Indonesian Journal of Veterinary Sciences
Vol. 2. No. 2 September 2021, Page. 53-57
DOI:10.22146/ijvs.v2i2.82599, ISSN 2722-421X (Online)
Available online at https://jurnal.ugm.ac.id/ijvs

Acute Toxicity Test of Tabar Kedayan Root Extract (*Aristolochia Foveolata Merr.*) In Swiss Strain Female Mice (*Mus Musculus*)

Ika Nindya Irianti, Desti Ika Yanti, Agustina Dwi Wijayanti*

Departement of Farmakologi, Faculty of Veterinary Medicine, Universitas Gadjah Mada
Jl. Fauna No.2. Karangmalang Yogyakarta 55281
*Corresponding author, Email: tinabdy@ugm.ac.id

Received: April 22, 2021, Accepted: May 15, 2021, Published: September 1, 2021

[1], A

Abstract

The Tabar Kedayan (TK) is a plant known as an antidote for poison in the Dayak community. The part of the TK plant used widely for medication is the root. Empirically, it is known to have benefits as an antidote, anti-diarrheal, anti-pain, and anti-cancer. However, the toxicity of TK is unknown. This study was conducted to determine the toxic levels of TK root extract using the OECD 423 method. The toxicity test is a procedure used to determine the toxic levels of a chemical compound in the body through physical changes, behavior, and death of experimental animals. The experimental female mice used in the study were divided into two groups: the control group and the treatment group. Both groups contained three mice. The treatment group was given 300 mg/kg BW of TK extract orally, and the control group was given 10 ml/Kg body weight water. The treatment dose was then increased to 2000 mg/kg BW with new experimental animals because the treatment group did not show symptoms of acute intoxication at the first dose. The experimental animals were observed for 14 days, and the results showed no sign of symptoms of acute intoxication, so the TK root extract was categorized as category 5, which was not classified, with an LD50 > 2000-5000 mg/kg BW.

Keywords: acute toxicity text, LD50, Tabar Kedayan root extract, mice

Introduction

The Dayak people in Malinau District have a plant empirically believed to be a medicinal plant, namely the root of Tabar Kedayan (*Aristolochia foveolata* Merr.). Tabar Kedayan root is a plant-believed to be an anti-poison to neutralize insect poisons, snake venom, and venomous animal bites (Liwun, 2009). Tabar Kedayan root also has benefits for treating intestinal pain, gallbladder pain, arthritis, gout, and rheumatism, boosting the immune system, and reducing pain at the start of menstruation. The study by Sapri et al. (2016) showed that the root of TK has analgesic properties. Tabar Kedayan root extract doses of 25 mg/20 g BW and 50 mg/20 g BW have analgesic properties equivalent to 0.05% tramadol, while the 100 mg dose has more potent analgesic properties than 0.05% tramadol.

The TK root extract has many properties and benefits that the people of North Kalimantan empirically trust, so further testing is performed regarding the toxicity of the TK root extract before it is made into an herbal medicinal product. The acute toxicity test is the first before other toxicity tests are performed. The acute toxicity test is carried out by administering the dose according to standard instructions, then observing the first 4 hours after administration and continuing to observe toxic symptoms every 24 hours for 14 days using the Organization for Economic Cooperation and Development (OECD) 423 method. The OECD method is the test used to determine the potential for toxicity. Toxic symptoms were observed using female mice as experimental animals. Anonymous (2001) states female mice are more sensitive to toxicity exposure for toxicity tests. The toxicity test results in the LD50 form the basis for doses that cause toxic effects so people can determine the safe doses. There is no supporting data regarding acute toxicity tests on TK root extract, so research

is needed to obtain complete information regarding the safety category of drug doses and the potential for further toxicity tests.

Materials and Methods

This research was conducted at the Laboratory of the Department of Pharmacology FKH UGM, using experimental animals, namely female Swiss mice aged 2-3 months with an average weight of 28.5 grams. The materials in this study were tabar kedayan root Simplicia extracted with 70% alcohol solvent, aqua distillate, 1 ml syringe (OneMed®), latex gloves (Sensi®), masks (Sensi®), labels, plastic, aluminum foil, rubber, picric acid, filter paper.

