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The Effect of Pasote Breaking Water (*Dysphaniaambrosioides* L.) on Serum Blood Creatinine Levels of Wistar Rats (*Rattus norvegicus*)

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Abstract: ***Pasote plant (Dysphania ambrosioides. L) is a plant that is commonly found in North Sulawesi and has been widely used as herbal medicine. The purpose of this study was to determine blood serum creatinine levels as an indicator of kidney function in the administration of Pasote water extract to Wistar rats (Rattus norvegicus). The method used is the spectrophotometric method. The test animals were grouped into four groups. The normal group was given water, the test group was given 2 g, 4 g, and 8 g of Pasote steeping. The administration of Pasote tea was carried out for 34 days. Rat body weight was measured every day during the treatment and it was seen that a large weight gain occurred in the 2 g treatment group, while the control and 8 g groups showed almost the same growth. The blood serum creatinine test was carried out on days 14, 24 and 34. After measuring for 3 times the average data for the control group was 0.74 mg/dL, the test groups were 0.74 mg/dL, 0.78 mg/dL, and 0.74 mg/dL respectively, where the creatinine levels produced are normal values. These results indicate that the administration of steeping Pasote water for a month has an effect on the weight growth of rats but does not show a significant effect on rat blood serum creatinine levels.***

Keywords: Creatinine, Pasote, *Dysphania*, Kidney, Wistar, Rat

1. Introduction

The wealth of thousands of islands, ethnicities, religions and the use of different natural resources has enriched the Indonesian state. The use of traditional herbal medicines is also diverse and during the COVID-19 pandemic their use is increasing (Pandiangan & Nainggolan. 2020), but this should be a warning to the public because there are no standards for the use of herbal medicines from a safety perspective and it is impossible to determine the right dose. also. Some herbal medicines contain toxic species, allergens and heavy metals that intentionally or unintentionally cause drug poisoning as the cause of side effects of these herbal plants (Hussin. 2007).

The quality of medicinal plants is directly related to their active compounds. This component refers to the secondary metabolites of plants. However, herbal remedies containing other compounds are often neglected and poorly understood, and as such are generally difficult to produce a curative effect. In addition, the risk in medical treatment is that any drug has side effects, and these side effects cannot be eliminated as part of the pharmacological activity of the drug (Capasso *et al.*, 2003) and symptoms that may arise as a side effect of pharmacological actions are disturbances kidney function.

Kidneys play an important role in removing harmful or toxic substances and maintaining the balance of fluids and other substances in the body (Aditya, 2018). Kidney disease or disorders can be monitored by checking changes in blood creatinine (Pranata, 2013; Fitriani *et al.*, 2019). Observation of serum creatinine concentration of laboratory animals or rats (*Rattus novergicus*) can be a parameter to determine the side effects of medicinal plants in humans. In Indonesia, there are many herbal plants, especially the people of North Sulawesi who use a lot of alternative medicine, one of which is *Dysphania ambrosioides* L or what is called Pasote by the people of North Sulawesi in the Minahasa section. Several studies have reported the use of Pasote as an antioxidant, anticancer, and antihyperlipidemic (Pandiangan *et al.*, 2020; Pandiangan *et al.*, 2018). Capso *et al.* (2003) reported that side effects are an integral part of pharmacology, and to date there have been no studies reporting related Pasote nephrotoxicity (D. ambrosioides) on renal creatinine levels. Based on the description above, research on the effect of

steeping water Dysphania ambrosioides Lon blood serum creatinine levels in vivo in rats (Rattus novergicus) can become new knowledge for the community so as to reduce public concern regarding the side effects that may arise due to consuming Pasote as an alternative herb.

2. Materials and Methods

The research was conducted at the Biovina Herbal Sea Mitra Laboratory, during January 2022 to June 2022.

