Acute toxicity test and lethal dose (LD_{50}) from a combination of sambiloto extracts *(Andrographis paniculate)* and catfish oil *(Pangasius micronema* Blkr.*)* in mice

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Abstract. Sambiloto (*Andrographis paniculata*) contains andrographolide compounds, which have anti-inflammatory properties. On the other hand, catfish oil (*Pangasius micronema* Blkr.) contains DHA and EPA compounds known to be unsaturated fatty acids and have anti-inflammatory properties. Sambiloto extracts and catfish oil will be combined to determine the pharmacological effects caused by an acute toxicity test to determine the toxicity and LD_{50} value of the combination of extracts. This research is conducted experimentally, utilizing mice as test animals. The extract was administered for 14 days, and symptoms of toxicity were observed, including the number of animals that died, body weight, and the weight of the liver, kidneys, and heart. The data was then tested using the Thompson-Weil method and statistical tests using the one-way ANOVA test and continued with the Tukey post hoc test. The toxicity test results indicated toxicity symptoms at the highest dose, but the combination of extracts' LD_{50} value was pseudo-LD50, implying that test animals could theoretically still receive the highest dose value. The statistical analysis revealed no significant differences in the body and organ weight of the mice, indicating that this research could still use the combination of the two extracts up to the highest dose.

1 Introduction

Sambiloto (*Andrographis paniculata*) is a plant commonly found in tropical and subtropical areas and widely used as medicine. Sambiloto is known to contain andrographolide, which is known to act as an anti-inflammatory compound [1]. Apart from Sambiloto, catfish (*Pangasius micronema* Blkr.) is a type of fish that is low in fat, high in protein, and has polyunsaturated fatty acids such as DHA and EPA, which are also known to have beneficial effects. When consumed [1-2], it is anti-inflammatory.

Using more than one type of compound in combination should have a more significant effect than using just one type [3]. However, there is currently no supporting data regarding the pharmacological effects and safety level of the combination of the two ingredients when consumed. As a result, it is necessary to conduct further research on test animals to

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determine whether there are toxic effects to increase the product's safety and efficacy. The results of this study are expected to provide crucial information regarding acute toxicity and the values of the LD50 combination of bitter extract and catfish oil on test animals. This toxicity test aims to observe whether the pharmacological activity of a compound occurs within a short time after administration of a certain dose [4]. The findings of this research could potentially contribute to the development of safer and more effective pharmaceutical products.

Acute toxicity test is a way to detect toxic effects that appear within a short time after administering a substance in a single dose or repeated doses given within 24 hours [5]. Based on this test, qualitative data will be obtained in the form of toxicity symptoms that can be observed. Clinical symptoms resulting from administering the prescribed dose include tremors, heart rate conditions, coat condition, activity and behavior patterns, digestive system, weakness, seizures, and breathing slowing down [6]. In addition, to state the acute toxicity of a material, it can be determined by calculating the LD_{50}. Lethal Dose, is a dose that statistically can kill 50% of experimental animals. This is determined by giving the drug in varying or graded doses to a group of experimental animals, and each animal is given a single dose [7].

2 Materials and method

2.1 Materials

The materials used in the study include sambiloto leaves, catfish from the local market in Surakarta city, Central Java, 96% ethanol pharmaceutical grade repacking by PT. Nathin-Naturafit, Sragen, distilled water repacking by Arkan Medika Raya, Solo, corn oil (Tropicana Slim), Na-CMC powder repacking by Saba Kimia, Solo, and bentonite.

2.2 Research methods

2.2.1 Preparation of sambiloto extract

The sambiloto extract was obtained through a meticulous maceration method extraction process for 3 x 24 hours using 96% ethanol solvent with stirring every 24 hours. After 3 days of maceration, the sample was then filtered and concentrated using a hotplate until a thick extract was obtained. The macerate was then evaporated on a hotplate to obtain a thick extract which is then calculated the yield. The yield obtained was 6.08%. This careful and precise process ensures the reliability of the results.

2.2.2 Preparation of catfish oil

Catfish oil is produced using the wet processing method. The fish is cleaned, and the meat is separated from the bones. The fish meat is steamed using the double-jacketed method at 80°C for 1 hour. The fish oil on the top layer is collected, and the remaining pulp is filtered to extract the oil. The oil from both procedures was combined, and then 1% bentonite was added to act as a separator for the oil and contaminants, which were then separated.

2.2.3 Dilution of sample

The sample is diluted using two different materials, where sambiloto extract will be diluted using 0.25% Na-CMC solution while catfish oil will be diluted using corn oil.

2.2.4 Test animal preparation

The mice we used for the study were white Wistar strain mice.The test animals were divided into four groups, each with five animals. The test animals were acclimatized for one week while the feed frequency (BR1) and water consumption were controlled. Test animals' body weight was weighed weekly to monitor growth, health, and preparedness.

