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Toxicity of *Sida rhombifolia* L. 96% ethanol extract based on LD_{50} and macropathological examination of mice's organs

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ABSTRACT: The Indonesian people empirically use Sidaguri (*Sida rhombifolia* L.) in medicine, such as anti-hyperuricemia. Herbal-based treatment is currently much preferred. Drug metabolism, in general, mainly occurs in the liver, so the possibility of damage to this organ is high. This study aims to determine the acute toxicity category of 96% ethanol extract of sidaguri based on the LD_{50} value, changes in body weight, changes in organ weight, and macro pathology in the organs of male DDY mice in vivo. The method used in this research was experimental with a fixed dose design following the Indonesian Food and Drug Authority regulations regarding guidelines for in vivo non-clinical toxicity tests. The test group was divided into six dose groups (standard, 50, 300, 2000, 5000, and 15000 mg/kg Body of weight). Observations were made for 24 hours. Observations were continued for 14 days on death parameters, toxicity symptoms, body weight, and relative organ weights using five main organs (heart, lungs, liver, spleen, and kidneys, and pathological examination). The acute toxicity test results showed no death in all dose treatment groups. Macropathological analysis did not show abnormalities in organs in all groups of mice. LD_{50} value is more than 15000 mg/kg. Sidaguri 96% ethanol extract is safe and practically non-toxic.

KEYWORDS: DDY mice; In vivo; Sidaguri.

▪ INTRODUCTION

Since the dawn of human civilization, people have used natural remedies to treat various illnesses. These organic ingredients are effective in treating several human ailments [1]. Despite being used for a long time, conventional medicines are not entirely safe because they are foreign substances to the body. It is crucial to understand their possible toxicity. If the dose is relatively low, toxic effects on living things may or may not be obvious; the damage may only affect cells [2].

Indonesia is a tropical nation with a wealth of natural ingredients and raw materials for medicines. In Indonesia, more than 30,000 plant species have therapeutic qualities. Medicinal plants help battle numerous disorders to suit life's necessities [3]. The sidaguri plant (*Sida rhombifolia* L.) is a perennial or sometimes annual plant native to tropical and subtropic areas [4]. The stems have an erect to sprawling growth habit, characterized by branching, and attain a height ranging from 50 to 120 cm. The lowest portion of the stems possesses a woody composition. The leaves of the plant are diamond-shaped and exhibit a dark green coloration. These leaves are positioned alternately along the stem and measure between 4 and 8 centimeters in length [5]. Additionally, the petioles of the leaves are relatively short, measuring less than one-third of the total length of the leaves [6].

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Sida rhombifolia L. holds significant prominence among the twenty genera of Sida globally recognized for their therapeutic properties [6]. *S. rhombifolia* is recognized for its diverse array of therapeutic applications, including xanthine oxidase inhibition, anticholinesterase [7], anticancer, anti-inflammatory [8], antibacterial [9], antioxidant, antihyperglycemic, antimalarial, antiproliferation, anti-diabetes [10], wound healing, and analgesic activities are just a few of the biological activities that *S. rhombifolia* is known to possess. The bioactive substances found in *S. rhombifolia* are the source of this biological activity [11].

Although *S. rhombifolia* is widely used for the above-mentioned biological activities, no toxicological studies of the 96% ethanol extract of the plant have been reported previously. Drug metabolism mainly occurs in the liver, so the possibility of damage to this organ is enormous. So, data related to toxicity tests is essential. The tests to be carried out include an acute toxicity test up to a dose of 15000 mg/kg BW [12]. The acute toxicity test is a test to determine the potential toxicity of LD_{50} binding, assessing various toxic symptoms, spectrum of harmful effects, and mechanisms of death [13]. The purpose of acute toxicity testing is to detect the toxicity of a substance, determine target organs and sensitivity, obtain hazard data after acute administration, and obtain initial information that can be used to determine the dose level required for subsequent toxicity testing.