The tools used during the study were metabolic cages, drinking bowls, water baths (Julabo PC®), mortars, tampers, sonde (oral cannula), HI 301N magnetic stirrers (Hanna Instrument®), 1000 ml beakers (Pyrex®), 100 ml beakers (Pyrex iwaki® te-32), 50 ml Duran beaker (Schott®), 25 ml measuring cup (Pyrex®) 100 ml measuring cup (Pyrex®), 50 mm funnel (Herma®), glass stirrer, digital balance (Ohaus®), ram, knife, cutting board, tray, tin, oven (Jouan®), blender (Panasonic®), 1000 ml Erlenmeyer flask (Pyrex®), plastic pot, 10 ml volumetric flask (Pyrex iwaki®), glass jar, and dropper.

The TK root was simply washed and then drained. The TK root was cut into small pieces using a knife and then placed in a baking dish. The TK roots were air-dried for a day and then put in the oven at 55°C for a day. The root has been baked, blended, and then weighed to determine the weight of the simplicial. The simplicial powder of the TK root was put in two pieces of 1000 ml beaker glasses then 70% alcohol was added. The mixture of TK root Simplicia powder and 70% ethanol was stirred using a magnetic stirrer for 30 minutes and then allowed to stand for 24 hours. The mix of TK root Simplicia powder and 70% ethanol was filtered using filter paper and put in an Erlenmeyer. The filtered liquid was evaporated in a water bath at 55°C until a thick extract was obtained. The thick extract was put in a plastic pot. Evaporation of the extract was repeated for four days.

The experimental animals were divided into two groups: a treatment group of three mice and a control group of three mice. The control group was given distilled water at 10 ml/kg BW treatment group was given TK extract with an initial dose of 300 mg/kg BW orally. Experimental animals were observed intensively for 4 hours and continued every 24 hours for 14 days. Animals were kept for physical changes, behavior, and death. If the animals show no toxicity, the dose is increased to 2000 mg/kg BW, and then observed for 14 days. The experimental animals were analyzed quantitatively and qualitatively by observing behavior and mortality and then determining the toxicity category of LD_{50} regarding OECD guideline.

Result and discussion

An acute toxicity test is a test that is used to ensure the safety of a compound. Based on OECD 423, toxicity tests can provide information about the potential toxicity of a compound and its effect on several parameters such as age, sex, route of administration, and environmental factors. Toxicity tests can determine variables between species and between strains of microbial animals and the reactivity of animal populations. Physical observations include changes in skin and hair, eyes, mucous membranes, respiration, blood circulation, nervous system, and behavior patterns.

The quantitative data obtained from the acute toxicity test is the $_{LD50,}$ while the qualitative data is in the form of changes in behavior and symptoms of the toxic effects of the test compounds that appear. LD_{50} is defined as a single dose of a substance that is statistically expected to kill 50% of experimental animals. The LD_{50} data obtained is used to determine the toxicity potential of the acute compound relative to other compounds, or it can also be used to estimate doses for other toxicological tests (Donatus, 2005).

Classification of toxicity level categories from OECD 423 (2001) in Table 1.

a. Category 1 (0-5 mg/kg)
b. Category 2 (5-50 mg/kg)
c. Category 3 (50-300 mg/kg)
d. Category 4 (300-2000 mg/kg)
e. Category 5 (2000-5000 mg/kg Unclassified)

The LD_{50} value can be related to the ED_{50} value (a dose that can provide a therapeutic effect

Table 1. Globally harmonized classification system (GHS) category with $_{LD50}$ value

The number of test animals per step	Number of dosages (mg/kg BW)	Number of test animals	GHS category (mg/kg BW)	LD_{50} *cut-off* (mg/kg BW)
3	5	2-3*	Category I (>0-5)	5
3	50	2-3*	Category II (>25-50)	25-50
3	300	2-3*	Category III (>50-300)	200-300
3	2000	2-3*	Category IV (>300-2000)	500 (if 3*) 1000 (if 2*) 2000
3	2000	0-1*	Category V (>2000-5000)	2500 (if 1*) 5000 (if 0*) (unclassified)
3	5000	0*	Category VI (unclassified)	5000 (if 0*) (unclassified) [1], A

(*)test animals show toxic/death symptoms

in 50% of the population) to determine the IT (Therapeutic Index). The therapeutic index is the ratio between the dose that can cause death and the dose that can cause a therapeutic effect. The drug is considered relatively safe if the ratio between LD50 and ED50 is large. In addition, the LD_{50} is useful for determining the relationship between dose and the emergence of toxic effects such as behavior and death, knowing symptoms of acute toxicity which are useful in diagnosing poisoning, fulfilling regulatory requirements if the test substance is to be developed into a commercial drug (Priyanto, 2009).

