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IN VIVO ACUTE TOXICITY OF ALOIN EXTRACT ON MALE WHITE MICE (Mus musculus)

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Abstract

Motivation/Background: Aloin has many benefits potential as a medicine. However, it is necessary to carry out toxicity tests on experimental animals as a pre-screening test. Toxic effect parameters were seen from changes in body weight, tremors, convulsions, salivation, weakness, walking backward, walking on the stomach, death, and levels of SGOT and SGPT in experimental animals. This study aims to evaluate the in vivo acute aloin toxicity test results in mice (Mus Musculus) regarding potential toxic symptoms and animal death. Method a sample of 27 mice were separated into three groups for this investigation: the control group, the treatment group I at a dose of 2500 µg/kg BW, and the treatment group II at an amount of 5000 µg/kg BW, and 5 mice in the preliminary test. This study employed a laboratory experimental test method using a pre-post control group design study. The results is based on observations of experimental animals, it concluded neither the preliminary test nor the toxicity test revealed any toxic signs. Neither the initial test nor the toxicity test resulted in any observed animal fatalities. Before and after aloin administration, changes in body weight were significantly different. Treatment group II, treatment group I, and the control group's SGOT levels were classified from highest to lowest, with subsequent values of 22.4 U/L, 21.9 U/L, and 19.9 U/L. The highest value of 35.7 U/L, 32.1 U/L, and 26.2 U/L in the treatment groups II, I, and the control group was used to rank the SGPT levels. The conclusions there were no toxic symptoms and no mortality in experimental animals, so it can be concluded that aloin did not cause harmful effects in mice, so it was categorized as a pseudo-lethal dose.

Keywords : Toxicity test; lethal dose: toxic effect; acute toxicity

1. Introduction

Aloin or commercially known as barbaloin is one of the main components of phenolic compounds found in Aloe Vera. The aloin content in Aloe Vera is 0.099 mg - 3.1 mg per 100 g in fresh condition. The molecular weight of aloin is 418.39 g/mol with the molecular formula $C_{21}H_{22}O_9$. Aloin is found in the yellow layer located between the skin and flesh of Aloe Vera. Aloin can be obtained by extracting aloe vera plants (Aloe Vera). Gel extraction and processing of aloe vera plants must be optimized properly. This aims to avoid changes in the composition of nutrients that can change the physiological properties of the aloe vera plant.(1-3)

Aloin's properties as an antioxidant have potential benefits, as it has a higher inhibition rate than the standard inhibition rate of vitamin C. Aloin in the pharmaceutical world is also useful for relieving constipation and intestinal wall injuries, improving gastric work and suppressing the population of intestinal microorganisms, and regulating gastric acidity. However, if there is a large amount of aloin in food, it can cause side effects in the form of obstacles to the reabsorption of water and electrolytes and can cause irritation to the mucous membranes. In addition, a high enough aloin content in food can cause irritation of the digestive tract and stomach cramps. This proves that aloin has potential as a drug, but there are several things that need to be reviewed from the side effects that aloin can cause.(4-7)

Traditional medicines consumed must be scientifically proven for their efficacy and safety of use in humans. The stages of developing traditional medicines into phytopharmaceuticals begin with selection, preclinical tests (toxicity and pharmacodynamics

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tests), standardized preparations, and clinical trials. Recently, many studies have been conducted on plants whose chemical content is thought to be efficacious and potentially medicinal. However, although these ingredients do have good potential, any material used in humans must not be harmful or have no toxic effects. Moreover, if the material has the aim to be used as an alternative treatment that is curative and preventive. Tests that can be done to measure the toxic properties of an ingredient to cells are through toxicity tests.(8-10)

