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Acute toxicity of ethanol extracts of *Gynura divaricata* L. leaves on DDY mice

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Abstract

Gynura divaricata (L.) DC., a traditional medicinal herb, has been used for the treatment of many diseases such as bronchitis, pulmonary tuberculosis, pertussis, toothache, rheumatic, arthralgia, uterine bleeding and diabetes in folk medicine. Despite its widespread use, studies on its toxicological characteristics are still very limited. The aim of this research is to observe the acute toxicity of ethanol extract of *Gynura divaricata* on Deutscland Denken Yoken (DDY) mice. The acute toxicity of ethanol extract of *Gynura divaricata* leaves was evaluated by giving it orally to DDY mice at single doses of 625, 1250, 2500, and 5000 mg/kg bodyweight. Animals were observed for mortality, body weight changes, macroscopic organ and relative organ weight for the next 14 days, and analyzed with ANOVA and Duncan post hoc test ($p<0.05$). The results shown ethanol extract of *Gynura divaricata* leaves did not caused deaths. The gross necropsy analysis did not reveal changes of the organs. Ethanol extract of *Gynura divaricata* also did not cause significant change in relative organ weight. In conclusions, ethanol extract of *Gynura divaricata* leaves acute administration were safe at the dose used ethnomedicinally and practically non-toxic. Higher doses may involve toxicological risks.

Keywords: *Gynura divaricata*; Acute toxicity; DDY mice; Practically non-toxic

1 Introduction

Many plants produce chemicals that can be extracted and utilized to make medications, or the plants themselves can be used as a pharmaceutical. However, the potential modifications generated by indiscriminate herb use can possibly harm normal tissues. *Gynura divaricata* (GD) is a traditional medicinal plant that is easy to obtain, can grow in all seasons, and has many benefits [1]. Some of the pharmacological activities of GD are hypoglycemic, antihypertensive, hypolipidemic, antiproliferative, antioxidant, and antitumor activities. GD appears to have obvious hypoglycemic and hypolipidemic effects, according to both traditional medicine and modern studies [2,3,4,5].

The phytochemical content of the GD leaves are polyphenols, flavonoids, steroids, tri-terpenoids, terpenoids and quinones [3]. GD also rich in chlorogenic acid and its derivatives that can effectively alleviate hyperglycemia and hyperinsulinemia while also improving pancreatic function, demonstrating that GD can be used as a viable food or drug for diabetes treatment by efficiently restoring pancreatic function [3,5].

However GD also contain hepatotoxic and carcinogenic substance which is pyrrolizidine alkaloids [6]. The cytotoxicity of GD was displayed weak activity at a concentration of 100 mg/mL [7]. Acute toxicity study of GD has not been carried out. Therefore, this study aims to observe the effect of acute administration of GD on DDY mice. Observations were made include the effect on weight and manner of death, macroscopic organ and relative organ weight after administration of single dose of GD.

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2 Material and methods

The research was conducted at the Animal Laboratory, Faculty of Medicine, Universitas Padjadjaran Bandung West Java Indonesia, which was held from August until November 2020.

2.1 Sample collection

GD leaves were obtained from Bumi Herbal Dago Farm, Bandung, Indonesia and identified as GD by the School of Life Sciences and Technology of Bandung Institute of Technology.

2.2 Preparation of ethanol extract of GD

Two kg of GD leaves were cleaned, crushed, and dried at a temperature of 70^{o}C. Before the maceration procedure was used, the dried leaves were ground in an electronic grinder at room temperature. Then, for 72 h, 1.3 g powder was mixed with 300 mL 95% ethanol, and the mixture was shaken for 5–10 minutes every 8 hours. The macerate was then removed from the dregs and filtered. The maceration process was carried out again and again until the macerate was clear. A rotary evaporator (Buchi®) was used to concentrate the ethanol extract [3].

2.3 Animal preparation

Ethical approval was obtained from the research ethic commission team of Universitas Padjadjaran Bandung West Java, Indonesia No.668/UN6.KEP/EC/2020. Ethical aspect of the research is based on three principal of Russel and Burch in Guide for the care and use of laboratory animal, they are reduction, replacement, and refinement [8].

