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The Effect of Sambiloto (*Andrographis paniculata Nees.*) and Kejibeling (*Strobilanthes crispa*) Leaves on Uric Acid Levels of White Rats (*Rattus norvegicus*)

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Abstract

This study aims to seek answers to four research questions, (1) Do the extracts of Sambiloto (*Andrographis paniculata Nees.*) and Kejibeling (*Strobilanthes crispa*) leaves affect the blood uric acid levels of white rats (*Rattus norvegicus*)? (2) Do different dose concentrations of Sambiloto (*Andrographis paniculata Nees.*) and Kejibeling (*Strobilanthes crispa*) leaves extracts affect the blood uric acid level of white rats (*Rattus norvegicus*)? (3) And do time exposures of Sambiloto (*Andrographis paniculata Nees.*) and Kejibeling (*Strobilanthes crispa*) leaves extracts affect the blood uric acid level of white rats (*Rattus norvegicus*)? (4) Do the interactions of the variables in this study affect the blood uric acid of white rats (*Rattus novergicus*)? This laboratory experimental research design used twenty-eight white rats, divided into two negative and positive control groups and six treatment groups. Rats were acclimatized for fourteen days and were fasted before the data collection. The data are blood uric acid levels of the blood samples taken from the cut made at the tip of the tail. The variables of the study are the extracts (two) of the leaves, the different levels (three) of dosages of the extract, and the length times (four) exposures of the animals after being given the extract orally. Univariate Statistical Analysis was used to analyze the data. The results of the analysis show that there are no statistically significant differences in the effect of the different kinds of leaves extracts (p = 0.994), the different dosages (p = 0.574), the length of times of exposure (p = 0,112), and also in the interactions of the variables on the levels of the uric acid, Dose*Time with p = 0.895, Dose*Group with p = 0.266, Time*Group with p = 0.511, but in the interaction of Dose*Time*Group is significant with p = 0.047. However, on the descriptive study, the results show that the leaves extracts, the different dose concentrations used, and the length of times of exposure show that the uric acid levels were lowered. Thus, this study concluded that Sambiloto (*Andrographis paniculata Nees.*) and Kejibeling (*Strobilanthes crispa*) leaves extracts to lower the blood uric acid levels of white rats (*Rattus novergicus*).

Keywords: Sambiloto, Kejibeling, Uric Acid, White Rats.

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INTRODUCTION

Health is a very important thing in life. However, in line with the times, especially in the field of technology, it is the main factor that causes the lifestyle of modern society in society. Therefore, people are increasingly turning to consuming instant or ready-to-eat food without considering the side effects (Septianto et al., 2020).

Uric acid is acid in the form of crystal as the end product of purine metabolism, one of the nucleic acid components found in the nucleus of a cell of the body. Uric acid is an end product formed from purine (adenine, guanine) and is produced in the tissue that contains xanthine oxidase, especially in the liver and small intestine. Uric acid is absorbed by the mucosa of the intestine, enters the blood vessel, flows into the kidneys then excreted through urine. Uric acid level for man is < 7.0 mg/dL and woman is <6.0 mg/dL.

When the synthesis of uric acid is too much, and the excretion through the kidney is less, the uric acid level in the body will rise, uric crystals, which are difficult to be dissolved in the body's liquid, will precipitate in the joints and tissues and will cause sore. Uric acid has a role as an antioxidant if the amount is not too much in the blood, but when it is too much in the blood, then it will act as a prooxidant.

The use of traditional medicine in Indonesia is part of the life of the Indonesians. The advantage of using traditional or natural medicines is that the side effects are relatively small. This can also be used as new knowledge in herbal medicine. Medicinal plants have been used since ancient times. Knowledge in the use of medicinal plants for prevention and overcoming disease is needed (Pranaka et al., 2020).

Sambiloto (*Andrographis paniculata Nees.*) and Kejibeling (*Strobilanthes crispa*) are just a few of the many medicinal plants used by people. Sambiloto leaves have a bitter taste, and they contain an active substance called Andrographolide at about 2.39%. Sambiloto leaves also contain glucose orthosiphones, essential oils, saponins, polyphenols, flavonoids, saponins, potassium salts, and myoinositol (Paramitha & Rahamisa, 2016). Some researches show that Sambiloto leaves extract (Anggraini, 2009) and Sambiloto roots (Septianingsih et al., 2012) lower blood uric acid concentration,

Kejibeling (*Strobilanthes crispa*) is a plant used for diabetes, kidney stones, cholesterol, ulcers, and Uric Acid. One of the nutrient content found in the leaves is sodium which functions to increase the extracellular fluid of the blood. Researches show that those Kejibeling leaves contain polyphenols, saponins, alkaloids, which have anti-diabetic, cholesterol, and uric acid properties (Nonci et al., 2016), the extracts of Kejibeling (Rakhman, n.a) and infuse of Kejibeling (Anggraeini, 2018) lower blood uric acid levels. Noviandari, Inayah (2017) said that Kejibeling lowers blood uric acid concentration, although the effect is lower than Allopurinol.

