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RESEARCH ARTICLE

Acute toxicity study of the ethanolic extract of *Eleutherine bulbosa Urb* in Wistar rats

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Abstract

Introduction: *Eleutherine bulbosa Urb* is a plant species with medicinal properties, including anti-inflammatory, widely relied upon in traditional practices. For this reason, the present research was intended to assess and, thus, ensure the safety of this plant for conventional medicinal purposes using a toxicity test study. **Methods:** The acute toxicity test of the ethanolic extract of *E. bulbosa Urb* (EEEB) used the method adopted from the Organization Economic Cooperation and Development (OECD) guidelines 425 for testing Wistar rats. **Results:** During 14 days of the acute toxicity study, there were no significant changes in rat weight, no mortality, and no signs of toxicity after the oral EEEB administration at 2000 mg/kg body weight (bw). The limit test showed that the LD50 of EEEB was higher than 2000 mg/kg bw. **Conclusion:** EEEB has low toxicity because its LD_{50} is higher than the limit test results.

Introduction

More than 30,000 types of plants and 1,000 types of medicinal plants have been used in the traditional medicinal industry in Indonesia. Medicinal plants are, in general, forest plants that have been grown in yards and hereditarily used as traditional medicine since the era of ancestors. Recently, they have been widely developed as Indonesian traditional herbal medicine, namely "jamu", standardised herbal medicine, and phytopharmacy (Anam *et al*., 2013). "Bawang Dayak" (*Eleutherine bulbosa Urb.*) is an example of medicinal plants, nutritious for health but still scarcely used in community medicine. This plant is commonly found in South Kalimantan island, where the locals already use it as traditional medicine. Its bulbs are widely used for several therapeutic purposes. *E. bulbosa* Urb. effectively reduces cholesterol (Anjar, 2016) and has antihypertensive, immunomodulatory, and anti-inflammatory activities (Muthia & Astuti, 2018; Paramita & Nuryanto, 2018; Rauf *et al.*, 2018). The bulb extracts contain flavonoids, phenolics, saponins, and tannins (Andi *et al.*, 2013; Pratiwi *et al.*, 2013; Muthia *et al.,* 2021).

Acute toxicity testing is a preclinical test aiming to measure the toxic effects (degree of toxicity) of a compound or chemical that occurs immediately or shortly after it is delivered orally as a single dose or repeatedly within 24 hours (WHO, 2004). It is designed to quantitatively measure the Lethal Dose 50 (LD_{50}) of a substance. Its parameter includes the mortality of the test animals (Dipasqual, 2001). Medicinal plants must go through various testing processes for the safety of their consumption, one of which is the acute toxicity test (Syamsul *et al.*, 2015). As *E. bulbosa* Urb has many therapeutic and non-therapeutic properties, it is necessary to test its acute toxicity.

Methods

Plant collection and sample preparation

The *Eleutherine bulbosa Urb* plants were collected from Banjarbaru, South Kalimantan, and determined at the Herbarium Bogoriense, Biology Research Center, Indonesian Institute of Sciences (LIPI) Bogor, with the registration number 2244/IPH.1.01/If.07/XII/2019. The bulbs were separated, cleaned, washed, cut into small pieces, and dried by aeration. Afterwards, the dried bulb samples were ground to fine powders, which were later sieved using mesh number 16 and stored in closed containers.

Bulb extract preparation

The bulb powders obtained from the previous procedure were extracted by maceration for 24 hours, using 96% ethanol as the solvent (DepKes, 2014). The resulting filtrate was filtered, and the residual pulp was macerated twice using the same maceration procedure and solvent. The ethanolic extract was evaporated in a rotary evaporator at 45^{o}C and a water bath until a fixed weight was reached.

Approval from the animal ethics committee

The acute toxicity test was performed on seven healthy non-pregnant female Wistar rats aged 2-3 months old and weighing about 100-200 grams. The procedures involved were conducted after receiving approval from the institutional ethical committee University of Surabaya No: 141/KE/X/2020.