▪ MATERIALS AND METHODS

Materials

Sample Test

96% ethanol extract of sidaguri (*S. rhombifolia* L.) derived from simplicia leaves of sidaguri (Determination No. 766/UN2.F3.11/PDP.02.00/2021 by Biology Departement FMIPA, Indonesia University) obtained from the Research Institute for Spices and Medicinal Plants (BALITTRO, Bogor).

Animals Test

Male mice DDY strain, aged 8-10 weeks, weighing ± 30 grams, 30 mice from Faculty of Veterinary Medicine, Bogor Agricultural Institute (IPB).

Acute toxicity test

Fixed-dose following the Indonesian Food and Drug Authority no.7 of 2014 regulations concerning guidelines for In Vivo Nonclinical Toxicity Tests. Oral route of administration using a gastric probe. The test animals fasted for ± 16 hours. However, drinking water may be provided. The test animals were weighed and given the test preparation orally (a single dose) with a gastric probe. The test group was divided into six groups, namely the standard group, the amount was 50 mg/kg Body of weight (BW), the amount was 300 mg/kg BW, the amount was 2000 mg/kg BW, the dose was 5000 mg/kg BW, and the quantity was 15000 mg/kg BW. Then, observations were made for 24 hours. After treatment, the feed can be given again after 3-4 hours [14].

Observations were continued for 14 days: Death and symptoms of toxicity: behavioural changes, skin and hair, mucous membranes and eyes, respiration, circulation, nervous system, somatomotor activity, tremors, convulsions, salivation, diarrhoea, lethargy, sleep, and coma; Body weight before and after treatment; Weighing the relative weights of 5 main organs: heart, lungs, liver, spleen, and kidneys; relative weight, i.e., absolute organ weight divided by body weight; and Pathological examination: macroscopic organ changes necropsied at the experiment's end [15].

▪ RESULTS AND DISCUSSION

Death and toxicity symptoms

Table 1 shows that there were no deaths in all dose treatment groups. Symptoms of toxicity were observed in the 1st hour at a dose of 15000 mg/kg BW, namely hair standing, rapid breathing, and decreased activity in general. In addition, symptoms of decreased activity were also observed in mice at a dose of 5000 mg/kg BW (1 head). There was death in the standard group mice (1 tail) on the first day after treatment. This could be because the mice used were not specifically pathogen-free, so they could experience pain/lesion that was innate to the animal, as shown in Table 1.

Table 1. Observation of toxicity symptoms of male mice strain DDY.

Group	Observation time				
	15th minute	1st hour	4th hour	24th hour	14th day
Normal	-	-	-	One dead mouse	-
Dose 50 mg/kgBW	-	-	-	-	-
Dose 300 mg/kgBW	-	-	-	-	-
Dose 2000 mg/kgBW	-	-	-	-	-
Dose 5000 mg/kgBW	One mouse of hair standing, eyes narrowed, silent, fast breathing	-	-	-	-
Dose 15000 mg/kgBW	Two mice with standing hair, three mice with narrowed eyes, silent, fast-breathing	Two mice had narrowed eyes and fast-breathing	-	-	-

Body weight

There was a decrease in the average body weight of mice at doses of 50, 2000, and 15000 mg/kg BW in the range of 1 gram. Mice at doses of 300 and 5000 mg/kgBW experienced an increase in average body weight, but the growth rate in body weight was lower than that of the standard group, as shown in Table 2. These results indicate that sidaguri extract slightly stimulates mice's appetite and may not irritate the gastrointestinal tract directly [6]. Acute stress in rats can significantly increase the body weight of animals. The response of rats to acute stress may cause two different possibilities: weight gain in obese-prone rats or constant body weight in resistant rats [16].

Table 2. Body weight of mice during observation.