Alloxan, a stable free radical, is a substrate that is structurally a simple pyrimidine derivative. It is introduced as alloxan hydration in aqueous solutions. The name alloxan is derived from the combination of the words allantoin and oxalurea (oxaluric acid). It can be treated intravenously, intraperitoneal, and subcutaneously. It induces the release of calcium ions from mitochondria which results in a disrupted cell oxidation process. The release of ions from the mitochondria results in a homeostatic disorder which is the beginning of cell death (Yuriska, 2009).

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RESEARCH METHODOLOGY

This research is an experimental laboratory research that uses lab tools and materials to conduct the experiment and in collecting data. These are the experimental stages.

Experimental animals preparation

Twenty-eight white rats used, 8-10 weeks old and reared for 14 days, were used and undergone an acclimatization process. The rats were divided into eight groups. The negative control group consisted of 2 individual rats. The positive control group consisted of 2 individual rats. The treatment group consisted of 6 groups, each of 4 individual rats.

Simplicia preparation

The raw materials were obtained from the campus garden and herbal plant shops, 1 kg wet weight of each plant was prepared. The leaves were cleaned using running tap water. The leaves were then sliced ± 2 mm thick and dried in the sun for 7 hours. The dried ingredients are then powdered using a blender and sieved using a 50 mesh sieve. The powder, called Simplicia, was then stored in a dim room and protected from sunlight.

Extracts preparation

The 160 gr of Simplicia obtained were extracted by soaking them into 800 mL of 96% ethanol as solvent (this procedure was done three times). The solution was shaken using a rotator shaker for ± 24 hours, then filtered using a 0.45-micrometer filtrate filter paper. The results were then stored in an Erlenmeyer flask. This extraction process is carried out three times. The resulting macerate is then solidified using a vacuum rotary evaporator and oven at 50°C for 15 minutes. The yield of concentrated Sambiloto extract was 43 g, and the Kejibeling extract was 40 g.

Extract dosage calculation

According to (Kasmawati et al., 2015), the effective dose level in mice is 0.28 g/kg BW. Therefore, the dose for mice in this study used a conversion factor from mice to mice with an average mouse bodyweight of 20 g and an average rat bodyweight of 200 g. From the conversion table, it is found that the conversion factor is 7.0. Therefore, according to the formula: 0.28 g x 7.0 = 1.96 g / kg BW. Therefore, the doses used is (1.96 g x 1000 g)/5 = 392 mg (dose conversion from mouse to white rat)

Based on the calculation of the standard dosage above, the researchers determined the dosage in this study with three concentrations, namely dosage I 196 mg (below standard), dosage II 392 mg (standard), and dosage III 588 mg (above standard). The dosages were then dissolved into 10 mL CMC.

Alloxan preparation

In this procedure, 1 g of alloxan was dissolved in 10 mL NaCl 0.9%. The positive control and the treatment groups were induced 0.25 mL of alloxan.

Secondary metabolite compounds test

Secondary metabolites test was carried out using the Farnsworth (1966) method on eight groups of active compounds, namely alkaloids, flavonoids, quinones, saponins, tannins, polyphenols, triterpenoids, and steroids.

The treatment

Rats fasted for 18 hours, and then blood samples were taken for data prior to treatment. Control negative rats are not induced with alloxan, while control positive are rats induced with alloxan, and treated rats were induced with alloxan and extracts of the different plants and dosage. Alloxan was given only once 48 hours before the first treatment of extract on day 0. Extract treatments were given 18 hours before taking the blood samples. During this time, the rats are being fasted. Data were taken on days 1, 5, 10, and 14.

Test animals in group 1 (negative control), group 2 (positive control), and group 3-8 (treatment groups) were fasted for 18 hours and checked for blood uric acid levels (T_0). The test animals in the positive control group and the treatment groups were then induced with 0.25 mL of alloxan for 72 hours, including 18 hours of fasting, and their blood uric acid levels were checked, namely (T_1). After taking blood uric acid levels (T_1), the animals in the treatment groups were treated as follows: group 3 were treated with 2 mL of 196 mg, group 4 with 2 mL of 392 mg, group 5 with 2 mL 588 mg, all with Sambiloto leaves extracts, and group 6 with 2 mL 196 mg, group 7 with 2 mL 392 mg, and group 8 with 2 mL 588 mg all with Kejibeling leaves extracts.