Acute toxicity test

As per the OECD Guidelines 425 (Up-and-Down Procedure) (OECD, 2001; OECD 2008), the test rats were kept in a standard condition for 15 days. The limit test for single peroral administration was conducted at 2000 mg/kg body weight (bw). The test rats were given no food three to four hours before the administration but had ad libitum access to water. After the prepared extract was given to one female rat, it was closely observed for any toxic effects in the first 30 minutes, 4 hours, and then regularly (at an interval of 24 hours) for 14 days. Food was provided one to two hours after the administration. If this test rat survived the procedure, the extract was given to four additional rats at the same dose and under the same conditions. These five test rats were labelled as the treated group. However, if it died, the main test to calculate the LD_{50} of responses was initiated. If three animals died, the limit test was terminated, then the main test was performed. The LD_{50} was greater than 2000 mg/kg bw if three or more test animals survived the procedure. Apart from the treated group, the experiment also used two other test rats as the control (vehicle-treated group). This group was given 1% carboxymethyl cellulose (CMC) gel orally, then, like the treated group, monitored for any toxic effects in the first 30 minutes, four hours, and at a regular interval of 24 hours for 14 days. The test rats that survived were examined for the onset of toxic reactions; their weights were also monitored and documented until the end of the study (OECD 2001; OECD 2008). The LD_{50} was computed in the Acute Oral Toxicity (AOT) 425 StatPgm software. After the experiment, the test rats that survived were anesthetised and sacrificed for histopathology.

Statistical analysis

The body weights were expressed as mean±SD, and the statistical significance between the treated and control groups was analysed using an independent-samples t-test on SPSS version 16. $p \leq 0.05$ reflected statistically significant differences.

Results

Behavioural pattern and body weight

Table I shows the test rats' weights in the control and treated groups. Table II presents the behavioural pattern of these rats observed after administering the ethanolic extract of *E. bulbosa Urb.*

Table I: Mean weight of the test rats in control and treated groups in the 14-day acute toxicity test

Treatment	Body weights (g)			
	Day 0	Day 1	Day 7	Day 14
Control	154.7±27.08	155.5±23.3	173.5±6.3	189.5±2.1
EEEB	153.8±17.7	147.8±15.6	169.8±12.6	173.6±17.8

Value provided as mean±SD (n=2) for control; (n=5) for treated group

Table II: Toxicity symptoms in control and treated groups in the acute toxicity test

Parameters	Symptoms of Toxicity							
	30 minutes		4 hours		24 hours		14 days	
	CG	TG	CG	TG	CG	TG	CG	TG
Fur & Skin	N	N	N	N	N	N	N	N
Eyes	N	N	N	N	N	N	N	N
Respiration	N	N	N	N	N	N	N	N
Convulsions	NF	NF	NF	NF	NF	NF	NF	NF
Tremors	NF	NF	NF	NF	NF	NF	NF	NF
Diarrhea	NF	NF	NF	NF	NF	NF	NF	NF
Mortality	NF	NF	NF	NF	NF	NF	NF	NF

CG = Control Group; TG = Treated Group; N = Normal; NF = Not Found

Acute toxicity test results

All rats used in the toxicity test of the ethanolic extract of *Eleutherine bulbosa* Urb., which were administered at 2000 mg/kg bw, showed no symptoms of toxicity and survived until Day 14 of the observation, meaning that the LD_{50} of this extract is higher than 2000 mg/kg bw. At this state, the LD_{50} fell into category 5: no symptoms of toxicity at a dose of 2000 mg/kg bw. Screenshots of the acute toxicity test results in the AOT 425 StatPgm software are presented in Figure 1.

Histopathological results

Treated rats were given the ethanolic extract of *Eleutherine bulbosa* Urb at a limit dose of 2000mg/kg bw. Liver and heart histopathological sections can be seen in Figure 2.

