be able to extend the reaction time to heat mice compared with controls. This is evidenced by the higher dose of the extract given to the test animals, the more the long reaction time. The reaction time dose of 14 mg/20 g BW extract provides the highest effect in reducing pain in mice caused by heat. From the results of LSD test showed that the extract at a dose of 14 mg/20 g BW is the most significant doses in prolonging the reaction time of mice so that it can function as an analgesic.

The highest decrease was obtained in the extract at a dose of 14 mg/20 g BW. From the overall results of this study, it was found that the data generated by the analgesic effect of the leaf extract of Sisik naga longer than tramadol. It can be seen from the effects of maximal decrease in the average number of test animals to extract responses which were seen to decrease from 30-120 min with tenure longer. The maximum effect of a decrease in the average number of responses which was given tramadol test animals at 120 min began to disappear.

Figure 4 show that the extract at a dose of 14 mg/20 g BW provides a sharper decline than other doses. The higher the dose used, the effect produced is also increasing. Pain response after induction thermic shown with a lick and jump. This is because pain receptors located in the skin and a specific mediator for this is prostaglandin. Parameters measured in the induction of pain are the reaction time, i.e., the interval between administrations of the painful stimulus until a response. This period may be extended by a drug is efficacious as analgesic. An extension of time can be used as a benchmark in evaluating analgesic activity [7].

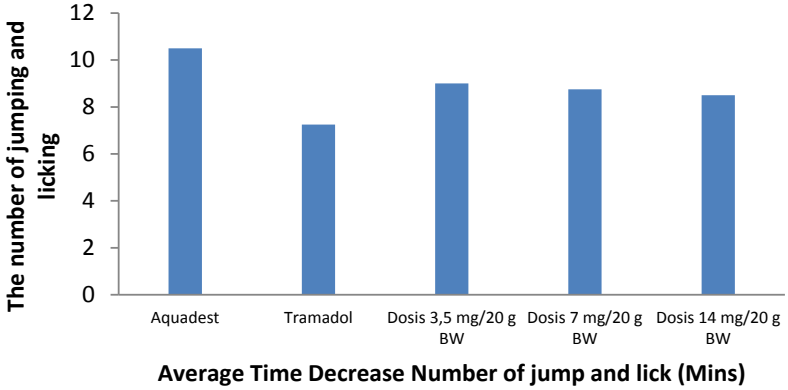

Figure 4. Graph of average time decrease number of jump and lick (Min).

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Induction heat used in this study is the constant temperature 55ºC, since the critical temperature is the average time a person starts to feel pain is 45ºC. Temperatures above 45ºC start to cause damage to the tissues resulting in the appearance of pain. The pain caused due to heat very close relation with the ability to heat the tissue damage. Stimulus heat will stimulate pain receptors that are sensitive to hot or cold temperature extremes of pain receptors thermo sensitive. These receptors pain will continue both through the fiber type C that has no myelin [8].

Analgesic effect of this extract might be caused by the presence of some chemical content in the leaf dragon scales such as flavonoids and tannins which act as analgesics [9]. Responses varied from each of the animals in each test group were very likely to occur. Various internal factors such as species, genetic, sex, age as well as external factors such as food and the environment will greatly affect the response of test animals. Female white mice were more sensitive to pain than male mice.

Based on the results of this test showed that groups of test animals which were given the aqueous extract of Sisik naga leaves showed a decrease in the average number of responses after the pain stimulus being administered the extract. Aqueous extract of Sisik naga leaves with 3 variations of the administered dose showed an analgesic effect.

CONCLUSION

From the research that has been conducted in regarding test the analgesic effects of Aqueous extract of Sisik naga leaves can be concluded that the aqueous extract of Sisik naga leaves on dose 3,5mg/20 g BW, 7 mg/20 g BW and 14 mg/20 g BW has an analgesic effect on female white mice. Extract at a dose of 14 mg/20 GBW has the most effective analgesic effect compared to other doses.

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