Toxicity test is a test to observe the pharmacological activity of a compound that occurs in a short time after exposure or administration in a certain dose. The purpose of the toxicity test is to determine the toxic effect produced by a single dose of a mixture of chemicals on experimental animals as a pre-screening test. The principle of toxicity testing is that bioactive components always become more toxic when given at high doses and will become drugs at low doses. The acute toxicity test is carried out in a short time, namely 24 hours after oral administration of the test preparation in a single dose. Then the presence or absence of toxic effects or death is observed. Observations made can last for 7-14 days after treatment. The quantitative benchmark that is often used to express a lethal or toxic dose is the lethal dose 50 (LD50).(9,11-13)

LD50 determination is the initial stage to determine the safety level of materials that will be used by humans by determining the amount of dose that causes 50% death in test animals after a single dose. There are several commonly used LD50 calculation methods, including the Thompson and Weil method. Determination of the LD50 value with this method uses a table made by Thompson and Weil. The experiment must meet several requirements, namely: the number of test animals must be the same in each treatment group, the dose interval is a fixed multiple, and the number of treatment groups is at least 4 groups.(11,14,15)

This study was conducted in vivo, using mice (Mus Musculus) as experimental animals given a single exposure to graded doses. Observations made include the number of animals that die and clinical symptoms that occur in the first 30 minutes and 24 hours after the administration of aloin, and observed for 14 days after the administration of aloin extract. Then the observation continued by looking at the levels of SGOT and SGPT mice. The selection of aloin in this study is because of its content which is commonly used as ingredients for phytopharmaceutical preparations (11,16-18).

Until now, research on the acute toxicity test of aloin in vivo in mice (Mus Musculus) has not been known. Therefore, researchers feel it is important to conduct an aloin toxicity test conducted in vivo in mice. Parameters of toxic effects are seen from changes in body weight, tremors, convulsions, salivation, weakness, backward walking, walking on the stomach, death, and SGOT and SGPT levels of test animals. Researchers hope that the results of this study can be a reference to determine the safety of its use in mice, prevent toxicity or poisoning, can be a source of information for similar studies, and can obtain scientific information if it will be developed into a standardized herbal medicine preparation or phytopharmacaegative correlation.

2. Methods

This research is a pre-posttest control group design research, namely data processing is carried out before and after research treatment. The data taken are changes in body weight, toxic symptoms, death, LD 50 value of aloin, aloin toxicity, SGOT and SGPT levels. This study used observational research, namely test treatment on 5 groups of mice: a. Group I was given aloin at a dose of 1000 µg/kg BW

b. Group II was given aloin at a dose of 2000 µg/kg BW
c. Group III was given aloin at a dose of 3000 µg/kg BB
d. Group IV was given aloin at a dose of 4000 µg / kg BB
e. Group V was given aloin at a dose of 5000 µg / kg BB

Observation of clinical symptoms and mortality in the first 30 minutes and 24 hours after aloin administration. Observations were continued once a day for 14 days after treatment. If

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there is a death of experimental animals, the dose limit that will be used in the toxicity test is determined. If there is no death of experimental animals, SGOT and SGPT levels are examined at the end of the observation period.

The research sample used male white mice (Mus musculus). The inclusion criteria for male white mice used are mice aged six to 8 weeks with a body weight of 20-40gr. Male white mice are healthy and active during the treatment given. The exclusion criteria in this study are if the male white mice experience disability during the treatment given. The sample amounted to 27 mice which were divided into 3 groups. Each group amounted to 9 mice. The division of research groups is as follows:

- Negative control group (K-): mice are given distilled water
- Treatment Group (P1): mice were given aloin at a dose of 2500 µg/kg BW
- Treatment Group (P2): mice were given aloin at a dose of 5000 µg / kg BW

Male white mice were acclimatized for 1 week, then treatment according to the group began to be given. Taking research indicators begins with the observation of clinical toxic symptoms and death in the first 30 minutes and 24 hours after aloin administration. Observations were continued by monitoring once a day for 14 days after treatment. The body weight of the experimental animals was evaluated twice, namely before treatment and after treatment. Then observations were made of toxic symptoms and death for 14 days. Toxic symptoms that appear are tremors, convulsions, salivation, weakness, backward walking, and walking using the stomach. SGOT and SGPT levels were checked at the end of the observation period, i.e. after 14 days. Measurement of blood serum SGPT and SGOT activity was carried out using the principles of the kinetic method established by the International Federation of Chemical Chemistry (IFCC) using a UV-Vis spectrophotometer.