Tools and Materials
The tools used in this research include: Biosystems BTS-350 semi-automatic, analyzer, Miyako brand kitchen blender, Tray, Dispo, Rat drink pacifier, Measuring cup, Drinking glass, Scissor, Tea bag, Stove, Kitchen pan, Mouse surgical table, Pot, Scalpel, Gloves, Blood serum (EDTA) tube, A place to eat Rat, Digital scales, Tissue, Jar, Tweezers, Ointment pot

The materials used in this study consisted of: Water, Pasote leaves (*Dysphania, ambrosioides* L.), Chloroform, Creatinine kinase reagent A, Creatinine kinase reagent B, B552 animal feed.

Methods

Sampling
The sample used was taken in the village of Noongan, Langowan District, Minahasa Regency. The samples taken were all parts of the Pasote plant

Sample Preparation
This procedure follows a modified Pandiangan *et al.*, (2018). 10 kilograms of fresh Pasote are taken and then cleaned and removed from the roots, then washed and dried without direct sunlight. After drying, the paste was weighed and roughly blended.

Furthermore, Pasote which has become coarse powder is put into a tea bag as much as 2 g/tea bag. The tea bags were divided into glasses according to the treatment, namely 1 bag for the 2 g treatment, 2 bags for the 4 g treatment, and 4 bags for the 8 g treatment. Each glass is given 100 mL of boiling water like how to make tea, then allowed to stand until the water turns slightly brown in color. The Pasote brew that has been finished is then allowed to cool enough to be put into a rat's drinking bottle.

Test Animal Preparation
The test animals used were Wistar rats (*Rattus norvegicus*), 2-3 months old, with an initial body weight of 140-185 gs, as many as 12 tails. Before the experiment was conducted, the Rat was acclimatized for 7 days at first. During the adaptation period, the rats were given water and food every day, namely feed from a mixture of corn and basic food and their body weight was weighed. Adapted rats were divided every 3 rats per treatment group.

Treatment of Test Animals
Rat that had finished the adaptation period were then given different treatments according to the test group. During the test period, every day the rats were given the same feed as during adaptation and their body weight was weighed. Tests on rats were carried out for 34 days ad libitum, with the distribution of test group as follows:

Table 1: Distribution of Rat Treatment Groups

Group Treatment			
Control	Treatment 1	Treatment 2	Treatment 3
Drinking Water/ Aquades (100 mL)	Given steeping Pasote 2 mg/100 mL	Given steeping Pasote 4 mg/100 mL	Given steeping Pasote 8 mg/100 mL

During the treatment, the steeping water given to the Rat was changed every day. Provision of steeped Pasote water for treatment groups 1, treatment 2 and treatment 3 was made as a substitute for drinking water for rats. During the treatment, the morphology of the rats was observed and weight was measured, to see the physical changes and growth of the rats.

Examination of Rat Blood Serum Creatinine Levels
Examination of rat blood serum creatinine levels was carried out using the Biosystems BTS-350 semi-automatic analyzer. The examination was carried out three times, namely on day 14, day 24 and day 34. Each examination took one rat from each treatment group. The examination procedure was carried out the same for three examinations. First, the Rat were weighed before surgery. The rats were then euthanized by placing them in a jar containing a tissue that had been poured with chloroform.

The Ratwere then dissected quickly before blood clots occurred. The blood taken from the heart of Rat because this part contains the most liquid blood. 3 mL of blood was taken using a dispo, then put into a serum blood tube. The same method was repeated for other Rat to be examined on the same day. The collected blood samples were then centrifuged at 3000-4000 rpm for 15 minutes to obtain blood serum. After that, 0.1 mL of blood serum was mixed with 1 mL of reagent A and 1 mL of creatinine kinase reagent B. After the blood serum and reagent samples were mixed, the samples were then tested in a spectrophotometer with a wavelength above 400 nanometers (visible) at 37°C. After waiting for a while, the results of the creatinine levels will be immediately visible on the spectrophotometer monitor, with the measured parameter being rat blood serum creatinine levels.

