JURNAL BIOMEDIKA
Volume 14 No. 02 September 2021
P-ISSN: 1979 - 035X (printed) & E-ISSN: 2302 – 1306 (online)
DOI: https://doi.org/10.31001/biomedika.v14i2.1083

Subacute Toxicity Test of (*Scaevola taccada* Gaertn.) Roxb Leaf Extract on Kidney and Liver Function in Rats *(Rattus norvegicus*)

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ARTICLE INFO

Article History:
Received: September, 2020
Revise: June, 2021
Accepted: July, 2021

ABSTRACT

Beruwas laut leaf (*Scaevola taccada* (Gaertn.) Roxb has pharmacological effects such as anti-inflammatory, analgesic, antioxidant, antidiabetic and anticancer. Although beruwas laut leaf have many advantages, safety is the main requirement that herbal medicine must have. This research was aimed to prove sebacute of beruwas laut leaf (*Scaevola taccada* (Gaertn.) Roxb.) about the function of kidney and liver. This research used 20 rats were devided into 4 groups. Group 1 as a control group and groups 2,3, and 4 as an experimental group by administration of beruwas laut leaf extract with dose 200 mg/kgBB, 400 mg/kgBB, and 600 mg/kgBB. The extract was made using maceration and sebacute toxicity testing was carried out for 14 days. After giving the extract, some of rats had diarrhea. The results showed significant effect to increase levels of ureum and CGT after administration of dose 400mg/kgBB. Moreover, administration of dose 600 mg/kgBB caused significant effect in liver biomarkers and kidney (GGT and ureum). It was concluded that ethanol extract of beruwas laut leaf (Scaevola taccada (Gaertn.) Roxb.) with dose 200mg/kgBB showed safe but toxic to kidney and liver with dose 600mg/kgBB.

Keywords: sebacute toxicity; beruwas laut leaf, kidney; liver.

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INTRODUCTION

Traditional medicine that developed in Indonesia is a legacy from our ancestors. When conventional medicine has not yet entered this country, Indonesian people are more familiar with traditional healing methods, where traditional medicine is obtained from information from generation to generation as well as from various experiments on various kinds of plants that thrive in Indonesia. Indonesian traditional medicine usually comes from natural ingredients that are around the living environment (Murtie, 2013).

Indonesia is a country rich in biodiversity. Various plants thrive in this country. Some of them have been used by the community as traditional medicine. However, its effectiveness and safety have not been fully supported by research. Natural resources of traditional medicine are national assets that need to be explored, researched, developed and optimized for use (Depkes RI, 2000).

The use of traditional medicines that utilize natural ingredients in healing various diseases is more favored by the community. One of the plants or natural ingredients that are used by the community as medicinal ingredients is the leaf of the sea rib (Scaevola taccada (Gaertn.) Roxb.). Sea shellfish (Scaevola taccada (Gaertn.) Roxb.) is a plant that has been studied, its various pharmacological effects include anti-inflammatory, analgesic, antioxidant, anti-diabetic and anti-cancer. People in Pinrang district, South Sulawesi Province, call this plant by the name of Sawi Laut and have used this leaf to treat DM (Diabetes Melitus) or commonly called diabetes (Rahmawati, 2013).

The chemical components or content of this marine-based plant are in the form of alkaloids, flavonoids, saponins, steroids and glycosides (Rahmawati, 2013). The increasing use of traditional medicines by the community encourages the need for further research and development with the aim of making them safer and more effective. Although the leafy leaf plant (Scaevola taccada (Gaertn.) Roxb.) has many benefits, safety is the main requirement that herbal medicines must have. To determine the safety of using an herbal medicine, a toxicity test is needed (BPOM, 2014). One of the tests carried out for the safety of drug use is toxicity testing. Sub-acute/sub-chronic toxicity test is a test whose purpose is to evaluate the effect of compounds given to experimental animals repeatedly (Hendriani, 2007).