Group	Body weight of mice (grams)	
	Early (1st day)	End (14th day)
Normal	30.8 ± 2.49^{b}	33.0 ± 2.94^{abc}
Dose 50 mg/kg BW	35.8 ± 2.28^{a}	34.2 ± 1.789^{abc}
Dose 300 mg/kg BW	30.4 ± 1.52^{b}	31.0 ± 1.23^{a}
Dose 2000 mg/kg BW	36.2 ± 4.08^{a}	35.4 ± 3.51^{bc}
Dose 5000 mg/kg BW	35.8 ± 1.48^{a}	36.2 ± 2.28^{b}
Dose 15000 mg/kg BW	33.8 ± 2.68^{ab}	32.2 ± 1.30^{ac}

Note: Data is presented in the form of mean ± SD.
Different superscripts (a, b, c, d) in the same column showed significant differences ($P<0.05$).

Relative organ weights

The relative weight of organs is a parameter that can provide a general picture regarding the effect of administering the test material. The size of the enlarged or shrunk organ can be known, although it cannot be used as a benchmark in determining organ function damage or repair. The results showed that the relative weights of the liver and right kidney in the treatment group were generally much higher than the standard group. However, in the 2000 mg/kg BW dose group, the relative weight of the liver was smaller than in the standard group. Previous research shows that the increased burden on the heart, kidneys, and liver after administration of high doses of the extract may indicate some toxic effects. In addition, therapeutic bioactive plant products may contain substances that act as toxins in humans [6]. These results were then correlated with the results of the macropathological examination, as shown in Table 3.

Table 3. Relative organ weight of mice during observation.

Group	Relative organ weight (g)					
	Heart	Lungs	Liver	Spleen	Right kidney	Left kidney
Normal	0.42 ± 0.03^{a}	0.84 ± 0.18^{a}	4.93 ± 0.06^{bc}	0.44 ± 0.05^{a}	0.64 ± 0.06^{d}	0.70 ± 0.04^{a}
50 mg/kgBW	0.42 ± 0.04^{a}	0.81 ± 0.17^{a}	5.57 ± 0.49^{a}	0.48 ± 0.24^{a}	0.76 ± 0.08^{abc}	0.81 ± 0.07^{a}
300 mg/kgBW	0.42 ± 0.06^{a}	0.71 ± 0.12^{a}	5.34 ± 0.33^{ab}	0.34 ± 0.10^{a}	0.81 ± 0.24^{ab}	0.72 ± 0.13^{a}
2000 mg/kgBW	0.49 ± 0.16^{a}	0.78 ± 0.18^{a}	4.74 ± 0.22^{c}	0.32 ± 0.12^{a}	0.68 ± 0.60^{cd}	0.69 ± 0.06^{a}
5000 mg/kgBW	0.48 ± 0.07^{a}	0.80 ± 0.22^{a}	5.60 ± 0.61^{a}	0.43 ± 0.15^{a}	0.73 ± 0.60^{acd}	0.75 ± 0.06^{a}
15000 mg/kgBW	0.52 ± 0.15^{a}	0.93 ± 0.30^{a}	5.66 ± 0.22^{a}	0.45 ± 0.53^{a}	0.83 ± 0.10^{b}	0.84 ± 0.16^{a}

Note: Data is presented in the form of mean ± SD.
Different superscripts (a, b, c, d) in the same column showed significant differences (P<0.05).

