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Acute Toxicity of Pulai Stem Bark (*Alstonia scholaris* (L.) R. Br.) Extract in Female Wistar Rats

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ABSTRACT

Pulai plants (*Alstonia scholaris* (L.) R. Br.) are often found and easily available in Kalimantan. Empirically it is used for traditional medicine, but data on the safety of Pulai plants is still limited. This study aims to determine the acute toxicity analysis of the ethanol extract of Pulai stem bark (*Alstonia scholaris* (L.) R. Br.) on clinical conditions, LD50, and blood cell components in female Wistar rats. Pulai stem bark was extracted by the maceration method using 70% ethanol solvent. acute toxicity testing based on the guidelines of the Indonesian Food and Drug Supervisory Agency (BPOM). The test animals consisted of six groups, namely the 5000 mg dose group, the 3500 mg dose group, the 2500 mg dose group, the 1500 mg dose group, the 500 mg dose group, and the control group. Observation of clinical conditions and LD50 was carried out for 14 days. The collection of blood cell components was carried out before treatment and on the 14th day. The results of physical observations and the deaths of the test animals showed that administration of ethanol extract of Pulai stem bark up to a dose of 5000 mg did not cause death in the test animals but caused abnormal clinical symptoms such as silence, excitatory passivity, weak reflexes, flabby muscle tone, bradypnea, a weaker pulse, pyloreaction hairs, red nose. For the body weight of the test animals before treatment until the 14th day, there was no significant difference. The results of blood cell component testing showed that the ethanol extract of Pulai stem bark at a dose of 3500 mg–5000 mg could reduce the number of leukocytes.

Keywords: Acute toxicity, *Alstonia scholaris.*

INTRODUCTION

Indonesia has tropical forest areas that are rich in a variety of flora. The potential of this flora can support various kinds of needs, especially in the field of medicinal plants. The use of natural materials, especially those derived from plant materials, for the purpose of treating diseases has been known to mankind since ancient times. The use of traditional medicine in Indonesia is widely accepted by the community, but the safety and effectiveness of traditional medicine have not been fully supported by research (Yusuf et al., 2018).

One of the plants found in Indonesia that is used as traditional medicine is the Pulai tree (*Alstonia scholaris* (L.) R. Br.). Pulai plants are plants that are often found and easy to find in Kalimantan. Since ancient times, the efficacy of the Pulai as a medicinal plant has often been used by the community, especially its leaves and bark, as the people's choice in traditional medicine (Halimah et al., 2021). Based on research, the bark of the Pulai tree contains tannins, quinones, alkaloids, and flavonoids (Arifuddin, 2018). According to Dey (2011), the traditional medicine of this Pulai plant is used to cure asthma, malaria, dysentery, diarrhea, epilepsy, skin diseases, and snake bites. Arulmozi (2010) and Pankti (2012) reported that Pulai is used to treat beriberi and shortness of breath, while the sap is used to treat wounds, tumors, and rheumatism (Mayor & Wattimena, 2022). Whereas in the Tanah Bumbu district, the bark of Pulai stems is often used in traditional medicine to treat diarrhea, wound healing, and malaria.

Traditional drug toxicity tests need to be carried out to assess the safety of the traditional medicines being tested. A toxicity test is a test to detect the toxic effect of a substance on a biological system and to obtain typical dose-response data from the test preparation. The data obtained can be used to provide information regarding the degree of danger of the test preparation if exposure occurs to humans, so that the dosage can be determined for human safety (BPOM RI, 2020).

MATERIALS AND METHODS

a. Materials

The tools used in this study were pipettes, beakers, hotplate stirrers, measuring cups, 10 ml volumetric flasks, maceration vessels, analytical balances, blood analyzers, rat cages, oral probes, and rotary evaporators. The materials used in this study were Wistar strain female rats with a body weight of 100–200 grams obtained from rat farms, bark from Pulai stems obtained from Marga Mulya Village, Sungai Loban District, Tanah Bumbu Regency, Na.CMC, and 70% ethanol.

b. Extraction

The extract was made using the maceration method. A total of 100 grams of pulai bark powder was weighed, put into a glass beaker, and added to 1 liter of 70% ethanol. After 24 hours, the solution was filtered using filter paper to obtain a liquid extract from the Pulai bark. The liquid extract from the ethanol bark of the Pulai stem was collected and evaporated to dryness using a rotary evaporator to obtain a concentrated ethanol extract of the Pulai stem bark.

c. Na.CMC 0.5%

A 0.5% Na.CMC solution was prepared by weighing 500 mg of Na.CMC into 10 ml of hot distilled water and then allowed to stand for 15 minutes until it was clear and resembled a gel. It was then stirred until it became a homogeneous mass and diluted in a 100 ml volumetric flask with distilled water up to the mark.