Adult males (average weight 20±5 g) DDY mice were taken from the Animal Laboratory, Faculty of Medicine, Universitas Padjadjaran, Bandung, Indonesia. They were kept in well-ventilated cages at ambient conditions (temperature of 22 ± 3°C, 30-70% relative humidity, with a 12-hour dark/light cycle), with the same maintenance techniques and strict supervision were done during stage [8]. Each cage contained equal number of mice. Experiments were started after the animals acclimating for a week.

2.4 Acute toxicity test

Animal experiment divided randomize into five groups, each consisting of five mice. The control group was given only distilled water, four treatment groups were given the dosage of ethanol extract of GD with a dose of 625, 1250, 2500, and 5000 mg/kg body weight. The dose in this study was calculated using National Agency of Drug and Food Control, Republik Indonesia number 7 of 2014 recommendations with minor adjustments on dose toxicity tests for traditional materials (Generally Recognized As Safe/GRAS) by the level of toxicity of five for GD, which is a dose of 5 g/kg, implying that the calculation is practically non-toxic. The dosage was given orally in experimental animals, once during the test [9].

2.5 Mortality analysis

Every day until day 14 of the experiment, experimental animals were observed for toxic symptoms to check if any mice died. Mice that died were dissected immediately to determine the cause of death [10].

2.6 Body weight analysis

Every day till day 14th, the body weight of each mouse was recorded. To observe the effect of the test material on the growth of body weight, the data of average weight changes is compared between grups.

2.7 The relative organ weight (ROW)

The internal organs, such as the liver, spleens, lungs, heart, kidneys, and testes were examined in detail on day 15th, after animal experiment were sacrificed. They were examined for any abnormalities as well as the presence of any lesions caused by the ethanol extract of GD treatment. The organs were then carefully dissected, cleaned of any fats and weighed. The relative organ weight (ROW) of each organ was then calculated according to the following equation [11]:

$$ROW = (\text{absolute organ weight (g)} \times 100) / \text{body weight of mice on sacrifice day (g)}$$

2.8 Statistical analysis

Analyze the number of dead animals to determine the value of LD50, which is calculated according to the third edition of the Indonesian Pharmacopoeia. This method is used to evaluate the acute toxicity potential of ethanol extract of GD according to the criteria of M. N. Gleason (1964). The weight data and ROW were analyzed by ANOVA followed by Duncan's post-hoc test.

3 Results and discussion

The results showed that the administration of a single dose of ethanol extract of GD doses 625 to 5000mg/kg body weight did not cause death after 14 days of observation in both the control and treatment groups. From the results of this study, it can be determined that the LD50 value is more than 5000mg/kg body weight. Hence, ethanol extract of GD is included in the practically non-toxic category according to National Agency of Drug and Food Control [9]. Traditional medicine can be said to be safe if there is no death in the acute toxicity test in experimental animals after administration of a single dose of extract up to the 14 days observation.

Table 1 shows that the results of the comparison of weight changes analyzed in the treated group did not show a significant difference compared to the control group (p = 0.096).

Table 1 Body weight of mice in the acute toxicity study of the treated and control groups

Groups	Initial body weight (g) ±SD	Final body weight (g) ±SD	Body weight changes	P
Control	21.00 ± 1.04	25.60 ± 2.06	4.60 ± 1.54	0.096
GD 625 mg/kb	21.34 ± 1.02	23.23 ± 1.17	1.89 ± 0.80	
GD 1250 mg/kg	22.17 ± 0.94	24.00 ± 2.47	1.89 ± 2.90	
GD 2500 mg/kg	23.00 ± 0.61	25.17 ± 2.84	2.17 ± 2.76	
GD 5000 mg/kg	22.20 ± 1.17	23.26 ± 0.88	1.06 ± 0.76	

GD: Gynura divaricata

In this study, in addition to observing death and body weight, mice were also sacrificed on the 15th day to take vital organs from the mice. The organs taken were the heart, liver, kidneys, lungs, spleen and testes. These organs were observed macroscopically to assess whether there were abnormalities in these organs characterized by changes in shape and color. The results showed that there were no changes in the shape or color of the organs observed in either the control group or the treatment group (Table 2).