Macropathology examination

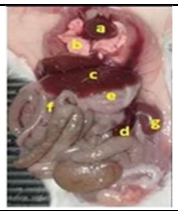
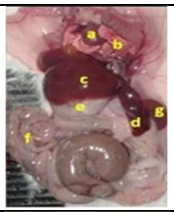
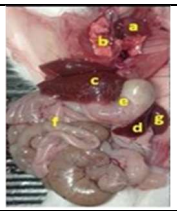
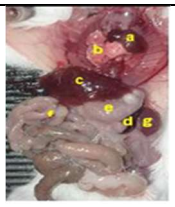
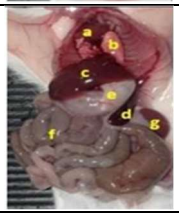
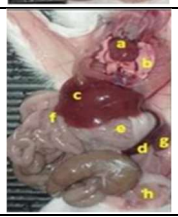
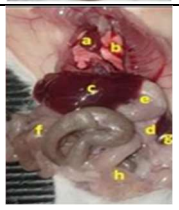
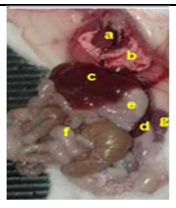
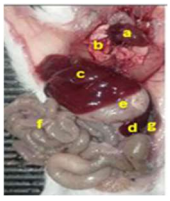
The acute toxicity test results were evaluated based on the dose range in Table 4 [16].

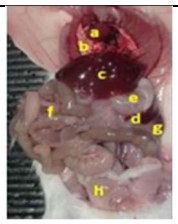
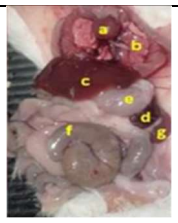
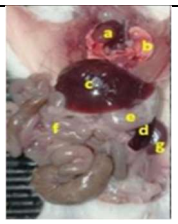
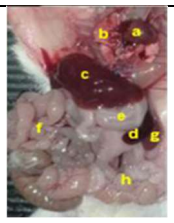
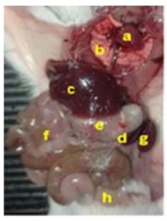
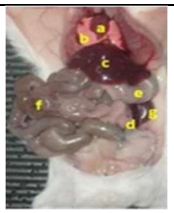
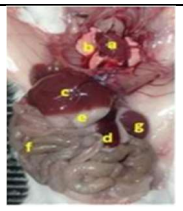
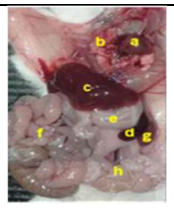
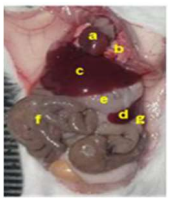
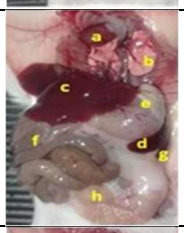
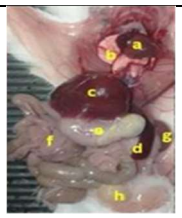
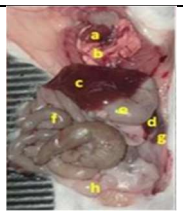
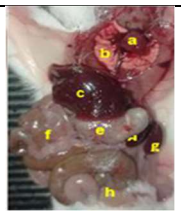
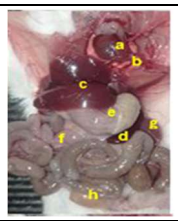
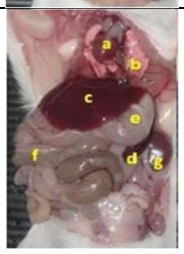
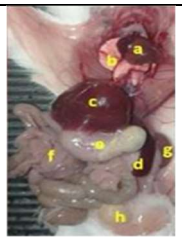
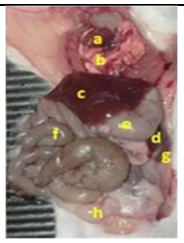
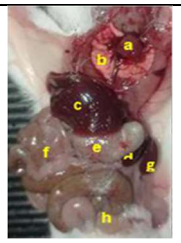
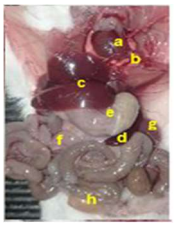
Table 4. Toxicity of compound on LD_{50}.