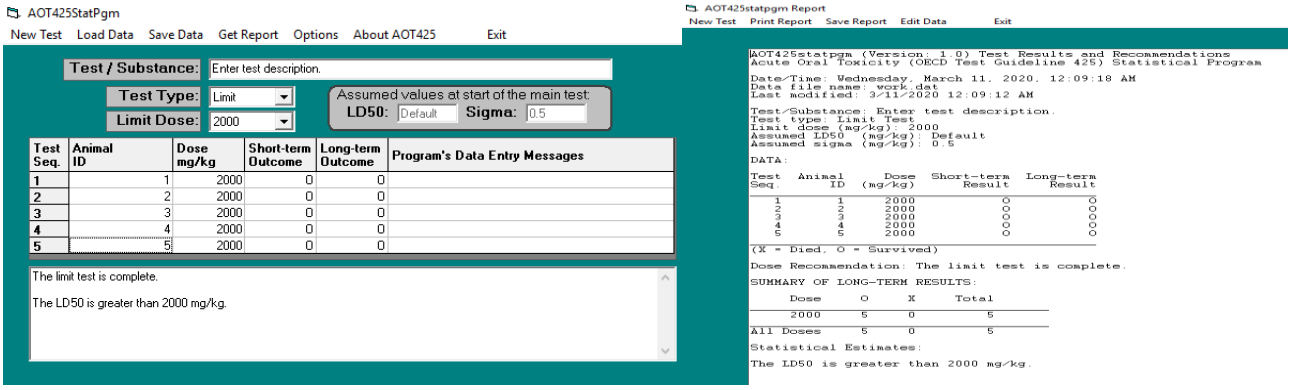
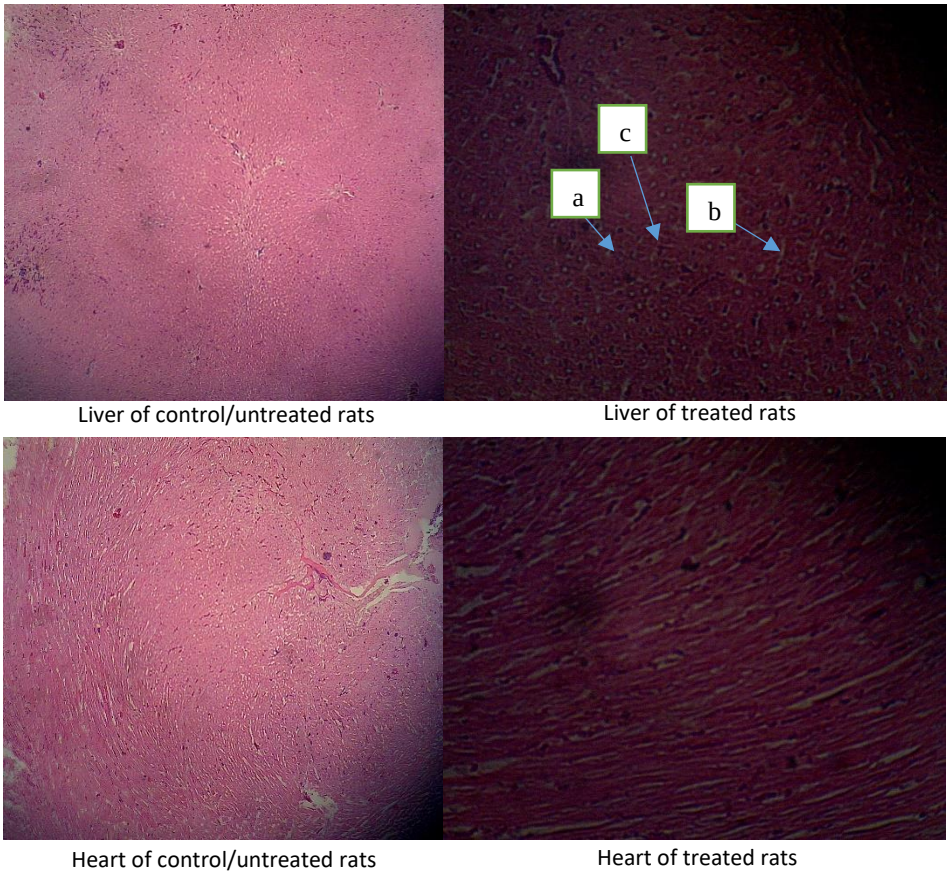


Figure 1: The AOT 425 StatPgm window for the acute toxicity test results



Liver of control/untreated rats

Liver of treated rats

Notes:

a) Normal hepatocyte
b) Hydropic degeneration
c) Hepatocyte necrosis

Heart of control/untreated rats

Heart of treated rats

Figure 2: Liver and heart histopathological sections of the control and treated rats (Hematoxylin and Eosin staining, 40x10 magnification)

Discussion

All test rats survived the toxicity study of the ethanolic extract of *Eleutherine bulbosa* Urb at a dose of 2000 mg/kg bw. The observations were made in the first 30 minutes up to 4 hours after the extract administration and then periodically for 14 days, meaning that the resulting LD_{50} of the ethanolic extract was from a dose higher than 2000 mg/kg bw.

As seen in Tables I and II, the control group gained weight throughout the 14 days of the toxicity study, while the treated group experienced fluctuating weights until the end of the observation. Weight changes are considered the manifestation of the toxic effects of a substance (Jothy *et al.*, 2011; BPOM 2014). Generally, the decrease in the weight of the body and internal organs are simple and sensitive indices of toxicity after exposure to toxic substances. Changes in body weight are indicators of drug and chemical adverse effects, considered significant if the loss is 10% from initial body weight (Vaghasiya *et al.,* 2011). The average body weight was analyzed using the statistical independent-samples t-test on SPSS, and no significant differences in body weight were found between the control group and the treatment group. The independent t-test of these weights resulted in a sig (2-tailed) value of 0.533, with $p > 0.05$. Wati *et al.* (2018) confirm no body weight change in the acute toxicity test, suggesting normal body metabolic processes (Klaassen, 2018). The acute toxicity of the ethanolic extract of *Eleutherine bulbosa* Urb was assessed in mice after orally administered at 1000, 2000, 3000, 4000, and 5000 mg/kg BW. These doses neither caused mortality nor show signs of toxicity (Hanh *et al.*, 2018).

