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Acute Oral Toxicity Evaluation of Extract Bajakah *(Spatholobus littoralis* Hassk) in Mice

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ABSTRACT: One potential biodiversity as traditional medicine is bajakah (*Spatholobus littoralis* Hassk.). Bajakah is an herbal plant that has been used in the Borneo region, Indonesia. However, there are not enough data regarding the toxicity of this plant. Therefore, in this study, we aimed to determine acute toxicity in the form of a lethal dose value of 50% (LD_{50}) and the effect on behaviour and organ macroscopic after administration of *S. littoralis* methanol extract. The LD_{50} test used 50 male mice divided into five groups of 5 mice each. The treatment groups were divided based on the concentration of the given extract, namely; 0, 1, 10, 100, and 1000 mg/g BW. Observations were made for 14 days after treatment, and then surgery and macroscopic observations were made on the organs of mice. The results showed that the LD_{50} value for replication A and B was 17.78 and 68.13 mg/Kg BW, respectively. Macroscopic observations found damage to the liver, heart, and stomach. The macroscopic assessment by ANOVA and Kruskal Wallis test showed that in the replication A there was a toxic effect on the stomach, which was marked by a significant difference in weight compared to the control at the test level of $p < 0.05$. These data revealed that the methanol extract of *S. littoralis* lignum is categorized as very toxic, and therefore, it requires special attention in its use. However, further research needs to be done to support the results of this study.

KEYWORDS: *Spatholobus littoralis* Hassk; LD_{50}, herbal medicine; acute toxicity; lethal dose.

1. INTRODUCTION

Herbs have a broader meaning, namely all types of plants and their parts containing one or more active ingredients that can be used in therapy (Sofowora et al., 2013). The use of herbal medicines has become a tradition in Indonesian society and several other parts of the world because it is safer than synthetic drugs (Karimi et al., 2015; Zhang et al., 2015). Therefore, research on natural drug sources has been carried out to obtain certain active compounds and reduce environmental waste (Fawwaz et al., 2018; Fawwaz, et al., 2021; Fawwaz et al., 2019).

To ensure safety in clinical use, traditional medicine is necessary to conduct further research on its acute toxicity test. Acute toxicity is the toxic effect that occurs briefly 24 h after administration in a single dose (Erhirhie et al., 2018; Parasuraman, 2011). At a certain dose, a compound still has a probability of toxicity in the body. Acute toxicity test is one of the toxicological evaluations of herbal extracts. The acute toxicity potential value as measured by the lethal dose 50 (LD_{50}) is the parameter used in the acute oral toxicity test (Adamson, 2016).

One potential biodiversity as traditional medicine is bajakah (*Spatholobus littoralis* Hassk.). Bajakah is a herbal plant originating from Borneo Province, Indonesia, which has not been spread to other areas (Kraatje). The Indonesian Institute of Sciences (LIPI) exhibited that S. littoralis has a high antioxidant content that captures free radicals (Kraatje). A previous study reported that Spatholobus sp contains flavonoids as the main bioactive compounds. Those flavonoids such as prunetin, formononetin, gallocatechin, epicatechin, catechin, calycosin and genistein (Shuwei & Lijiang, 2006; Wang et al., 2011). Genistein and gallocatechin have already been demonstrated as effective for osteoblast cell proliferation and cancer therapy (Fawwaz et al., 2014; Lambert et al., 2008; Lecumberri et al., 2013).

The Dayak community in Central Kalimantan has used this plant as a traditional medicine to treat various diseases, including cancer. The recognition of several Dayak tribespeople who recovered from cancer symptoms after using the *S. littoralis* herb has raised the name of this plant as one of the traditional plant's natives to Indonesia. Until now, the use of *S. littoralis* herbs is increasing so that more comprehensive research is needed to ensure safety in its use. Therefore, in this study, we aim to evaluate the acute toxicity potential of the extract of *S. littoralis*.

Here, we performed the acute toxicity potential of *S. littoralis*. The methanolic extract was obtained from the lignum of *S. littoralis* and evaluated the LD_{50} toward mice. The results of this study can be used as a complement for further research.

2. MATERIALS AND METHODS

2.1. Chemicals

The solvents and other chemicals were of analytical grade. The sodium carboxymethyl cellulose (Na CMC) was purchased from Sigma Chemicals Co. (St. Louis, MO, USA). Deionized water was obtained through a Millipore-Q50 Ultrapure water system (Sartorius). The glassware used was the analytical grade.

2.2. Collection of plant material

S. littoralis lignum was obtained from Petung Penajam, East Borneo, then dried and made into powder. The sample was confirmed by the Division of Botany, Pharmacognosy and Phytochemistry Laboratory, Universitas Megarezky, Makassar, Indonesia. The samples were dried at room temperature. After being dried, the samples were mashed and sieved to obtain a powder with a smaller particle size.