The principle of the subchronic oral toxicity test is that the test preparation in several dose levels is given to several groups of test animals with one dose per group and then the toxic effect is observed which leads to the death of the experimental animals (Lu, 1995). One of the problems that are often caused by drugs that are not monitored for use are acute kidney failure and acute liver failure. Acute kidney failure refers to a condition when the kidneys are damaged suddenly, so they cannot function. (Makris K & Spanou L, 2016). While failing. Acute liver disease can be caused by long-term use of drugs (Paracetamol, isoniazid), amanita phalloides mushroom, hepatitis B cytomegalovirus, Wilson's disease, Reye's syndrome (Widyati, 2014). The aim of this study was to determine whether the leaves of the sea nut (Scaevola taccada (Gaertn.) Roxb.) did not cause toxic effects on kidney and liver function, by looking at the values of blood urea and GGT.

MATERIALS AND METHODS

The equipment used includes a stirring rod, porcelain cup, beaker (pirex®), PLC series centrifuge, measuring cup (pirex®), watch glass, oral needle (cannula), measuring flask (pirex®), micropipette, capillary tube, iron horn spoon, blood tube, effendort tube, analytical balance (O'Hauss®), animal scale (Hang Viet Nam®), glass jar, rotary evaporator , syringe (Terumo®). The materials used include aquadest, picric acid, betadine, 70%

ethanol, technical ether, handscoon, cotton, sea nut leaf (Scaevola taccada (Gaertn.) Roxb.), blood samples, tissue, blood urea reagent and GGT reagent.

Sampling

Leaf ribs (Scaevola taccada (Gaertn.) Roxb.) were obtained from Mellenreng Beach, Bangko Hamlet, Panaikang Village, East Sinjai District, Sinjai Regency, South Sulawesi. The leaf samples of sea worms in this study were taken using the criteria for green, young, fresh and whole leaves.

Sample Extraction

The dried sea rib leaves were weighed as much as 500 grams, put into a vessel, then extracted by maceration method using 70% ethanol as solvent. In the meseration process, the sample was moistened with 70% ethanol until completely submerged for 15 minutes, after that it was added to 2 liters with 70% ethanol at room temperature for 3 x 24 hours while stirring occasionally.

Test Animal Setup

The experimental animals used were male white rats (Rattus nervicus) weighing 200-250 grams, acclimatized for 1 week in order to adapt to their environment.

Sub acute toxicity test

Animals were grouped randomly, namely the negative control group and the test group. Each group consisted of five tails. Group 1 as a control and groups 2, 3, and 4 as a test group were given a dose of marine nut leaf extract at a dose of 200 mg/kgBW, 400 mg/kgBW, and 600 mg/kgBW. The treatment of giving the negative control group and the test group was given for 14 days, with initial blood measurements on the first day and final blood measurements on the 14th day.

Biomarker measurement

Examination of blood urea biomarkers and GGT were performed before and after 14 days of extract administration. Blood samples were taken through the orbital vein, then the blood was centrifuged for 20 minutes at a speed of 2000 rpm. Then serum was taken and blood urea and GGT levels were measured using the Humalyzer 3500.

Data analysis

The data obtained from this test begins with a normality test using the Shapiro-Wilk test and continues with the One-Way ANOVA test. Furthermore, the Tukey HSD Post hoc test was conducted to determine the differences between groups.

RESULTS AND DISCUSSION

Rat Blood Biomarker Measurement Results

Before and after the administration of the leaf extract of the sea nut (Scaevola taccada (Gaertn.) Roxb.) blood was drawn through the orbital vein on the first day before treatment and on the 14th day after the extract was administered. The results of the examination of kidney biomarkers can be seen in Figure 1, and liver biomarkers in Figure 2.

Figure 1 shows that the blood urea of the rats did not increase significantly after being given the ethanol extract of the leaves of the sea nut (Scaevola taccada (Gaertn.) Roxb.) 200mg/kgBW compared to the control rats. However, after being given a dose of 400 mg, the blood urea of the rats increased significantly. Giving a dose of 600 mg/kg Sea Beruwas even increased blood urea significantly compared to the control group and the group that was given the extract in a low dose of 200 mg/kgBW.