Data analysis
The data obtained were tabulated and analyzed by means of variance (Anova) followed by the BNT test at a 99% confidence level. Statistical calculations of the data were carried out using the SPSS program.

3. Results and Discussion

Sample Preparation into Stew
Sampling was carried out in Langowan, Minahasa. Samples are quite easy to find around residential areas, both those that grow wild or those that are intentionally planted by residents. In Langowan, residents use Pasote as a complementary ingredient for spices and vegetable mixtures.

Figure 2: The process of taking Pasote samples in the residents' yard (a), taking Pasote in the residents' gardens (b).

In the sample preparation process based on the modified Pandiangan *et al.*, (2018) guide, there were no obstacles found in making steeping. The sample which was originally taken as much as 10 Kg decreased in weight after drying, but it crumbled well when blended.

Figure 3: The process of making Pasote steeping. (a) weighing of Pasote powder to be packed in tea bags. (b) Pasote is ready to be brewed. (c) Pasote brewed with boiling water. (d) brewing Pasote in drinking teats given to Rat.

Pasote brewed water has a distinctive and strong odor that almost resembles the smell of mint and fragrant, with a bland to quite strong taste depending on how much Pasote is brewed. Pasote brewing water also differs in color depending on the amount of Pasote that is brewed. Steeping 2 g of Pasote is light yellow, steeping 4 g is yellowish brown, and steeping 8 g is dark brown like tea.

Rat Growth Observation

After acclimatization for 7 days, the body weight of the Rat was continuously monitored to see the growth of the Rat during the treatment period. The growth of Rat was determined by using data on the body weight of Rat. Measurement of body weight of rats was carried out before giving Pasote, and for 34 days giving Pasote water steeping. The growth of Rat can be seen in the Figure 4, the average weight of the following Rat

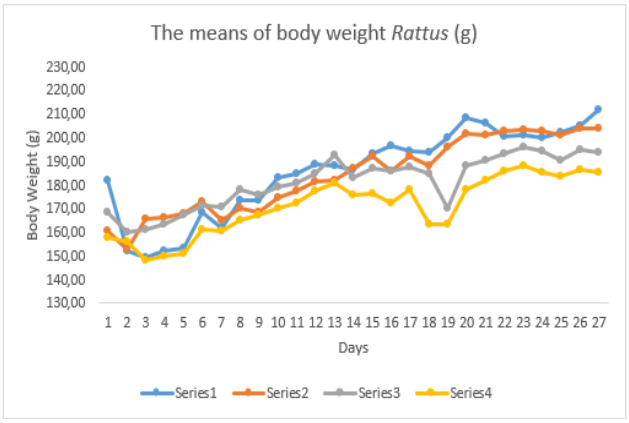

Figure 4: Graph of growth of Wistar rats (*Rattus novegicus*) by observing body weight (g) of rats for 27 days treated with Pasote (*Dyshania ambrosioides*) steeping water series 1 = control (Aquades), series 2 = 2 g, series 3 = 4 g, Series 4 = 8 g

Table 2: The results of the measurement of body weight of white rats (g) before and after being given treatment

Treatment	Initial Average Weight (g)	Final Average Weight (g)	Weight gain (g)
Control (Aquaes)	179 ± 6.05	212± 8.48	33
1 bag (2 g)	153± 8.08	204± 15.55	51
2 bag (4 g)	160.33± 17.55	194± 5.65	33.66
4 bag (8 g)	156± 16.37	185.5± 6.36	29.5

Body weight measurements were carried out in this study to see whether or not there was an effect of giving Pasote extract on weight gain in white rats. Data on the weight gain of white rats during this period can be seen in Table 2. Based on the weight data before the extract treatment (initial data) and after the extract treatment for 34 days (final data) there was a fairly high increase in body weight.

Figure 4: The appearance of the Rat that experienced the highest weight gain.