2.3. Sample preparation

A total of 750 g of *S. littoralis* lignum powder was put into a maceration vessel, and then methanol 500 mL was added until the powder was submerged. The container is tightly closed and left for two days at room temperature, stirring occasionally. After two days, the extract was filtered and re-macerated using methanol to obtain the final extract. The extract obtained was collected, concentrated with a rotary evaporator, and then stored in a desiccator until a thick extract was obtained. The suspension of the *S. littoralis* lignum extract was made by weighing the extract according to a particular concentration and then suspended with 0.5% w/v Na CMC to 10 mL.

2.4. Animals

The male mice (*Mus musculus*) were housed in a cage with food and water maintained at a constant temperature (23 – 25 ◦C) with a 12 h light/dark cycle. A total of 50 mice with a bodyweight of 20 – 30 grams. Animal experiments were conducted following laboratory guidelines for the care and use of animals of Universitas Megarezky. After getting approval with reference number: 001.C/07.091056/VII/2021 from the animals' ethics committee of Universitas Megarezky, Makassar, Indonesia, the study was performed.

2.5. Acute toxicity test

The acute toxicity test was measured, as previously reported, with a modification (Saleem et al., 2017). Mice were divided into five groups; each group consisted of two replications; each replication consisted of five mice. Group I as negative control and group II – V as the treatment group. The mice were kept without food for 3–4 h prior to dosing but had access to water ad libitum. The dose was administered to a single dose according to body weight. Group I was given a 0.5% w/v Na CMC solution, group II – V was given an extract suspension with a concentration of 1, 10, 100, and 1000 mg/g BW, respectively. This treatment was carried out with two replications, namely replication A and B. The animals were closely observed for the first 5, 10, 15, and 30 min, followed by 1, 2, 3, and 4 h. The criteria for observing toxic effects include diarrhea, urination, respiratory changes, aggressive behavior, and decreased movement activity. The calculation of the LD_{50} value was based on the number of dead mice in each treatment group for 14 days. Surgery was performed for the dead mice, and the liver, kidneys, heart, stomach, and intestines were weighed. Observations were continued for 14 days, while on the 15th day for mice that still survived, surgery was needed to weigh the organs, after which the LD_{50} value was calculated using the Weil method (Weil, 1952).

2.6. Data analysis

Determination of the LD_{50} value was obtained using the Thompson and Weil formula (Weil, 1952). This method has a relatively high level of confidence and is a method that is often used because it does not require a large number of experimental animals. This method also uses a list of LD_{50} calculations so that the results obtained are accurate. Toxic effect data were taken from mice that showed abnormal symptoms after administration of the extract suspension compared to controls. While the LD_{50} data was taken from the number of dead and living mice in each group then tabulated and calculated using the Thompson and Weil formulas. The data obtained were statistically processed using SPSS. The analysis carried out was homogeneity test and normality test. The groups' statistical analysis was analyzed using one-way ANOVA followed by the Kruskal Wallis multiple comparison test. $p \leq 0.05$ was considered statistically significant.

3. RESULTS AND DISCUSSION

Extraction by maceration obtained thick extract as 97.95 g from 750 g of sample. The percentage of extraction can be seen in **Table 1**. The extract was then suspended with 0.5% w/v Na CMC and made in several dosages for acute toxicity test.

Table 1. The extraction result of *S. littoralis* lignum by maceration

S. littoralis **lignum powder (g)**	**Methanol (mL)**	**Extract (g)**	**Yield (%)**
750 g	500 mL	97.95 g	13.06%

3.1. Acute toxicity test

Group one as a negative control using 0.5% w/v Na CMC solution exhibited no mortality. The treatment group was observed with special attention for the first 5 min until four h. Observations were recorded at the time intervals throughout the study period, i.e., 14 days. Replication A showed that the administration of methanol extract of *S. littoralis* lignum obtained mortality data based on **Table 2**. Death of mice occurred at all doses starting from the smallest dose of 1 mg/g BW with total mortality of one mouse. Three mice died at doses of 10, 100, and 1000 mg/g BW, respectively. There were six dead mice on the first day and only one dead mouse on the second day. On the fifth day, there were two dead mice; and on the fourteenth day, there was one dead mouse.

Table 2. Acute toxicity potential of methanolic extract of *S. littoralis* lignum

Groups	The Number of mice	Dose (mg/g BW)	Mice mortality	
			Replication A	Replication B
I	5	0	0	0
II	5	1	1	1
III	5	10	3	2
IV	5	100	3	2
V	5	1000	3	1

Similar to replication A, death occurred at a 1 mg/g BW dose in replication B of one mouse. There was one dead mouse on the first, sixth, and eighth days, respectively. Meanwhile, on the twelfth day, there were two dead mice. On the thirteenth day, there was one mouse died. For the rest of the other mice, observations were still carried out until day 14th. The percentage of mice mortality can be seen in **Figure 1.**