Figure 2 shows that the blood GGT examination of rats has not increased significantly after being given the ethanol extract of the leaves of the sea nut (Scaevola taccada (Gaertn.) Roxb.) at a dose of 200mg/kgBW.

However, after administration of a dose of 400 mg/kg, the extract of Beruwas Laut triggered an increase in GGT, giving a higher dose even triggering a significant increase in biomarkers of liver damage when compared to control rats and rats given the extract in low doses (200 mg/kg).

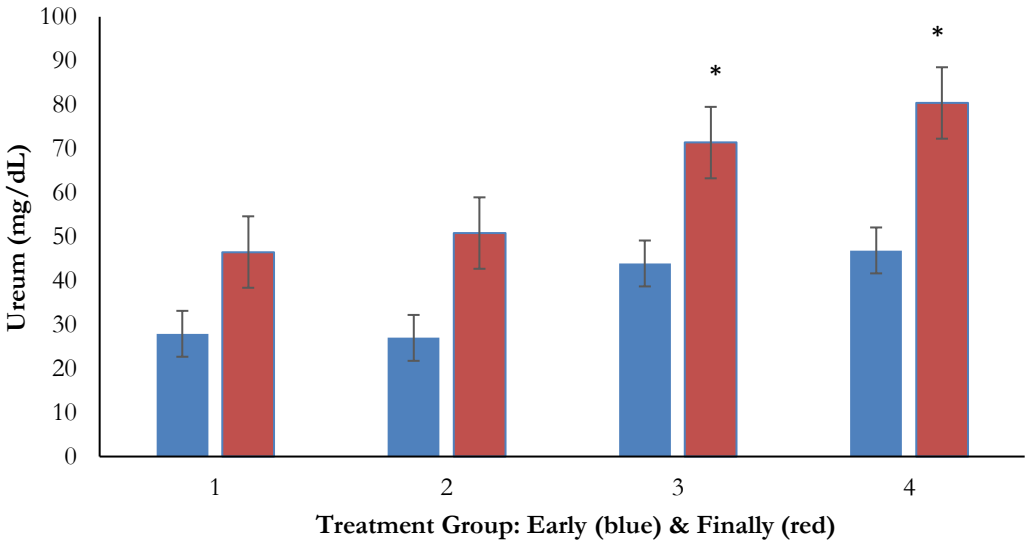

Figure 1. Urea value before (initial) and 14 days (end) treatment of experimental rats. Note: 1 = control, 2 = extract 200 mg/kg, 3 = extract 400 mg/kg, 4 = extract 600 mg/kg

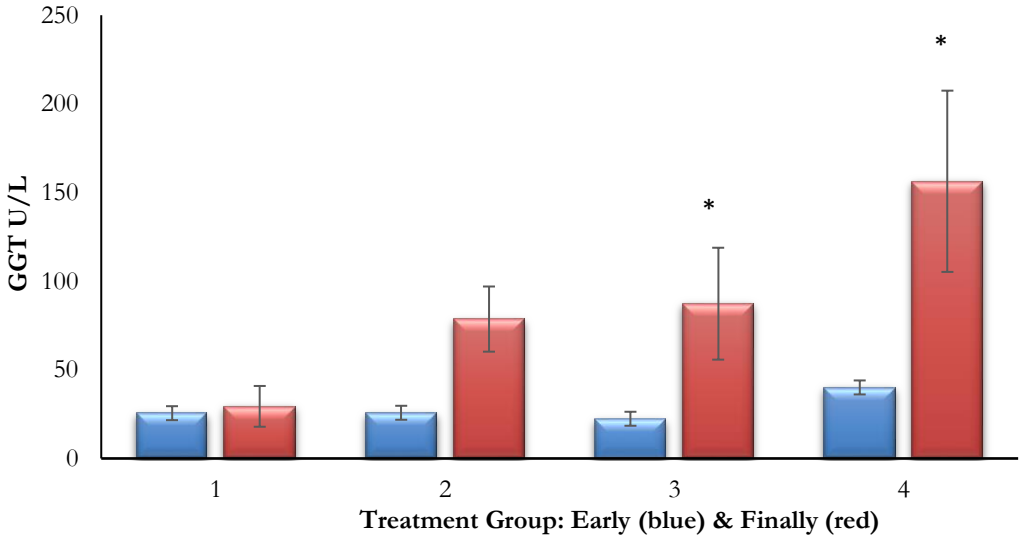

Figure 2. Urea values before (initial) and 14 days (final) treatment of experimental rats. Note: 1 = control, 2 = extract 200 mg/kg, 3 = extract 400 mg/kg, 4 = extract 600 mg/kg