Uric acid levels test

The rats' tails were cut about 0.5 mm, the blood was dripped on a uric acid measuring strip, and the results were displayed on the GCU meter screen that was used. The measurement results obtained are used as data in this study. The steps above were performed for every rat used in this study.

Data collection

The treatment groups were given the extract every day with feeding for three days, and then on the 4^{th} day, the rats fasted, and on the 5^{th} day, blood samples were taken to check the blood uric acid levels of the rats for T_2. Then proceed with feeding for three days and the giving of the extract. On the 9^{th} day, the rats fasted, and on the 10^{th} day, blood was taken to check the blood uric acid levels of the rats for T_3. After that, feeding for two days and giving the extract; on the 13^{th} day, the rats fasted, and on the 14^{th} day, the blood was taken to check the blood uric acid levels of the rats for T_4.

Data analysis

In this study, the data obtained were analyzed using statistical calculation of ANOVA (Analysis of Variants) Univariate. Sambiloto (*Andrographis paniculata Nees.*) and Kejibeling (*Strobilanthes crispa*) leaves extracts, different concentrations of extract dose, and the time period of data collection as independent variables and blood uric acid levels of white rats (*Rattus novergicus*) as the dependent variable. Data collection in this study uses three replications.

RESULTS AND DISCUSSIONS

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In the study of medicinal plants, it is important to know the content of their biological and phytochemical compounds. In this study, the biological and phytochemical screening of the plants was done using Farnsworth's (1966) testing active compound method. The results of the screening show that Sambiloto (*Andrographis paniculata Nees.*) and Kejibeling (*Strobilanthes crispa*) leaves extracts are shown in Table 1 below.

Table 1: Secondary Metabolite Compound Test Results

No	Compound Groups	Sambiloto Leaves	Kejibeling Leaves
1	Alkaloids	-	-
2	Flavonoids	-	√
3	Saponins	√	√
4	Tannins	√	-
5	Quinones	-	-
6	Polyphenols	√	√
7	Triterpenoids and Steroids	-/-	√/√

Table 1 above shows that Sambiloto leaves (*Andrographis paniculata Nees*.) Simplicia contains saponins, tannins, and polyphenols, while Kejibeling leaves (*Strobilanthes crispa*) Simplicia contains flavonoids, saponins, polyphenols, triterpenoids, and steroids. However, both leaves extracts don't have Alkaloids and quinones.

Thus, it is concluded that these two plants contain active biological and phytochemical compounds and as plant-derived medicinal and, although they have been known, can be utilized for medicinal activity.

Further in the study and in determining the blood uric acid levels as affected by the extracts of Sambiloto (*Andrographis paniculata Nees.*) and Kejibeling (*Strobilanthes crispa*) leaves extracts, it was found that, descriptively, the blood uric acid levels were affected. The mean measurements collected, using three replicates, are shown in Table 2 below.

Table 2: Mean Blood Uric Acid Levels Measurements

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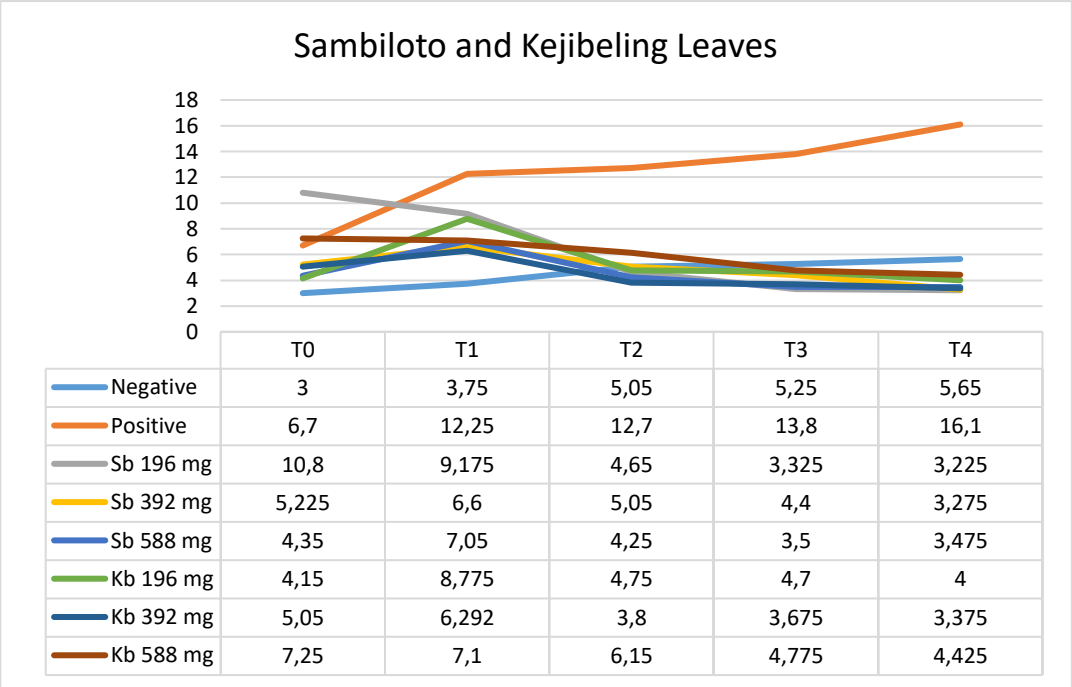