Balazs (1970) suggested that there were toxic symptoms that could be observed in experimental animals in Table 2.

dose of 300 mg/kg BW. This dose was chosen because there is no information regarding the toxicity of the tabar kedayan root extract. The test compound was administered orally to three mice in the treatment group. Three female mice in the control group were treated with the same volume

Table 2. Toxic symptoms that can be observed in experimental animals

Organ system	Observation and inspection	General intoxication symptom
Central nervous system and somatomotor	Behavior	Changes in behavior toward observers, unusual vocalizations, restlessness
	Movement	Twitches, tremors, ataxia, catatonia, paralysis, convulsions, forced movements
	Reactivity to various stimuli	Excitement, passivity, anesthesia, hyperaesthesia
	Cerebral and spinal reflects.	Weak, none
	Muscle Toni	Stiffness, softness
Autonomy nervous system	Pupil size	Miosis, mydriatic
	secretion	Salivation, lacrimation
Respiration system	rate of breath	Bradyspnea, dyspnea
Gastrointestinal system	Intestine problems	Diarrhea, constipation, flatulence, contractions
	Feces consistency and color	Shapeless, black color
Skin and coat	Color, wholeness	Softness, redness, blistering, pylorrection
Mucosal membrane	Conjunctiva, cavum oris	Congestion, bleeding, cyanosis, yellowness
Eye	Palpebral	
	Eyeball	Exophthalmos
	Clarity	Opacity
etc	General condition	Abnormal body condition

Table 3. Results of physical, behavioral, autonomic, and somatomotor observations of TK root extract at 300 mg/kg BW and 2000 mg/kg BW.

Parameter	Observation	Aquades 10 ml/kg BB		300 mg/kg BB		2000 mg/kg BB	
		4 hours	14 days	4 hours	14 days	4 hours	14 Days
Physical appearance	Hair Color	-	-	-	-	-	-
	Eyes color	-	-	-	-	-	-
	Urine color	-	-	-	-	-	-
Behavior	Grooming	-	-	-	-	-	-
	Restless	-	-	-	-	-	-
	Passive	-	-	-	-	-	-
	Tremor	-	-	-	-	-	-
	Convulsions	-	-	-	-	-	-
Autonomy nervous system	Defecation	-	-	-	-	-	-
	Urination	-	-	-	-	-	-
	Piloerection	-	-	-	-	-	-
	Diarrhea	-	-	-	-	-	-
	Lacrimal activity	-	-	-	-	-	-
Somatomotor nervous system	Walking	-	-	-	-	-	-
	Body position	-	-	-	-	-	-
	Extremity position	-	-	-	-	-	-

Explanation: (+) show intoxication symptoms, (-) no symptoms

of distilled water. The results did not find any toxic symptoms or death in mice during the observation period. The dose was then increased to 2000 mg/kg BW and observed for 14 days. Tabar kedayan root extract was administered orally to three different mice. The results also found no toxic symptoms or death, as seen in Table 3.

Observation of physical parameters for acute toxicity testing of TK root extract doses of 300 mg/kg BW and 2000 mg/kg BW did not cause toxic symptoms. The physical parameters observed were hair, eye, and urine. The results of group observations before and after the experimental animal treatment showed that all mice have clean and white hair color. No visible dirty and dull hair was shown during the experiment. According to the results of Fitria's research (2019), unhealthy mice show physical and behavioral changes, namely coarse hair, dirty tails, and muzzles, due to reduced activity and grooming behavior. Observation of eye color showed that the mice had clear, red healthy eyes before and after treatment. According to Sirois (2016), mice have porphyrin in the Harderian gland, which causes a red color in the mice's eyes. The clinical signs of restless behavior, passivity, tremors, and convulsions in this study were observed visually.