LD_{50}	Classification
< 5 mg/kg	Super toxic
5 – 50 mg/kg	Extremely toxic
50-500 mg/kg	Highly toxic
500 - 5000 mg/kg	Moderately toxic
5000 – 15,000 mg/kg	Slightly toxic
> 15,000 mg/kg	Practically non-toxic

The test preparation's pharmacokinetic trajectory encompasses intestinal absorption and hepatic metabolism. The metabolic by-products are disseminated throughout the body via systemic circulation to various organs, such as the heart, kidneys, spleen, and lungs [12]. A macropathological examination is performed to see the organ's presence or absence of abnormalities. The macropathological examination results showed no abnormalities in the internal organs of all mice at the end of the observation, as shown in Table 5.

Table 5. Results of macropathological examination of mice's organs.

Group	Condition of Mice's Organs					Result
	1	2	3	4	5	
Normal					-	NA
Dose 50 mg/kg BW						NA

Group	Condition of Mice's Organs					Result
	1	2	3	4	5	
Dose 300 mg/kg BW						NA
Dose 2000 mg/kg BW						NA
Dose 5000 mg/kg BW						NA
Dose 15000 mg/kg BW						NA

Information : NA = No Abnormality, a = heart, b = lungs, c = liver, d = spleen, e = stomach, f = intestines, g = kidney, h = urine bag

Based on all the test results above, it is known that the LD_{50} value is more than 15000 mg/kg BW. So it can be concluded that the 96% sidaguri (*S. rhombifolia L.*) ethanol extract test sample is included in the practically non-toxic category [16]. These results align with previous studies Based on the OCED procedure, it may be inferred that sidaguri extract is categorized as non-toxic due to its acute toxicity dose limit, which is commonly considered 5000 mg/kg BW. Typically, higher doses are deemed unnecessary without any recorded fatalities at this threshold [6].

▪ CONCLUSION

The toxicity test results showed that the LD_{50} value of 96% ethanol extract of sidaguri (*S. rhombifolia* L.) was more than 15,000 mg/kg BW. Sidaguri 96% ethanol extract is safe and practically non-toxic.

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Author contributions: Concept – D.N., S.K., S.A., D.R.; Design – D.N.; Supervision – S.K., S.A., D.R.; Resources – D.N., S.Z., D.F.; Materials – D.N.; Data Collection and/or Processing – D.N., D.F.; Analysis and/or Interpretation – D.N., S.Z., D.F.; Literature Search – D.N., S.Z., D.F.; Writing – D.N., D.F.; Critical Reviews – S.K., S.A., D.R.

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REFERENCES

[1] S. Sam, "Importance and effectiveness of herbal medicines," *J. Pharmacogn. Phytochem.*, vol. 8, no. 2, pp. 354–357, 2019.

[2] J. Jumain, S. Syahruni, and F. Farid, "Uji Toksisitas Akut dan LD50 Ekstrak Etanol Daun Kirinyuh (Euphatorium odoratum Linn) Pada Mencit (Mus musculus)," *Media Farm.*, vol. 14, no. 1, p. 28, 2018, doi: 10.32382/mf.v14i1.82.

[3] I. M. Sumarya, I. W. Suarda, N. L. G. Sudaryati, and I. Sitepu, "Benefits of biopharmaca products towards healthy Indonesia," *J. Phys. Conf. Ser.*, vol. 1469, no. 1, 2020, doi: 10.1088/1742-6596/1469/1/012133.

[4] M. Rafi *et al.*, "Identification of Sida rhombifolia from Its Related Plants Using Thin-Layer Chromatographic Analysis," *Indones. J. Chem.*, vol. 23, no. 1, pp. 21–32, 2023, doi: 10.22146/ijc.73077.

[5] N. S. Aminah, E. R. Laili, M. Rafi, A. Rochman, M. Insanu, and K. N. W. Tun, "Secondary metabolite compounds from Sida genus and their bioactivity," *Heliyon*, vol. 7, no. 4, 2021, doi: 10.1016/j.heliyon.2021.e06682.

