Jurnal Kedokteran Hewan June 2022, 16(2):62-66
P-ISSN : 1978-225X; E-ISSN : 2502-5600 DOI: https://doi.org/10.21157/j.ked.hewan.v16i2.19310

SUBACUTE TOXICITY TEST OF BEACH CABBAGE (*Scaevola taccada* (Gaertn.) Roxb.) LEAF EXTRACT ON HISTOPATHOLOGICAL CHANGES IN THE LIVER AND KIDNEYS OF WHITE RATS

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ABSTRACT

The purpose of this study was to evaluate the toxic effects of beach cabbage leaves (*Scaevola taccada* (Gaertn.) Roxb.) by observing the histopathological changes in the livers and kidneys of male white rats. This study used 20 white rats which were divided into 4 groups: 1 control group and 3 groups receiving treatments in the form of *Scaevola taccada* leaf ethanolic extract (STLEE) in graded doses (200 mg/kg BW, 400 mg/kg BW, and 600 mg/kg BW). Toxicity test was performed by administering STLEE for 14 days. The results of daily observations showed that the experimental animals experienced diarrhea. Meanwhile, histopathological observations showed the occurrences of hydropic degeneration, fat degeneration, necrotic liver cells, and dilatation of Bowman's capsule in the animals' kidney cells. It was concluded that STLEE at a dose of 200 mg/kg BW is safe to use, but is toxic at a dose of 600 mg/kg BW, both to the livers and kidneys.

Key words: beach cabbage leaves, kidney, liver, *Scaevola taccada*, subacute toxicity

ABSTRAK

Penelitian ini bertujuan mengevaluasi efek toksik daun beruwas laut (Scaevola taccada (Gaertn.) Roxb.) dengan melihat perubahan histopatologi hati dan ginjal tikus putih jantan. Dalam penelitian ini digunakan 20 ekor tikus putih yang dibagi ke dalam 4 kelompok, 1 kelompok kontrol dan 3 kelompok pemberian ekstrak etanol daun beruwas laut (EEDBL) dosis bertingkat (200 mg/kg bobot badan, 400 mg/kg bobot badan, dan 600 mg/kg bobot badan). Pengujian toksisitas dengan pemberian EEDBL dilakukan selama 14 hari. Pengamatan harian menunjukkan hasil hewan uji mengalami diare. Hasil pengamatan histopatologi menunjukkan terjadinya degenerasi hidropik, degenerasi lemak, nekrotik pada sel hati dan terjadinya dilatasi pada kapsula Bowman pada sel ginjal. Disimpulkan bahwa EEDBL dengan dosis 200 mg/kg bobot badan aman untuk digunakan, namun bersifat toksik pada dosis 600 mg/kg bobot badan baik pada organ hati dan ginjal.

Kata kunci: daun beruwas laut, ginjal, hati, Scaevola taccada, toksisitas subakut

INTRODUCTION

World Health Organizing (WHO) reported that 80% of the population in developing countries and 65% of the population in developed countries prefer traditional medicine (Oktarlina 2018). According to Oktarlina (2018), around 40% of the total Indonesian population also prefer traditional medicine, with 70% of whom living in rural areas. Traditional medicine that utilizes natural ingredients for healing various diseases is popular in the community because they are easily obtainable and have low side effects (Joshua 2016). Beach cabbage (*Scaevola taccada* (Gaertn.) Roxb.) is one of the plants commonly used by the community for traditional medicine. Empirically, it has been used by the Pinrang community in South Sulawesi to treat diabetes, hypertension, and eye infection (Kosman 2012). The chemical content of a beach cabbage plant includes alkaloids, flavonoids, saponins, steroids, and glycosides (Rahmawati 2013).

The increasing use of traditional medicine has not yet been fully understood. It is often considered safe and less hazardous for the body. However, several studies revealed that some types of traditional medicine have toxic properties (Aminullah *et al*. 2019).