The results of body weight measurements of white rats (Table 2) showed that there was an increase in body weight of white rats. The highest increase in body weight was seen in white rats treated with a dose of 2 g/100 mL with a final body weight of 204 g (Figure 3) from an initial body weight of 153 g. This shows that the administration of steeping Pasote also has a good effect on the growth of rats in addition to feeding, as evidenced by an increase in body weight during treatment. Although the rats from each group experienced significant weight gain, it could be observed

that the rats remained agile and showed no signs of difficulty breathing or physical pain.

Results of Examination of Rat Blood Creatinine Levels

After being given Pasote tea steeps, the rats in each treatment group were checked for blood creatinine levels on day 12, day 24 and day 34. Each time, one rat was taken from each group to measure serum creatinine levels. Examination of serum creatinine levels was carried out by taking rat blood samples from the heart. The process of rat surgery was carried out as described in the research method.

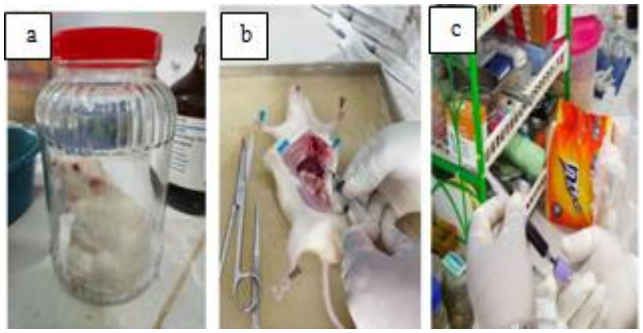

Figure 5: The surgical process of Rat. (a) rats in a jar containing a tissue containing chloroform. (b) rat surgery to take blood from the rat heart. (c) rat blood taken with dispo was put into the EDTA serum serum blood tube

After the rat blood was taken, it was then examined using the Biosystem BTS 350 semiautomatic Analyzer by centrifugation at a speed of 3000-4000 rpm for 15 minutes with a wavelength above 400 nanometers (visible) to obtain blood serum. The results of each examination are read automatically on the monitor and recorded as follows:

Table 3: Results of Measurement of Serum Creatinine Levels (mg/dL)

Measurement Day	Control	1 bag (2 g)	2 bag (4 g)	4 bag (8 g)
14	0,92	0,91	0,72	0,81
24	0,61	0,62	0,71	0,70
34	0,70	0,80	0,80	0,70

On day 14, the serum creatinine levels of the four groups showed that there was no significant difference between the results of the control group and the 2 g treatment group, as shown in the following Figure 6 below this.

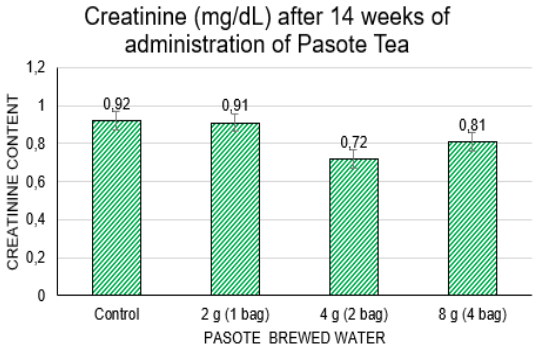

Figure 6: Figure of the results of measuring rat serum creatinine levels on day 14

On day 24, the control group and the 2 gtreatment group actually experienced a decrease in serum creatinine levels by 0.61 mg/dL and 0.62 mg/dL:

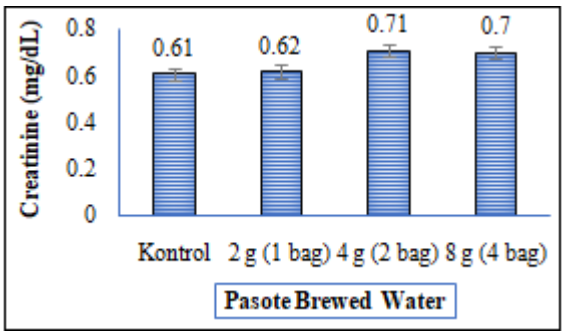

Figure 7: Figure of the results of measuring rat serum creatinine levels on day 24.