[6] A. J. P. Assam, J. P. Dzoyem, C. A. Pieme, and V. B. Penlap, "In vitro antibacterial activity and acute toxicity studies of aqueous-methanol extract of Sida rhombifolia Linn. (Malvaceae).," *BMC Complement. Altern. Med.*, vol. 10, p. 40, 2010, doi: 10.1186/1472-6882-10-40.

[7] S. H. Mah, S. S. Teh, and G. C. L. Ee, "Anti-inflammatory, anti-cholinergic and cytotoxic effects of Sida Rhombifolia," *Pharm. Biol.*, vol. 55, no. 1, pp. 920–928, 2017, doi: 10.1080/13880209.2017.1285322.

[8] F. C. Rodrigues and A. F. M. de Oliveira, "The genus Sida L. (Malvaceae): An update of its ethnomedicinal use, pharmacology and phytochemistry," *South African J. Bot.*, vol. 132, pp. 432–462, 2020, doi: 10.1016/j.sajb.2020.04.030.

[9] D. Debalke, M. Birhan, A. Kinubeh, and M. Yayeh, "Assessments of Antibacterial Effects of Aqueous-Ethanolic Extracts of Sida rhombifolia's Aerial Part," *Sci. World J.*, vol. 2018, 2018, doi: 10.1155/2018/8429809.

[10] S. Kumar, P. K. Lakshmi, C. Sahi, and R. S. Pawar, "Sida cordifolia accelerates wound healing process delayed by dexamethasone in rats: Effect on ROS and probable mechanism of action," *J. Ethnopharmacol.*, vol. 235, pp. 279–292, 2019, doi: 10.1016/j.jep.2018.07.003.

[11] A. H. Karomah *et al.*, "UHPLC-Q-Orbitrap HRMS-based Untargeted Metabolomics of Sida rhombifolia Leaves and Stem Extracts," *HAYATI J. Biosci.*, vol. 30, no. 4, pp. 770–778, 2023, doi: 10.4308/hjb.30.4.770-778.

[12] E. O. Erhirhie, C. P. Ihekwereme, and E. E. Ilodigwe, "Advances in acute toxicity testing: Strengths, weaknesses and regulatory acceptance," *Interdiscip. Toxicol.*, vol. 11, no. 1, pp. 5–12, 2018, doi: 10.2478/intox-2018-0001.

[13] M. Kifayatullah, M. S. Mustafa, P. Sengupta, M. M. R. Sarker, A. Das, and S. K. Das, "Evaluation of the acute and sub-acute toxicity of the ethanolic extract of Pericampylus glaucus (Lam.) Merr. in BALB/c mice," *J. Acute Dis.*, vol. 4, no. 4, pp. 309–315, 2015, doi: 10.1016/j.joad.2015.06.010.

[14] N. A. Pratiwi, R. Susanti, and N. U. Purwanti, "UJI TOKSISITAS AKUT EKSTRAK ETANOL BIJI BUAH CEMPEDAK (Artocarpus champeden L.) TERHADAP TIKUS BETINA (Rattus norvegicus L.) GALUR WISTAR," *J. Kesehat. Khatulistiwa*, vol. 8, no. 2, p. 1, 2022, doi: 10.26418/jurkeswa.v8i2.54182.

[15] A. K. Nisa, R. Choesrina, and F. Lestari, "Studi Literatur Uji Toksisitas Akut Pada Beberapa Ekstrak Tumbuhan," *Pros. Farm.*, vol. 6, no. 2, pp. 694–698, 2020, [Online]. Available: http://dx.doi.org/10.29313/.v6i2.23683

[16] N. Rossiana *et al.*, "Urgency longterm oil sludge biophytoremediation: Acute, subchronic toxicity on liver and kidney rats," *Environ. Technol. Innov.*, vol. 19, p. 100766, 2020, doi: 10.1016/j.eti.2020.100766.