	T0	T1	T2	T3	T4
Negative	3	3,75	5,05	5,25	5,65
Positive	6,7	12,25	12,7	13,8	16,1
Sb 196 mg	10,8	9,175	4,65	3,325	3,225
Sb 392 mg	5,225	6,6	5,05	4,4	3,275
Sb 588 mg	4,35	7,05	4,25	3,5	3,475
Kb 196 mg	4,15	8,775	4,75	4,7	4
Kb 392 mg	5,05	6,292	3,8	3,675	3,375
Kb 588 mg	7,25	7,1	6,15	4,775	4,425

Legend: Sb = Sambiloto, Kb = Kejibeling, T_0 before treatment, T_1 day 1 after treatment, T_2 day 5 after treatment, T_3 day 10 after treatment, and T_4 day 14 after treatment

Table 2 above shows that the blood uric acid levels in both the negative and positive control groups increase during the period of the experiment and data collection period from T_0 to T_4. However, the blood uric acid of the six treatment groups decreases during the experiment and data collection period from T_0 to T_4. Thus these results show that the two plants lower used in this study lower the blood uric acid of the animals.

To determine the significant effect of Sambiloto (*Andrographis paniculata Nees.*) and Kejibeling (*Strobilanthes crispa*) leaves extracts on the blood uric acid levels, statistical data analysis techniques were carried out using univariate statistical methods (ANOVA). The level of confidence is at $\alpha = 0.05$.

The results of the analysis, as shown in Table 3 below, shows that there was no significant effect of the two plants, Sambiloto (*Andrographis paniculata Nees.*) and Kejibeling (*Strobilanthes crispa*), with a *p*-value of .994 ($> \alpha = 0.05$), the three different concentration of dose with a *p*-value of .574 ($> \alpha = 0.05$), and different length of time exposure of the extract with a *p*-value of.122 ($> \alpha = 0.05$). In the interaction of the 3 variables used in this study on the blood uric acid levels, it was also shown that there is no significant effect of the dose and time with a *p*-value of .895 ($> \alpha = 0.05$), dose and group with a *p*-value of .266 ($> \alpha = 0.05$), time and group with a *p*-value of .811 ($> \alpha = 0.05$), however on the interaction of the 3 variables, the Time, Dose, and Group shows a significant difference with a *p*-value of .047 ($< \alpha = 0.05$).

Table 3: Univariate Analysis of the Difference of the Dose, Time, Groups, and the Interaction of the Variables on the Blood Uric Acid Levels

Source		Type III Sum of Squares	Df	Mean Square	F	Sig
Intercept	Hypothesis	3808.301	1	3808.301	681.681	.000
	Error	23.877	4.274	5.587[a]		
Dose	Hypothesis	23.369	2	11.684	.743	.574
	Error	31.460	2	15.730[b]		
Time	Hypothesis	46.579	4	11.645	2.028	.122
	Error	139.039	24.211	5.743[c]		
Group	Hypothesis	.001	1	.001	.000	.994
	Error	6.329	659	9.607[d]		
Dose*Time	Hypothesis	31.692	8	3.962	.395	.895
	Error	80.230	8	10.029[e]		
Dose*Group	Hypothesis	31.460	2	15.730	1.569	.266
	Error	80.230	8	10.029[e]		
Time*Group	Hypothesis	15.624	4	3.906	.389	.811
	Error	80.230	8	10.029[e]		
Dose*Time	Hypothesis	80.230	8	10.029	2.057	.047
*Group	Error	487.543	100	4.875[f]		

This research concluded that the Sambiloto (*Andrographis paniculata Nees.*) and Kejibeling (*Strobilanthes crispa*) leaves extracts, the different concentration of dose use, the length of time the treatments were given, descriptively lower blood uric acid levels in White Rats (*Rattus Norvegicus*), although statistically the effect is found to be not significant. In the interaction of each of the variables to the other, it has no significant effect, but the interaction of the three variables together gives a significant effect.

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