Table 2 Effects in GD treated and control groups on organ mascroscopically

Organ	Control	GD 625 mg/kg body weight	GD 1250 mg/kg body weight	GD 2500 mg/kg body weight	GD 5000 mg/kg body weight
Heart					
Liver					
Kidney					
Lungs					
Spleen					
Testes					

GD: Gynura divaricata

Table 3 shows that there was no significant difference in relative organ weight (p>0.05) between the treatment and the control groups. From the results of the analysis, ethanol extract of GD does not affect the relative organ weight of the heart, liver, lungs, kidneys, spleen and testes in mice. Based on the theory, in a toxicity test study, to determine whether the drug is toxic or not, that is by assessing the relative organ weight of experimental animals that have been given ethanol extract of GD at a predetermined dose and compared with the control group. The results of this study indicate that there is no difference in relative organ weight in the control group with the group given the treatment.

The results of this study are in line with previous research which showed that giving GD extract did not cause kidney function disorders [12]. However, the results of this study are different from previous studies that examined the effect of leaf extract of GD (*Gynura divaricata* L.) on SGOT and SGPT levels in female Sprague Dawley (SD) rats, a model of breast cancer. The results of previous study showed that administration of GD extract at a dose of 750 mg/kg/day for 14 days had a significant effect on the increase of SGOT levels and an insignificant effect on SGPT levels in the treatment group [13]. The results of a previous study conducted an oral acute toxicity test of the ethanol extract of Dewa leaves (*Gynura Pseudochina* L.) on the stomach conditions of male and female Wistar rats also shows that the content of flavonoids in GD at a dose of 1625 mg/kg/day given for 14 days has an inhibitory effect on lipoxygenase and cyclooxygenase which causes discontinuity in the gastric mucosal epithelium which can cause changes in the shape of the stomach organs [14].

There are several factors that cause differences in research results. One of them is that there is a group induced by a disease (breast cancer) in previous studies. It is suspected that the cause of death and organ disorder in this study was a disease induced in the group, besides that in the previous study GD ethanol extract was given with repeated doses, causing an increase in SGOT and SGPT levels in rats and causing death. In this study, each group was not affected or induced by any disease and the dose of GD ethanol extract in this study was carried out in a single dose, so that there were no deaths in each treatment group.

Table 3 Relative organ weight (ROW) of mice in the acute toxicity study of the treated and control groups

Organ	**Mean of ROW *(g)* ± *SD***					
	Control	**GD 625 mg/kg body weight**	**GD 1250 mg/kg body weight**	**GD 2500 mg/kg body weight**	**GD 5000 mg/kg body weight**	**p**
Heart	0.20± 0.04	0.22± 0.07	0.25 ± 0.05	0.24 ± 0.03	0.19 ± 0.11	0.530
Lungs	0.30 ± 0.74	0.24 ± 0.04	0.28 ± 0.06	0.28 ± 0.02	0.30 ± 0.08	0.607
Liver	1.94 ± 0.36	1.63 ± 0.09	1.86 ± 0.35	1.86 ± 0.48	2.06 ± 0.44	0.490
Kidney	0.50 ± 0.17	0.47 ± 0.07	0.48 ± 0.06	0.58 ± 0.13	0.51 ± 0.11	0.580
Spleen	0.62 ± 0.18	0.59 ± 0.17	0.58 ± 0.58	0.57 ± 0.08	0.60 ± 0.01	0.982
Testes	0.72 ± 0.36	0.63 ± 0.13	0.70 ± 0.26	0.83 ± 0.45	0.71 ± 0.17	0.877

In this study there were no changes in each organ observed, indicating that GD ethanol extract given to mice did not cause toxic effects, so it can be said that the traditional medicine of GD leaves is safe to use and belongs to the practically non-toxic group. Previous research showing an organ response to toxic substances has been confirmed by research by Praptiwi et al (2015) which showed that histopathological observations of administration of 2000 mg/kg of bisantraquinone compounds (+)-2.2'-episitoskirin A and (+)-1.1 '-bislunatin isolated from the endophytic fungus Diaphorte sp. In mice, it affects the tissue structure of organs, especially the liver and kidneys. Liver lesions show inflammation around the portal and central veins, fatty degeneration, and hepatocyte cell necrosis. Meanwhile, the kidneys experience protein leakage in the glomerulus which results in protein accumulation in the tubular lumen, degeneration and necrosis [13].

4 Conclusion

Ethanol extract of *Gynura divaricata* leaves acute administration were safe at the dose used ethnomedicinally and practically non-toxic.

Compliance with ethical standards

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Disclosure of conflict of interest

There is no conflict of interest.

Statement of ethical approval

This research has obtained ethical approval from the ethical commission of Universitas Padjadjaran Bandung West Java, Indonesia No.668/UN6.KEP/EC/2020.

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