After measuring on day 34, it can be seen that each treatment group showed creatinine levels with almost the same value, not significantly different as shown in the following Figure:

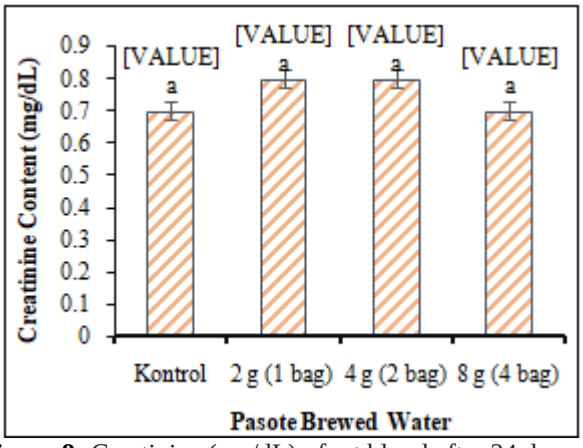

Figure 8: Creatinine (mg/dL) of rat blood after 34 days of drinking Pasote brewed tea

The measurement results on the 14th, 24th and 34th days were then averaged and it was seen that the four treatment groups showed no significant difference in serum creatinine levels in the following Figure:

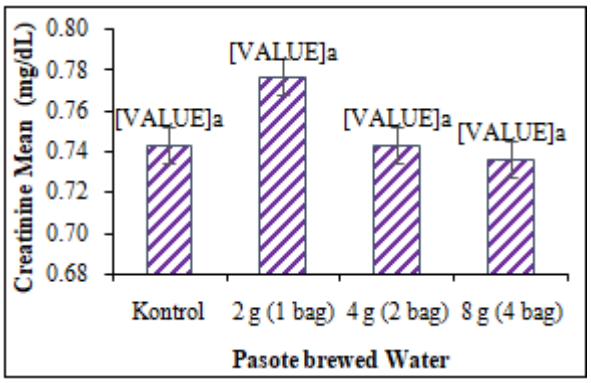

Figure 9: The results of the measurement of the average serum creatinine level after measurement on days 14, 24 and 34

The results of the measurement of serum creatinine levels were then analyzed using Anova variance and the following

results were obtained. Analysis of Variance was carried out using the SPSS Program at a 95% confidence level with the smallest Significant Difference further test.

Tests of between- Subject Effect
Dependent: Variable: Hasil

Source	Type III Sum of Square	Df	Mean Square	F	Sig.
Corrected Method	.068[a]	5	0.14	1.941	.221
Intercept	6.750	1	6.750	967.357	.000
Perlakuan	.003	3	.001	.140	.932
Kelompok	0.65	2	0.32	4.643	0.60
Eror	0.42	6	0.007		
Total	6.860	12			
Corrected Total	.110	11			

a. R Squared = .618 (Adjusted R Squared= .300)

From the results obtained, it can be seen that the value of each treatment group did not show a significant difference. Serum creatinine levels in rats showed values that were within normal limits. Previous research by Pandiangan et al (2019) stated that Pasote contains secondary metabolism, namely flavonoids which have high antioxidant activity. The antioxidant activity of Pasote is thought to reduce blood serum creatinine levels in rats. Morphological observations of Rat also showed that the Rat looked healthy and active. With these results, Pasote can be recommended as a functional drink to treat kidney disorders because it has been proven that there is no harmful effect on rat blood serum creatinine levels after administration of Pasote for 34 days. Pasote can also be recommended as a health drink because of its antioxidant levels which are good for the body.

4. Conclusion

Administration of Pasote for 34 days did not have a significant side effect on the kidneys of rats, it actually decreased serum creatinine levels and was good for growth, so it can be recommended for functional drinks.

Acknowledgments

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Author Profile

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