2.2.5 Acute toxicity test

All research protocols have been approved by the research ethics committee of Dr. Moewardi Surakarta Hospital, with number 1.996/XI/HREC/2023. In this study, 20 male mice strain Wistar 2-3 months old with a body weight of 20-35 grams were divided into 4 treatment groups, each of 5 male mice that were determined randomly.

- Control group: given aquadest
- Group 1: given a combination of 200mg/kg sambiloto extract and 500 mg/kg catfish oil.
- Group 2: given a combination of 400mg/kg sambiloto extract and 1000 mg/kg catfish oil.
- Group 3: given a combination of 800mg/kg sambiloto extract and 2000 mg/kg catfish oil

The combination of extracts was administered using an oral probe.

2.2.6 Determination of LD_{50}

The determination of the LD_{50} value in this study was carried out by Thomson and Weil method where the formula used is:

$$Log\ m = log\ D + d(f + 1) \quad (1)$$

where m : LD_{50} price, D : smallest dose used, d : log r (dose multiple), f : factor.
2.3 The Data Analysis

The body and organ weights between groups were analyzed using the SPSS program (Statistical Product and Service Solution) with a one-way ANOVA test, given that the data was normally distributed, the variances were homogeneous, and the samples were independent.

3 Result and discussion

This toxicity test research was carried out by looking at the clinical symptoms and toxic effects of the sambiloto extract combination *(Andrographic paniculata)* and catfish oil *(Pangasianodon hypophthalmus)* which can be seen from the LD_{50} value. Acute toxicity test observations were carried out for 14 days by observing symptoms of toxicity once a day [8].

Mice were also chosen because their anatomical and physiological structures are similar to human anatomical and physiological structures [8]. The mice used were male because they were not influenced by hormones. Before being given treatment, the mice were acclimatized for 21 days to adapt to the new environment and gain weight because when they arrived from the farm, they still weighed 15 grams. During the acclimatization period, mice were given pellets to eat and drink and placed in cages with a temperature of 23˚C. In this test, male mice were given the test material orally using a probe. The oral route was chosen because it is adapted to the route usually used in humans to consume a combination of sambiloto extract and catfish oil.

3.1 Toxicity observations

Observations for signs of toxicity were carried out every 30 minutes after administering the extract for four hours. After 24 hours and 48 hours of testing, one of the animals did not die. Based on Table 1.

Table 1. Toxicity observations.

Groups	Number of mice	Sambiloto dose (mg/kg BW)	Catfish oli dose (mg/kg BW)	Number of death
Control	5	0	0	0
Group I	5	200	500	0
Group II	5	400	1000	0
Group III	5	800	2000	0

The table presents the results of observations of LD_{50} values in mice that had been given a combination of sambiloto extract and catfish oil, there was no mortality rate at the three dose levels given. In the absence of a death rate for test animals, it shows that the f factor, namely the factor obtained from the Thompson and Weil table, is not obtained so that the LD_{50} value can not be calculated. By the criteria for acute toxicity tests carried out to assess LD_{50} based on previous research, if the maximum dose given does not cause the death of test animals, then the LD_{50} value declared false or not the real thing [7].

Table 2. Parameters observation.

Parameters	Observation in			
	Control	Group 1	Group 2	Group 3
Eyes	-	-	+	++
Digestive Tract	-	-	+	+
Neural Activity and Patterns Behavior	-	+	+	++
Cardio-vascular	-	+	+	++
Seizure	-	-	-	+
Tremors	-	-	-	++
Allergies (Itchy)	-	+	++	++
Fur	-	+	++	++

Apart from looking at the toxic effects caused by the death of test animals, in this study, the parameters used were observing abnormal symptoms. Observations for abnormal symptoms were conducted every 30 minutes after the extract was administered for 4 hours. During observations, it was found that test animals given the combination of extracts had abnormal symptoms. Observations of abnormal symptoms after dosing were mainly seen in group three, which had the highest dose. Table 2. shows that the abnormal conditions felt by the mice 30 minutes after administering the combination of extracts were weak and watery eyes, abnormal nervous activity and behavioral patterns, a fast heartbeat, and

allergies (itching) which was shown when the mice's hands tried to lick. the remaining combination of extracts, and the hair that stands up after treatment. After 24 hours, the abnormal symptoms that could be seen were the heart still beating fast and the mice's bodies trembling.