d. Extract Concentration Group

The ethanol extract of Pulai stem bark will be administered orally and divided into five different groups, namely 500 mg/kg, 1,500 mg/kg, 2,500 mg/kg, 3,500 mg/kg, and 5,000 mg/kg. The negative control used a 0.5% NaCMC solution.

e. Rats Preparation

The test animals were acclimatized for 7 days so that they could get used to adapting to the surrounding environment and avoid feeling threatened or stressed. Test animals were given food according to body weight 5-50 grams/100 grams/day and drank 10–12 ml/100 grams/day. Test animals were given 12 hours of light and 12 hours of darkness. The area of the cage per test animal is 20 cm high and 150 cm wide. Each cage is equipped with chaff, which is replaced every 3 days, a place to drink, and a place to feed. The test animals were fasted for 14–18 hours while still being given a drink before being given the test compound, with the aim of emptying the stomachs of the test animals so that the test compounds could have direct contact with digestion and were not affected by food.

f. Physical Observation and Death of Test Animals

In the acute toxicity test, observations of physical condition and death were carried out in the first 24 hours and continued once a day for 14 days to see delayed toxicity. In this study, the observations were made for 14 days. The physical criteria observed were the Central Nervous System and somatomotor, ANS, respiratory, cardiovascular, digestive tract, genitourinary, skin and fur, mucous membranes, and eyes.

g. Testing of Blood Cell Components

The process of taking the blood of white rats through the tail for as much as ± 0.5 ml before and after administration of the extract The blood sample taken is placed in a microtube containing ethylene diamine tetraacetic acid (EDTA). The amount of each is calculated using the Hematology Analyzer tool.

RESULTS AND DISCUSSION

Results

The results of physical observations on female rats given the ethanol extract of Pulai stem bark showed clinical symptoms. In the 24-hour observation, a dose of 5000 mg showed the most abnormal clinical symptoms. The clinical symptoms that arise can be seen in Table 1 and Table 2.

Table 1. Observation of Clinical Conditions 24 hours

	24 hours							
Groups	**CNS & Somatomotor**	**ANS**	**Respiratory**	**Cardiovascular**	**Digestive tract**	**Genitourinary**	**Skin and fur**	**Mucous membranes**	**Eyes**
1	Silent, excitatory passivity, weak reflexes, flaccid muscle tone	N	Bradypnea	Weaker pulse	N	N	Pyloreaction feathers	Red nose	N
2	Silence, excitatory passivity, flaccid muscle tone	N	N	N	N	N	Pyloreaction feathers	N	N
3	Silence, excitatory passivity	N	N	N	N	N	Pyloreaction feathers	N	N
4	Silent, weak reflexes	N	N	N	N	N	Pyloreaction feathers	N	N
5	Silent, sensitive to stimulation	N	N	N	N	N	N	N	N
Control	N	N	N	N	N	N	N	N	N

N: Normal

Table 2. Observation of Clinical Conditions 14 Days

	2-14 days							
Groups	**CNS & Somatomotor**	**ANS**	**Respiratory**	**Cardiovascular**	**Digestive tract**	**Genitourinary**	**Skin and fur**	**Mucous membranes**	**Eyes**
1	Silent, aggressive	N	N	N	N	N	Pyloreaction feathers	N	N
2	Silent, aggressive, biting	N	N	N	N	N	Pyloreaction feathers	N	N
3	Silent, aggressive, biting, scratching	N	N	N	N	N	N	N	N
4	Active, aggressive, biting, scratching	N	N	N	N	N	N	N	N
5	Active, aggressive, biting, scratching	N	N	N	N	N	N	N	N
Control	N	N	N	N	N	N	N	N	N

N: Normal

The results of observing the body weight of the test animals given the ethanol extract of Pulai stem bark showed that there was no significant difference between the body weights before and after treatment until the 14th day. The data can be seen in Table 3.

Table 3. Average Body Weight

Groups	Average Body Weight				
	Before Treatment	**Day 3**	**Day 7**	**Day 10**	**Day 14**
1	163,7 gram	171 gram	173,7 gram	175,2 gram	167,2 gram
2	172 gram	177,5 gram	181,2 gram	181,7 gram	172,5 gram
3	174 gram	179 gram	181,7gram	184,7 gram	175 gram
4	183 gram	184,2 gram	188,2 gram	188 gram	181,5 gram
5	164,7 gram	174 gram	174,5 gram	177 gram	169,5 gram
Control	174,5 gram	169,5 gram	184 gram	182,5 gram	174,25 gram

The results of the toxicity test of the ethanol extract of the bark of Pulai (*Alstonia scholaris* (L.) R. Br.) on female Wistar rats (*Rattus norvegicus* L.) given five different doses showed that none of the tested animals died in each group, or it can be said to be a pseudo LD50. The data can be seen in Table 4.