The data obtained from each indicator was processed using normality test and significance test, conducted by repeated measure test followed by Bonferonni post-hoc test.

The hypotheses in this study are:

- H0: There is no toxic effect on male white mice (Mus musculus) with acute toxicity test.
- H1: There is a toxic effect on male white mice (Mus musculus) with acute toxicity test.

3.Results And Discussions

This study was conducted by conducting preliminary tests and continued by conducting the main test, namely the toxicity test. The preliminary test aims to determine the toxic limit of aloin in mice. The results obtained from the preliminary test were that no mice died. Based on the results of the study, it is known that aloin does not cause toxic effects in mice so it is categorized into a pseudo lethal dose. Then proceed to conduct a toxicity test which aims to see the subtoxic symptoms experienced by mice. The subtoxic symptom parameters are seen from the SGOT and SGPT levels.

Qualitative Toxic Symptoms

Qualitative observations include several toxic symptoms that attack the central nervous system. The observations showed no toxic symptoms that can be seen in table

Table 1. Results of Observation of Toxic Symptoms in Preliminary Test

Group						
P1					-	-
P2	-	-	-	-	-	-
P3	-	-	-	-	-	-
P4	-	-	-	-	-	-
	Tremor	Seizures	salivation	Weakness	Way Back	Abdominal Walking

	-	-	-	-		
P5	-	-	-	-	-	-

Description: P1 = dose of 1000 µg/kg BW; P2 = dose of 2000 µg/kg BW; P3 = dose of 3000 µg/kg BW; P4 = dose of 4000 µg/kg BW; P5 = dose of 5000 µg/kg BW; (-) = no symptoms; (+) = showing symptoms.

Acute toxicity evaluation is not only about LD50, but also behavioral abnormalities., stimulation and motor activity of the test animals to get an idea of the cause of death. After conducting preliminary tests, then proceed with conducting toxicity tests. In the three observation groups, no toxic symptoms were found. The results of observations in the toxicity test can be seen in Table 2.

Table 2: Results of Observations of Toxic Symptoms in Toxicity Tests

Group	Tremor	Seizures	Salivation	Weakness	Way Back	Abdominal Walking
K	-	-	-	-	-	-
P1	-	-	-	-	-	-
P2	-	-	-	-	-	-

Description: K = Control; P1 = dose of 2500 µg/kg BW; P2 = dose of 5000 µg/kg BW; (-) = no symptoms; (+) = symptoms.

Number of Animal Deaths

Animal mortality was observed in the preliminary test group followed by the research group. In the preliminary test there were no deaths of experimental animals. Likewise, when the research group test was conducted. Based on this, the toxicity test will be continued. Animal mortality can be seen in tables 3 and 4.

Table 3. Number of mice that died in the preliminary test

Group	Number of Mice	Number of Deaths in 24 Hours	Number of Deaths in 14 Days
P1	1	-	-
P2	1	-	-
P3	1	-	-
P4	1	-	-
P5	1	-	-

Description: P1 = dose of 1000 µg/kg bw; P2 = dose of 2000 µg/kg bw; P3 = dose of 3000 µg/kg bw; P4 = dose of 4000 µg/kg bw; P5 = dose of 5000 µg/kg bw; (-) = did not show any death.

Table 4. Number of mice that died in toxicity test

Group	Number of Mice	Number of Deaths in 24 Hours	of Deaths in 14 Days
K	9	-	-
P1	9	-	-
P2	9	-	-

Description: K = Control; P1 = dose of 2500 µg/kg BW; P2 = dose of 5000 µg/kg BW; (-) = does not show any mortality

If the maximum dose does not cause death, then the LD50 is declared as pseudo LD50 (pseudo lethal dose).(11,14) In this study the dose of 5000 µg/kg BW was declared as a pseudo

lethal dose because it was the maximum dose that did not cause death in experimental animals.