The condition of the eyes is weak, or called ptosis, which occurs due to a decrease in motor activity in test animals [9]. Besides the eyes becoming weak, excessive tear production influences the autonomic nervous system [10]. Symptoms of abnormalities in the form of abnormal nerve activity and behavioral patterns occur because mice experience increased pain. These symptoms may be caused by depression of the central nervous system [10]. The ability of mice to lick their body parts with an increased frequency than usual indicates that mice experience increased pain. These symptoms may be caused by depression of the central nervous system [11]. The condition of the hair standing up can occur because the test animal is tense. The condition of the hair standing up called piloerection is controlled by the sympathetic nerve which functions as a temperature regulator [12]. Tremor is a condition where vibration occurs in test animals. Mice experiencing tremors after administration of the extract could occur due to the toxic effect of the test extract, which causes interference with the central nervous system in controlling muscle contractions, resulting in twitching or vibrations in the muscles [13].

3.2 Weight measurement

In this test, body and organ weight observations were carried out on the test animals. The mice's body weight was observed before being given the treatment, on the 7th day after being given the treatment, and on the 14th day after being given the preparation. Body weight data was obtained from the average body weight of 5 mice in each group. Observations of the mice's body weight can be seen in Table 3.

Table 3. Average animal body weight.

Groups	Mean (gram) ± Standard Deviation		
	Day 0	Day 7th	Day 14th
Control	27.30±1.49	27.92±1.63	30.38±1.07
Group 1	27.82±2.81	28.88±4.93	26.00±1.93
Group 2	27.92±2.01	28.76±3.47	28.56±2.71
Group 3	27.92±2.79	27.7±5.18	29.46±5.68

Based on the average value of body weight, it was seen that weight gain only occurred in the control group. From the average body weight values obtained, the first group that was given a combination of 200 mg/kgBW sambiloto extract and 500 mg/kgBW catfish oil experienced a decrease on the 14th day. In the second group, which was given a combination of 400 mg/kgBW sambiloto extract and 1000 mg/kgBW catfish oil, experienced a decrease on the 14th day. In the third group, which was given a combination of 800 mg/kgBW sambiloto extract and 2000 mg/kgBW catfish oil, experienced a decrease on the 7th day and increased again on the 14th day.

Observation of the body weight of the test animals was carried out for 14 days to determine the condition of the test animals after administration of the extract combination. Conditions that indicate an animal is sick are generally when body weight has decreased by more than 20% over 7 days or more. Test animals that experience fluctuating changes in body weight indicate illness or suffering after administration of the extract [7]. In this study, the weight loss in groups 1, 2, and 3 was a weight loss that did not indicate sick animals because the weight loss was still more than 20% of the initial weight. Several factors can cause changes in weight gain or loss. These factors include the content of

compounds in the treatment, internal factors, genes which are determining factors for traits inherited from the parent that regulate activities in the body, as well as external factors such as food, sunlight, activity, temperature, and the environment [8].

After 14 days, our analysis indicated that there was no significant difference in the body weight of the mice when comparing their weights before and after the treatment with the combination of extracts (p value ≥ 0.05), suggesting that the extracts did not have a measurable impact on the body weight of the mice over the duration of the study.

3.3 Organ weight measurement

On the 15th day, the test animals were dissected. In this surgery, mice's livers, kidneys, and hearts were taken to be weighed and macroscopically observed. Observations on the liver, heart, and kidneys were carried out because the liver is the largest and most metabolically complex organ in the body. This organ is involved in metabolizing nutrients and most drugs and toxicants. Observation data on mouse organs can be seen in Table 2.

Table 4. Average organ weight of mice

Group	Mean (gram) ± Standard Deviation		
	Liver	**Kidney**	**Heart**
Control	1.39±0.16	0.16±0.39	0.13±0.02
Group 1	1.14±0.25	0.14±0.05	0.12±0.04
Group II	1.54±0.35	0.18±0.03	0.14±0.02
Group III	1.24±0.23	0.15±0.03	0.11±0.06
Macroscopic observation	Shaped like wattle with dark red coloured	Shaped like nuts, coloured dark red	Shaped like seeds coloured dark red

According to the results, the average weight of the liver, heart, and kidney organs in all test groups of mice decreased. Figure 1 shows observations of each organ.