Further research and development need to be carried out with the aim that the use of beach cabbage leaves is safer and more effective. Based on the requirements set by Depkes RI (1994), traditional medicine must be safe, nutritious, and of high quality. Thus, to ensure the safety of the use of beach cabbage leaves as medicine, a toxicity test needs to be performed. The type of toxicity test that is commonly used is the subacute toxicity test, which is performed by giving multiple and repeated doses for 14-21 days (Rahardjo 2008). A toxicity test is designed to determine whether a substance in a biological system has toxic effects and to obtain specific dose-response data from the test preparation. The dose-response data obtained will be a source of information on the safe dose limit for use in medicine (BPOM 2014). Research by Nur (2018) has proven that the leaves of beach cabbage have analgesic and anti-inflammatory effects. However, little is known about the safe dose limit for its use. This study was therefore expected to be able to identify the safe dose of beach cabbage leaves and prevent toxicity.

MATERIALS AND METHODS

Sample Set up

The sample in this study was beach cabbage leaves collected from Mellenreng Beach, Bangko Hamlet, Panaikang Village, East Sinjai District, Sinjai Regency, South Sulawesi Province. They were purposively selected based on the following criteria: green, young, fresh, and intact.

Revised 08-03-2022, Accepted 16-04-2022

Sample Extraction

Five hundred grams of beach cabbage leaves in the form of dry powder were weighed and put into a vessel. Next, the extraction process was conducted using the maceration method with 70% ethanol as a solvent. The maceration process was carried out by dissolving the sample until it was completely submerged for 15 minutes and reached 2 liters. The sample was subsequently stored at room temperature for 3 x 24 hours and stirred occasionally.

Test Animal Set up

Based on the ethical approval protocol Number UH20100586 from the Medical Faculty of Hasanuddin University, the experimental animals used were male white rats (*Rattus nervegicus*) of the Wistar strain, weighing 200-300 g. Prior to the experiment, there was a one-week adaptation period for the animals.

Subacute Toxicity Test

Twenty male white rats were randomly divided into 4 groups: 1 control group and 3 test groups. Each group consisted of five rats. Group 1 was a negative control and Groups 2, 3, and 4 were test groups receiving *Scaevola taccada* leaf ethanolic extract (STLEE) at a dose of 200 mg/kg BW, 400 mg/kg BW, and 600 mg/kg BW. Treatments in the negative control group and the test groups were given for 14 days.

Histological Preparation

The harvesting of the animals' kidneys and livers was performed on day 15. Preparation and histopathological Hematoxylin-Eosin staining were conducted by referring to the procedure of the Maros Veterinary Center.

Observation of Histological Preparation

Histopathological images of the livers and kidneys of rats were observed using a light microscope with 40x magnification. The degree of degeneration and necrosis of each treatment was carefully examined here.

Data Analysis

Data analysis was carried out using a non-parametric test (Kruskal-Wallis) and the Post Hoc Mann Whitney test to evaluate the differences among the treatment groups. The scoring of liver tissue damage refers to the study of Setyaningsih *et al*. (2006), in which normal criteria include no hydropic degeneration, fat degeneration, and necrosis. The scores of the damage degree range from 0 (no damage), 1 (mild or there was 1 damage criterion between hydropic degeneration, fat degeneration, and necrosis), 2 (moderate or there were 2 damage criteria), to 3 (severe or there were 3 damage criteria). The scoring of kidney tissue damage refers to the study of Khalid *et al*. (2016) that employed the Endothelial, Glomerular, Tubular, and Interstitial (EGTI) scoring system. This scoring system is presented in Table 1 and Table 2.

RESULTS AND DISCUSSION

Results of Daily Observation

The results of the 14-day observation showed that the rats in each group experienced clinical symptoms of diarrhea. Groups 1 (aquadest) and 2 (STLEE 200 mg/kg BW) started having diarrhea on day 7. Meanwhile, the 400 mg/kg BW STLEE group and 600 mg/kg BW group began experiencing diarrhea on day 3. Table 3 and Figure 1 show daily observations of the rats' body

Table 1. Liver histopathological scoring system (Setyaningsih *et al*. 2006)

Damage	Score
No damage	0
Hydropic degeneration/ fat degeneration /necrosis	1
Hydropic degeneration + fat degeneration/necrosis	2
Hydropic degeneration + fat degeneration + necrosis	3