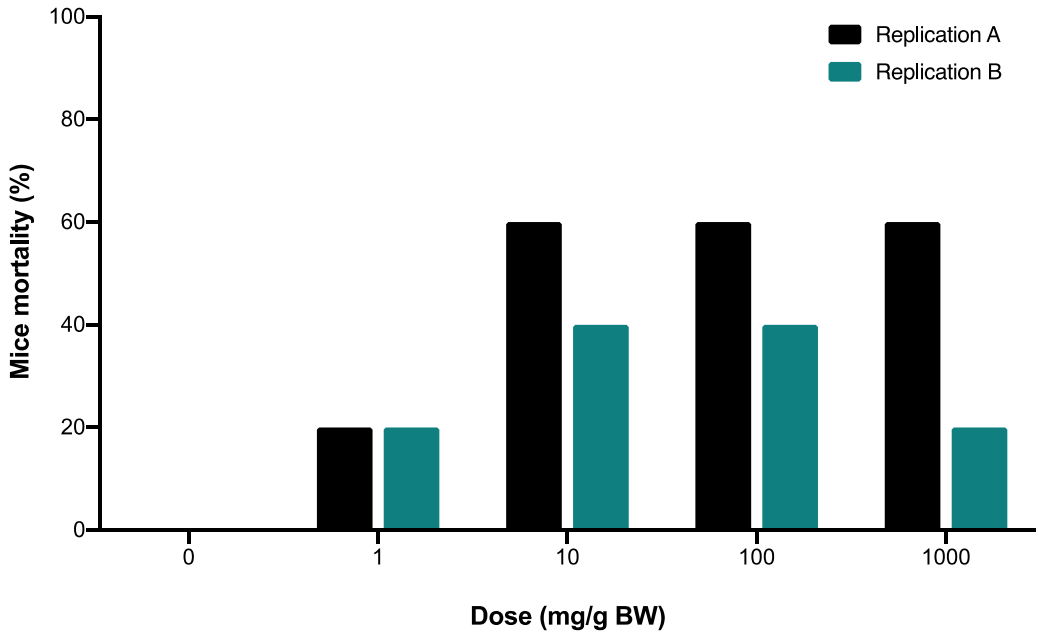

Figure 1. The percentage of mice mortality after treatment with extract of *S. littoralis* lignum

There are several possibilities that the mice died in the study, the first being caused by stress that can lower the immune system. Stress affects the body's immune system by stimulating cortisol and adrenaline secretion. Both affect the release of noradrenaline and sympathetic postganglionic nerve terminals in blood vessels and lymphoid organs (Carnevali et al., 2012; Leach & Suzuki, 2020). At the same time, the second factor could be caused by the high dose given to the mice so that there was a toxicity effect on the mice which resulted in death.

Observations were made on the emergence of symptoms of toxic effects that occurred. The symptoms shown are decreased diarrhea, urination, grooming, mydriasis, increased activity, and decreased movement activity. In group I (negative control), no toxic effects were seen. Meanwhile, in groups II to V, toxic symptoms appeared in decreased movement activity, grooming, diarrhea, urination, and mydriasis.

LD_{50} calculation is done using the Weil method (Weil, 1952). In replication A, the mortality data for mice were obtained at the dose, as shown in **Table 1**. The mortality factor 1, 3, 3, 3 was 0.25000, which can be seen in the Weil

table (Weil, 1952), with a dose multiple of 10 so that the LD_{50} value obtained was 17.78 mg/kg BW and is categorized as highly toxic because it is in the range of 5-50 mg/Kg (Weil, 1952). While in replication B, there were deaths with a mortality factor of 1, 2, 2, 1 of 0.83333, which can be seen in the Weil table, with a dose multiple of 10 so that the LD_{50} value obtained is 68.13 mg/kg BW and is included in the very toxic category because it is in the range of 50-500 mg/kg (Weil, 1952). Both replications exhibited that the methanolic extract of S. littoralis lignum is categorized as very toxic. The smaller the LD_{50} value, the more toxic the compound. Vice versa, the higher the LD_{50} value, the lower the toxicity (Raj et al., 2013).

3.2. Macroscopic observation

After 14 days of observation, the rest of the mice were anesthetized using chloroform. The test animals were immediately dissected to remove the organs of the heart, liver, kidneys, stomach, and intestines as previous study (Fawwaz, Mishiro, et al., 2021). Each organ was weighed and observed macroscopically. The macroscopic observations showed that all organs were damaged to all treatments.

The Kolmogorof-Smirnov normality test for both replications showed that the weight of the liver, kidneys, intestines, and stomach distributed normally. Hence, the analysis continued to the ANOVA test. Besides, the heart was not normally distributed with a significant assumption value ($p < 0.05$) and should be continued to Kruskal Wallis test. The Kruskal Wallis test on the heart showed not significant difference ($p > 0.05$), so there was no need for further tests. The ANOVA test for all organs in replication A showed that the gastric has a significant assumption value ($p < 0.05$), then continued with the further test. The BNT test found significant differences in the stomach against the negative control ($p < 0.05$). These results indicating that the damage in stomach was significant.

4. CONCLUSION

The methanol extract of *S. littoralis* lignum is categorized as very toxic. Physical observations on mice showed toxic symptoms in decreased movement activity, mydriasis, grooming, diarrhea, and urination. Macroscopic observations exhibited damage to the stomach.

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Conflict of interest: The authors declare that they have no conflict of interest.

Ethical Approval: The institutional animal ethics committee approved the study protocol at Universitas Megarezky, Makassar, Indonesia, with reference number: 001.C/07.091056/VII/2021.

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