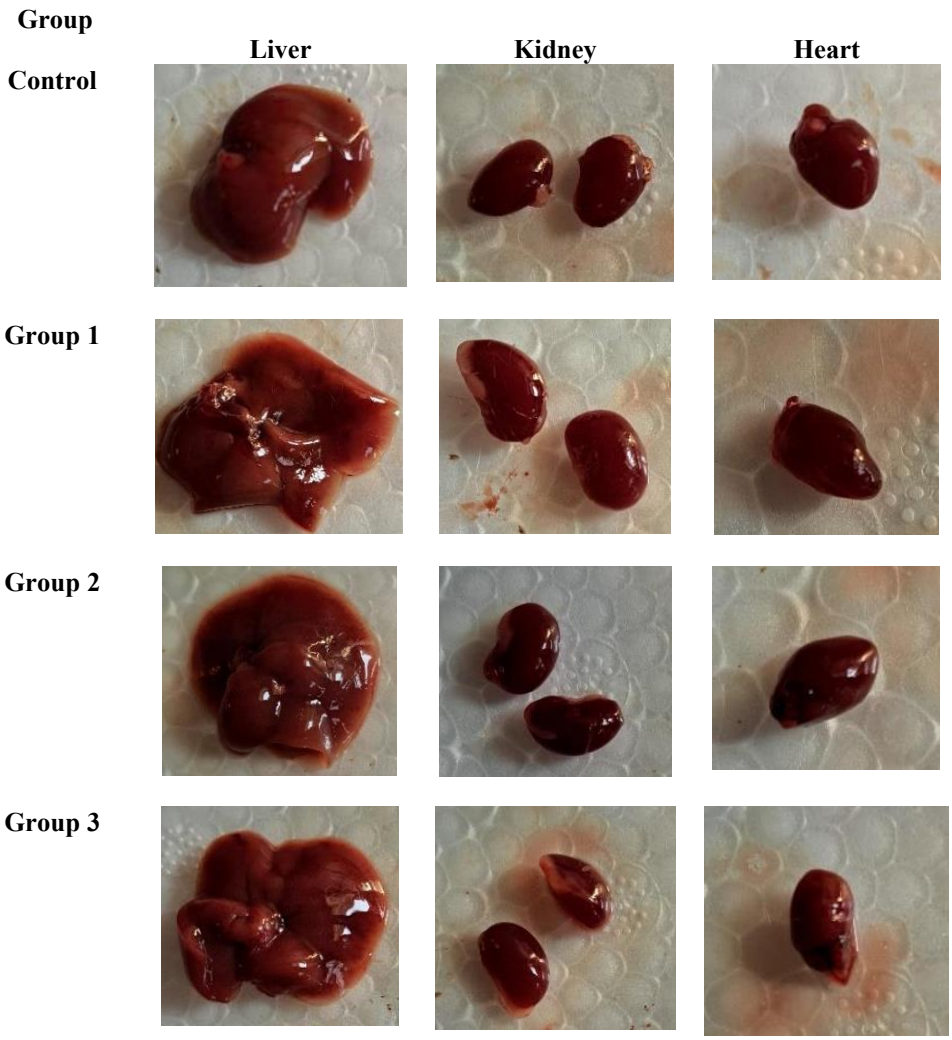

Fig. 1. Macroscopic organs after treatment.

Based on the table of average organ weights, the liver in group 1 has the lowest weight among the livers in other groups. This happened because one of the mice had the smallest liver weight among the 4 others in that group. The liver is an organ that can recover from enormous cell damage. Rodent liver weights vary. However, it is usually in the range of 2-3 grams in mice. Based on the data that has been obtained, the mice's livers weigh less than 2 grams. Based on macroscopic observations, the mice's livers are dark red like a wattle. The kidneys are the body's main excretory organ, removing metabolic waste substances that are no longer used and toxins. The kidneys of healthy mice weigh between 0.16 – 0.22 grams [14]. Based on the data that has been obtained, the kidneys in mice are between 0.16 grams and 0.184 grams. The heart is a vital organ that functions as a blood pump to meet the nutritional needs of oxygen and nutrients throughout the body. A normal heart has a brownish-red color with a smooth surface, while an abnormal heart will experience color changes and have a mottled surface [15]. Based on the data that has been obtained, the mouse heart weighs 0.123 to 1.37 grams.

This data found that $P < 0.05$, so the data was not homogeneous but the three organ weights were normally distributed. There was a significant difference in the organ weight data of mice. This shows no significant change in the liver, kidney, or heart weight compared to normal control organs. This study can be used for further research to determine the level of toxicity of the combination of Sambiloto leaf extract and catfish oil by conducting quantitative research through histopathological testing of mice organs to achieve a comprehensive assessment.

4 Conclusion

Based on the research that has been carried out, it can be concluded that toxic symptoms were caused after administering a combination of sambiloto extract and catfish oil at a dose of 200 mg/kgBW of sambiloto and 500 mg/kgBW of catfish oil, a dose of 400 mg/kgBW of sambiloto and 1000 mg/kgBW of catfish oil. As well as doses of 800 mg/kgBW of sambiloto and 2000 mg/kgBW of catfish oil, showed symptoms of toxicity in the form of eye disorders, disturbances in nervous activity and behavior, cardiovascular disorders, tremors, allergies, and disorders of the fur and skin. The LD50 value of the combination of sambiloto extracts and catfish oil is classified as pseudo-LD50, which means that the highest dose value can technically still be given to test animals.

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