Table 2. Kidney histopathological EGTI scoring system (Khalid *et al.* 2016)

Tissue type	Damage	Score
Tubular	No damage	0
	Loss of brush border (bb) in less than 25% of tubular cells. Integrity of basal membrane	1
	Loss of bb in less than 25% of tubular cells, thickened basal membrane	2
	(plus) inflammation, cest formation, necrosis up to 60% of tubular cells	3
	(plus) necrosis in more than 60% of tubular cells	4
Endhothelial	No damage	0
	Endhothelial swelling	1
	Endhothelial distruption	2
	Endhothelial loss	3
Glomerular	No damage	0
	Thickening of Bowman's capsule	1
	Retraction of glomerular turf	2
	Glomerular fibrosis	3
Tubulo/ Interstitial	No damage	0
	Inflammation, haemorrhage in less than 25% of tissues	1
	(plus) necrosis in less than 25% of tissue	2
	Necrosis up to 60%	3
	Necrosis more than 60%	4

weights. Measurements of serum glutamic pyruvic transaminase (SGPT) and serum glutamic oxaloacetic transaminase (SGOT) levels for the liver, creatinine, and urea levels for the kidneys were also conducted.

Results of Observations and Discussion of Rats' Histopathology

Histopathological observations refer to observations of the structure of the liver and kidney tissue after administration of beach cabbage leaf extract for 14 days. The extract was administered for 14 days because this period was the fastest time to see potential toxicity. During the administration, clinical symptoms were observed, and the results are shown in Table 3. Diarrhea was experienced by all white rats in the STLEE 400 mg/kg BW and STLEE 600 mg/kg BW groups. This might be due to the stress that the rats experienced during the administration of the test preparation. Based on daily observations for 14 days, it was found that the administration of a combination dose of extract may be the cause of toxic symptoms. However, in this study, the administration of three levels of dose concentration did not cause death in white rats. The histopathological results are illustrated in Figure 2 and Figure 3.

The histological structure of the rats' livers in the negative control group that only received aquadest and the 200 mg/kg BW STLEE group appeared normal with no damage (score 0). In the 400 mg/kg BW STLEE group, mild-moderate damage was found in the liver cells of the rats (score 1-2), characterized by hydropic and necrotic degeneration. The STLEE 600 mg/kg BW group showed mild-moderate damage to rat liver cells (score 1-2), characterized by hydropic degeneration and fat degeneration.

Based on the above results, the histopathological images of the livers showed an increase in changes at a dose of 400 mg/kg BW and 600 mg/kg BW. This indicates that the safe dose of beach cabbage leaves for use is 200 mg/kg BW, and a dose of 400 mg/kg BW can be toxic to the liver. This is in accordance with the results of Adri (2021) study in which the use of beach cabbage leaves at a dose of 200 mg/kg BW was safe for the liver, proven by the examination results of blood biomarkers of SGPT and SGPT parameters at normal levels. Hydropic degeneration occurred in the STLEE 400 mg/kg BW and STLEE 600 mg/kg BW groups. Hydropic degeneration is a condition that most frequently occurs when there is damage to cells. This condition is reversible, suggesting that the cells can return to normal. Cell damage occurs due to disruption in the active transport process which reduces the ability of cells to pump Na+ ions, causing the cells to swell and the concentration of K+ to leave the cell (Insani 2015). Hydropic degeneration is also defined as a condition in which the cytoplasm of the cell contains water and has cytoplasm if observed microscopically (Fahmi *et al*. 2015).