Body Weight Observation Results

In this study, the average body weight of mice increased during the study. No mice were found to be lazy to eat or inactive mice. The increase in mice body weight can be seen in table 5

Table 5 Average Weight Results of Each Group of Mice

Research Group	Before Treatment	After Treatment
K	30,50 ± 1,13	37,50 ± 1,13
P1	30,33 ± 0,97	35,33 ± 1,48
P2	30,50 ± 1,23	33,50 ± 1,13

Data from weighing the body weight of mice after treatment in each group were then analyzed statistically. The first test performed was the normality test using Shapiro-Wilk with the results of normally distributed data (p>0.05).

The test used to compare the body weight of mice before and after being given aloin is the Mann-Whitney U test with the results of p <0.05. This means that it can be concluded that there is a significant increase in body weight in the three research groups. These results are shown in table 6.

Results of Significance Value between Body Weight Before and After Given Aloin in Each Group

Signifikansi		
K	P1	P2
0.004	0.004	0.009

In this study, there was no weight loss, which is consistent with the absence of toxic symptoms in experimental animals.

SGOT levels

SGOT levels in the highest group of experimental animals were obtained in group P2, namely the group with a dose of 5000 µg/kg BW aloin. SGOT data is shown in table 7.

Table 7. SGOT levels of mice (Mus Musculus) post treatment

Group	Mice SGPT Level (U/L)
	Mean ± Std. Deviasi
K	20,8 ± 5,004
P1	21,9 ± 3,483
P2	22,5 ± 3,344

The data was then tested for normality with the results of normally distributed data (p>0.05). Then the analysis continued with the One Way ANOVA method with the result of p=0.630 (p>0.05). This means that there is no significant difference in SGOT levels between the control, treatment I, and treatment II groups.

This is related to liver damage that causes this enzyme to be released into the blood. SGOT values that are slightly above normal do not always indicate liver damage. It is suspected that not all increases in SGOT are the result of liver damage. SGOT levels can depend on how the blood is taken, the amount of blood serum obtained, the length of time the blood serum is stored before being examined, and the age of the experimental animal.(5,10,16) In this study, the limitation is that it does not pretest first, so it cannot be known with certainty how the condition of the SGOT value of mice before treatment.

SGPT levels

The highest SGPT levels were found in the treatment group II dose of 5000 µg/kg BW. This increase is in line with the increase in SGOT although statistically there is no statistical significance of the difference in SGOT values between groups. SGPT results are shown in table 8.

Table 8. SGPT levels of mice (Musculus)

	Mice SGPT Level (U/L)
	Mean ± Std. Deviasi
Group	
K	27,9 ± 3,810
P1	32,1 ± 8,343
P2	35,7 ± 2,711

The statistical test step carried out is to find the normality of the data distribution, with the results of normally distributed data (p>0.05). Then proceed with analyzing the data using the One Way ANOVA method with the result of p=0.008 (p.<0.05). Based on this, it can be concluded that there is a significant difference in the SGPT levels of mice between the control, treatment I, and treatment II groups.

The main function of SGOT and SGPT enzymes is as biomarkers or markers of liver disorders. However, the difference in SGOT and SGPT levels between the test group and the control group is not an indication of liver damage if it is still within the normal range in mice. If there is severe cell damage, there will be an increase in SGPT and SGOT levels simultaneously up to double even up to 20-100 times the normal level (19,20).

CONCLUSIONS

From the experimental results, it was revealed that the administration of aloin in doses up to 5000 µg/kg BW to experimental animals showed no significant toxic symptoms or incidence of death. This finding provides a strong basis to conclude that aloin does not produce significant toxic effects in mice and can be classified into the high quasi lethal dose category. This indicates that aloin may have a favorable safety profile in the mice organism within the dose range tested, providing potential implications for its use in various pharmacological and toxicological contexts that require dose evaluation

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