In the OECD 425 guidelines (2008), toxicity can be reflected by changes in skin, hair, and eyes. Other signs include lethargy, convulsions (seizures), tremors, diarrhoea, and death of the test animals. In this study, the test rats were examined for any of these toxicity symptoms. During 14 days of observation, no such manifestations were found in the test rats. Also, the administration of the ethanolic extract of *E. bulbosa* Urb bulbs at 2000 mg/kg bw did not cause mortality. The LD_{50} of this extract was found to be higher than 2000 mg/kg bw, which, according to the criteria for preparations set by BPOM RI (2014), is classified as "mildly toxic". For this reason, it is safe to suggest that relevant national or state agencies for food and drug controls, especially the ones in Indonesia, can authorise the mass production of preparations made of this ethanolic extract.

The histopathological sections presented in Figure 2 show that the treated rat's liver exhibited hydropic degeneration and no hepatocyte necrosis. Liver fatty degeneration is damage to hepatocytes marked by morphological changes and decreased organ function due to fat accumulation in the cytoplasm of liver cells, as apparent from the clear microscopic patches of fat. Similarly, the heart of the rats treated with the ethanolic extract at a limit dose of 2000mg/kg bw showed normal myocytes (Aiyalu & Ramasamy, 2016).

Conclusion

The LD_{50} of the ethanolic extract of *Eleutherine bulbosa* Urb is higher than 2000mg/kg bw, and no toxicity symptoms have been found.

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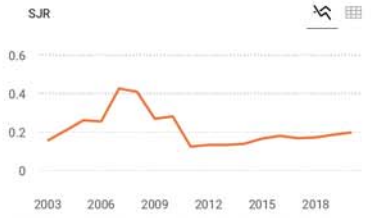

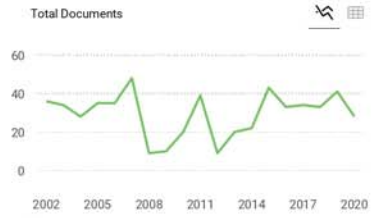

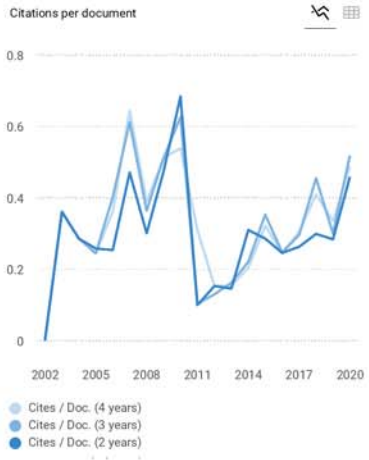

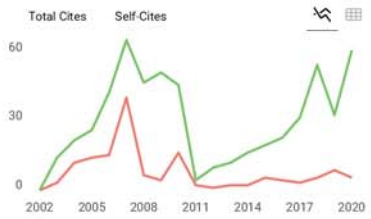

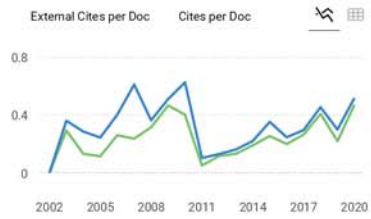

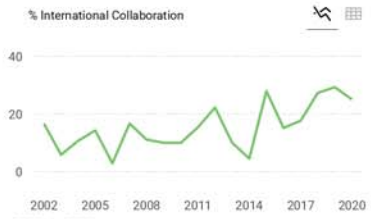

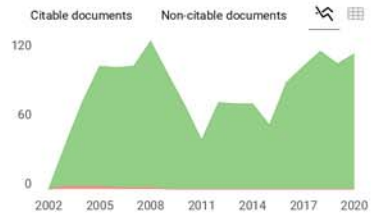

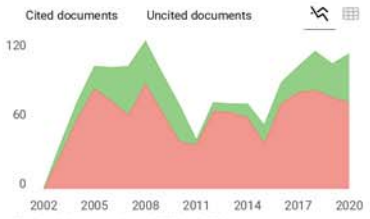

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