The STLEE 400 mg/kg BW group also experienced fat degeneration, a condition that describes hepatocytes with lipid accumulation in the cytoplasm (Kelly 1993; Fahmi *et al*. 2015). This happens because of the formation of free radicals that damage the lipid membrane or because of toxic materials. The liver has important roles in fat metabolism, synthesizing and transporting lipoproteins. Impaired protein and phospholipid synthesis have the potential to inhibit lipoprotein synthesis and secretion. Long-term fat

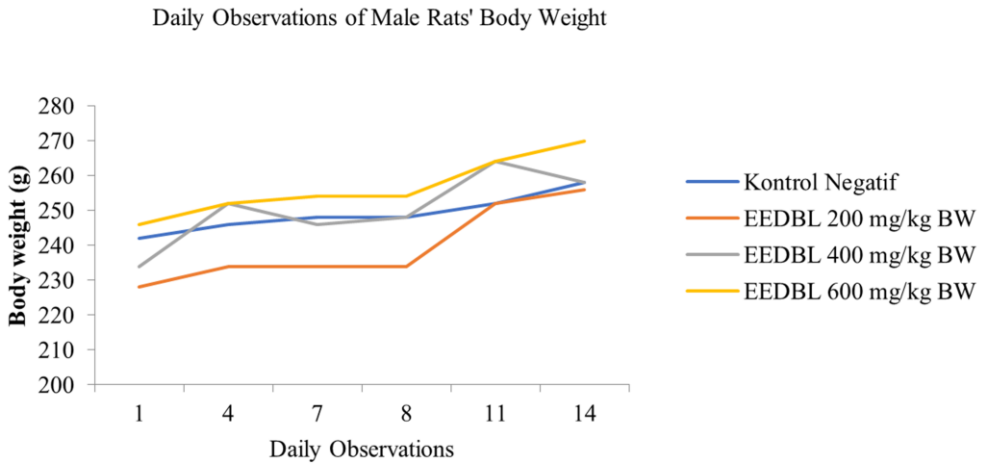

Figure 1. Diagram of daily observations of male rats' body weight for 14 days after the administration of *Scaevola taccada* leaf ethanolic extract (STLEE)

Table 3. The number of rats with clinical symptoms

Observations	Groups			
	Negative control (n= 5)	STLEE 200 mg/kg BW (n= 5)	STLEE 400 mg/kg BW (n= 5)	STLEE 600 mg/kg BW (n= 5)
Languid or tremor	0	0	0	0
Diarrhea	3	3	5	5
Pass away	0	0	0	0

STLEE= *Scaevola taccada* leaf ethanolic extract

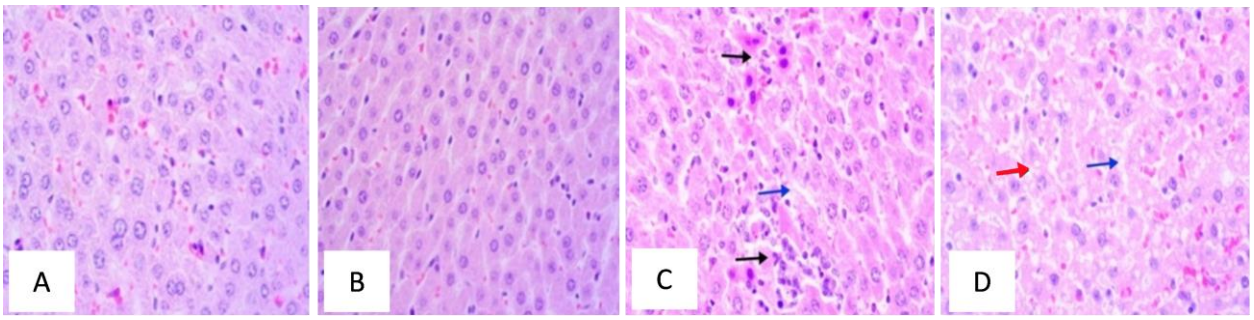

Figure 2. Histopathological result of white rats' liver. A= Negative control group; B= STLEE 200 mg/kg BW group; C= STLEE 400 mg/kg BW group; D= 600 mg/kg BW group; Blue arrow= Degeneration hydropic; Black arrow= Necrotic; Red arrow= Fat degeneration. HE 40x