Table 4. Acute Toxicity Analysis

Groups	Concentrations	Number of Dead Rats
1	5000 mg	0
2	3500 mg	0
3	2500 mg	0
4	1500 mg	0
5	500 mg	0
Control	Na CMC	0

The results of testing the blood cell components carried out on day 14 using a Blood Analyzer showed a decrease in the number of leukocytes at a dose of 3,500 mg–5,000 mg, and the results of the Mann-Whitney statistical test showed a significant difference. The data can be seen in Table 5 and Table 6.

Table 5. average WBC

Blood Data	1	2	3	4	5	Control
WBC	2,1 x 10^3/uL	2,2 x 10^3/uL	4,3 x 10^3/uL	4,3 x 10^3/uL	4,3 x 10^3/uL	4,7 x 10^3/uL

Table 6. Comparison WBC

Groups	1	2	3	4	5	Control
1	-	0,500	0,000	0,000	0,000	0,000
2	0,500	-	0,000	0,000	0,000	0,000
3	0,000	0,000	-	0,500	0,500	0,500
4	0,000	0,000	0,500	-	0,500	0,500
5	0,000	0,000	0,500	0,500	-	0,500
Control	0,000	0,000	0,500	0,500	0,500	-

Discussion

Based on the results of physical observation tests on female white rats (*Rattus norvegicus* L.), the ethanol extract of the bark of Pulai (*Alstonia scholaris* (L.) R. Br.) gave abnormal physical symptoms such as silence, excitatory passivity, weak reflexes, flaccid muscle tone, bradypnea, a weaker pulse, piloreactic hair, and a red nose. In the 24-hour observation, the 5000 mg test dose showed the most abnormal physical symptoms, while the 3500 mg–500 mg test dose only showed clinical symptoms in the CNS and somatotor as well as skin and fur. On the 2-14th day of observation, the test animals were still silent and aggressive when given a test dose of 2500 mg–5000 mg, while for the 500 mg–1500 mg dose, the test animals were active again.

The most common physical symptoms experienced were at a dose of 5000 mg. Physical symptoms such as silence, excitatory passivity, weak reflexes, and flabby muscle tone are probably caused by the content of flavonoid compounds contained in the bark of Pulai stems, where these compounds play a role in the central nervous system and can inhibit some protein kinase activities that make the test animals more insensitive, so that the test animals become more silent, and also play a role in decreasing the structure and function of nerve

cells, which can make the test animals more excitatory passive, weak reflexes, and flaccid muscle tone (Spencer, 2007). Another physical symptom that appears is a weaker pulse. This is probably caused by extracts from the bark of Pulai, which can inhibit calcium channels so that they can make the pulse weaker. This is in line with the slowing down of the respiratory rate, where calcium is part of the energy. Subsequent physical symptoms, namely pyloreaction and a red nose, were due to a hypersensitive reaction that was possibly caused by excess histamine release in the bodies of the test animals given the ethanol extract of Pulai stem bark (Simatupang et al., 2022).

In addition to observing clinical conditions, observations of animal body weight were also carried out. Weighing was carried out before treatment, on days 3, 7, 10, and 14. Based on the results of the normality test using the Shapiro-Wilk test, it showed a significance value of <0.05, so the data was not normally distributed. Furthermore, data on animal body weight was carried out by the Kruskal-Wallis test, and the results showed a significance value of $p > 0.05$, so there was no significant difference between animal body weights.

In observing the LD50, or death, of the test animals for 14 days, the results showed that none of the test animals died up to the test dose of 5000 mg. Based on the level of toxicity of a compound contained in the Food and Drug Supervisory Agency (2014), it can be said that the ethanol extract of Pulai stem bark (*Alstonia scholaris* (L.) R. Br.) is classified as practically non-toxic.

CONCLUSION

The conclusion of this study on the physical observations and mortality of the test animals, or LD50, showed that administration of ethanol extract of Pulai stem bark at a dose of 500 mg to 5000 mg did not cause death in the test animals or was practically non-toxic, but caused abnormal clinical symptoms such as silence, excitatory passivity, weak reflexes, flabby muscle tone, bradypnea, a weaker pulse, pyloreaction hairs, reddened noses, and for the body weight of the test animals before treatment until the 14th day there was no significant difference. The results of blood cell component testing showed that the ethanol extract of Pulai stem bark at a dose of 3500 mg–5000 mg could reduce the number of leukocytes.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

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