The behavior of the mice was expected, and they did not show agitated behavior. The group of mice treated at 300 mg/kg BW showed inactive behavior for a few minutes and then became active. The group of mice treated with 2000 mg/kg BW showed inactive behavior after 5 minutes of administration of TK root extract and then became active. The mice were active, and no nerve disturbance was observed, resulting in uncontrolled movements such as tremors and convulsions, as seen in Figure 1. According to the results of an acute toxicity study by Kumar et al. (2017), mice experiencing symptoms of toxicity will exhibit uncontrollable shaking leg behavior (tremor) and muscle nerves contracting and rapid relaxation (convulsions) due to the stimulus effect on the Central Nervous System (CNS). Tabar Kedayan root extract in this study does not interfere with the Central Nervous System (CNS), so it does not cause symptoms of behavior changes.

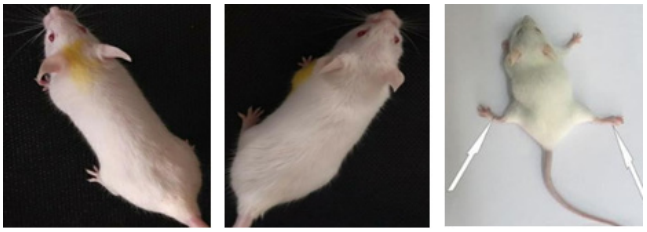

Figure 1. Observations of mice treated with 2000 mg/kg BW (left) and control mice with distilled water 10 ml/kg BW showed a normal gait and proportional limb position (middle) compared to mice given acrylamide 10 mg/kg BW experiencing muscle weakness and gait abnormal day 45 (right) (Lashein et al., 2018).

The OECD (2001) stated that female mice have a higher sensitivity to toxicity than male mice. Based on these results, TK root extract has a potential LD_{50} >2000-5000 mg/kg BW in Swiss female mice, and according to the Globally Harmonized Classification System (GHS) category in the OECD Guideline 423, it is included in category five or not classified.

Conclusion

Tabar kedayan root extract given to Swiss-strain female mice at doses of 300 mg/kg BW and 2000 mg/kg BW did not cause toxic effects and did not cause death. Tabar Kedayan root extract has an LD_{50} >2000-5000 mg/kg BW and belongs to category 5.

References

Anonim. 2001. *OECD Guideline for Testing of Chemicals, Acute Oral Toxicity-Acute Toxic Class Method* No. 423.

Balazs, T. 1970. *Measurement of Acute Toxicology*. Oxford: Blackwell Scientific Publication.

Donatus, I. A. 2005. *Toksikologi Dasar*. Yogyakarta: Laboratorium Farmakologi dan Toksikologi dan Farmasi Klinik Fakultas Farmasi Universitas Gadjah Mada

Fitria, L. 2019. Uji Toksisitas Oral Akut *Single Dose* Filtrat Buah Luwingan (*Ficus hispida* L.f.) pada tikus (*Rattus norvegicus* Berkenhout, 1769) galur Wistar. *Mangifera Edu*cation 4 (1). pp. 1–18.

Kumar, N., Pullaiah, C., P., Dhanunjaya and Reddy, D. 2017. Acute Toxicity Studies of Aqueous Seed Extract of Vigna Unguiculata In Albino Rats.*Innovare Journal of Ayurvedic Sciences* 5 (2). pp. 4–7.

Lashein, F. E. M., Amra, E.S.A., Saleem, A.A. and Badr, A.H. 2018. Ameliorative effect of bee venom and its extracted bradykinin-potentiating factor on neurological alteration induced by acrylamide and chips administration, *The Journal of Basic and Applied Zoology*. pp. 1– 13.

Liwun, N.M. 2009. Inventarisasi dan Identifikasi Tanaman Obat yang Digunakan oleh Suku Dayak Lundayeh di Kecamatan Muntarang Kabupaten Malinau Kalimantan Timur. *KTI Akademi Farmasi Samarinda*. Samarinda.

Priyanto. 2009. *Toksikologi Mekanisme, Terapi Antidotum, dan Penilaian Resiko*. Jawa Barat: Lembaga Studi dan Konsultasi Farmakologi

Sapri, Supriningrum, R., Warnida, H. 2016. Uji Daya Analgetik Ekstrak Etanol Akar Tabar Kedayan (*Aristolochia foveolata* Merr.) pada Mencit Putih Jantan dengan Metode Induksi Nyeri Panas. *Media Sains* 9 (1).

Sirois, M. 2016. *Laboratory Animal and Exotic Pet Medicine Principles and Procedures*. Second Edition. Elsevier. China. 98