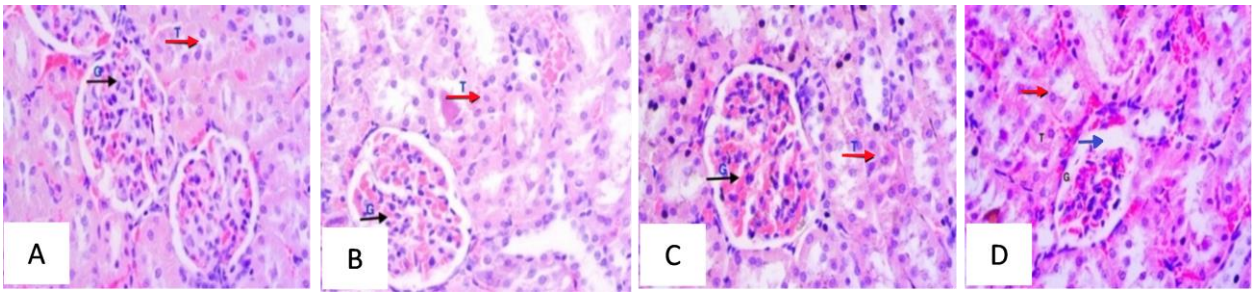

Figure 3. Histopathological result of white rats' kidneys. A= Negative control group; B= STLEE 200 mg/kg BW group; C= STLEE 400 mg/kg BW group; D= 600 mg/kg BW group; Red arrow= Tubulus; Black arrow= Glomerulus; Blue arrow= Dilatation of Bowman's capsule in the glomerulus. HE 40x

degeneration will lead to liver dysfunction, fibrosis, pigment accumulation, and nodular hyperplasia (Kelly 1993; Fahmi *et al*. 2015). Fat degeneration has a reversible nature, which means that cells can return to their original state. This is also often interpreted as the normal structure of cells before death because continued damage will be the beginning of necrosis. Necrosis is a morphological or structural change that takes place in cells with irreversible nature, in the sense that hepatocytes cannot return to their original state. Damage in liver cells can be the result of exposure to toxic substances or the intensity of exposure to a substance that continues to increase (Fahmi *et al*. 2015).

Histological structures of the rats' kidneys in the negative control group that only received aquadest, the 200 mg/kg BW STLEE group, and the 400 mg/kg BW STLEE group appeared normal with no damage (score 0). This is in accordance with Adri research (2021) which states that doses of 200-400 mg/kg BW of beach cabbage leaf extract are safe for kidneys, in which creatinine and urea levels are normal. The 600 mg/kg BW STLEE group showed mild damage to the kidneys (score 1), indicated by the dilatation of Bowman's capsule in the glomerulus leading to the shrinking of the glomerulus itself. The occurrence of Bowman's capsule dilatation is possible when the glomerular filtration membrane is damaged, resulting in increased capillary permeability that allows large molecules, such as proteins and red blood cells, to pass through the vascular wall (Mathlubi 2017).

Kidney histopathology based on the above results showed mild damage in the group receiving the extract with the highest dose of 600 mg/kg BW. This indicates that beach cabbage leaves are safe to use at the doses of 200 mg/kg BW and 400 mg/kg BW. In contrast, the 600 mg/kg BW dose is highly likely toxic to the kidneys.

Damage to liver and kidney cells found in this study might also be caused by the compounds present in the extract of beach cabbage leaves. One of the metabolites that likely have a toxic effect is flavonoid compounds. This is in accordance with the results of Sundaryono (2012) research stating that flavonoid compounds above 300 mg/kg BW can create toxic and teratogenic effects. Flavonoid compounds are widely known to have mechanisms as antioxidants, which in this case can lead to oxidative stress as a result of an imbalance in antioxidant content. Consequently, their cell number increases and interacts with cellular macromolecules. The interaction between antioxidants and cellular macromolecules can damage cells and lead to death (Aufia 2018).

CONCLUSION

Ethanol extract of beach cabbage (*Scaevola taccada* (Gaertn.) Roxb.) leaves with a dose of 200 mg/kg BW is safe to use. However, it becomes toxic at a dose of 600 mg/kg BW for both the liver and kidneys.

ACKNOWLEDGEMENT

We would like to thank our friends at the Phytochemical Laboratory and Biopharmaceutical Laboratory, Faculty of Pharmacy, Hasanuddin University, and the Maros Veterinary Center for their assistance in this research.

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