One of the problems that are often caused by drugs that are not monitored for use are acute kidney failure and acute liver failure. Kidneys are organs of the body that have an important role in regulating the volume and composition of fluids in the body, removing many medicinal products and waste products from metabolic processes. The liver is the largest gland in the body that acts as a bridge between the gastrointestinal tract and other organs in the body, because the liver plays a role in the defense of the body's metabolic homeostasis and the detoxification of toxic substances. substances used for coagulation of large amounts of blood and the liver secretes or excretes drugs, hormones and other substances.

Based on some of the important roles of the kidneys and liver, great attention is paid to safety, selection and adjustment of drug dosages are needed to maintain good kidney and liver function. To determine the safety of using a synthetic drug and herbal medicine, toxicity testing is required. The aim of this study was to determine whether the leaves of the sea nut (Scaevola taccada (Gaertn.) Roxb.) did not cause toxic effects on kidney and liver function, by looking at the values of blood urea and GGT.

In this study, 4 treatments were used for the test animals, namely a negative control group (aquadest), group II ethanol extract of sea nut leaves 200 mg/kgBW, group III ethanol extract of sea nut leaf 400 mg/kgBW, and ethanol extract of sea nut leaf 600 mg/kgBW. The use of aquadest as a negative control was due to the ethanolic extract of the leaves of the seahorse soluble in water, so it did not need to be made in the form of a suspension.

Serum urea levels will increase along with a decrease in the filtering ability of the glomerulus. Creatinine levels reflect the most sensitive kidney damage because it is produced and excreted constantly by the body. The higher the level of creatinine and urea enzymes, the higher the level of damage to kidney cells. The normal value of rat urea is 15-21 mg/dL.

GGT is mainly found in the liver, kidneys and to a lesser extent in the prostate, spleen and heart. The liver is considered a source of the enzyme GGT. This enzyme is a specific marker for liver function and cholestatic damage than alkaline phosphatase (ALP). GGT will increase if there is drug poisoning such as barbiturates, phenytoin and alcohol. Normal values for SGPT = 7-56 U/L, SGOT = 5-40 U/L, and male GGT <94 U/L and female GGT <70 U/L.

Based on the results obtained by statistical tests, it was shown that the ethanolic extract of sea nut leaves at a dose of 200mg/kgBW was declared safe for the kidneys and liver. However, if the dose is increased to 400mg/kgBW, liver function disorders are indicated by looking at the increase in levels (GGT), an increase in the blood urea value at that dose may also indicate metabolic disorders due to the administration of a dose of 400mg/kg. Meanwhile, giving a dose of 600mg/kg was seen to significantly induce an increase in blood urea levels and an increase in liver biomarkers. This indicates damage to the liver and kidneys due to the administration of high doses of marine extract. Previously, there were studies that showed that the leaf extract of the sea nut (Scaevola taccada (Gaertn.) at doses of 200 and 400 mg/kgBW effectively suppressed the increase in serum levels of glutamate pyruvate transaminase, serum glutamate oxaloacetate transaminase and alkaline phosphatase. prevent lipid peroxidation and restore antioxidant enzymes in cancer patients.However, with our research, it appears that a dose of 400 mg/kgBW has begun to be unsafe to use because it can trigger an increase in GGT and urea levels, and can even cause liver and kidney damage when used in a dose of 600 mg/kg body weight.

CONCLUSION

The ethanol extract of the leaves of the sea nut (Scaevola taccada (Gaertn.) Roxb.) at a dose of 200mg/kgBW was declared safe for the

kidneys and liver, but started to increase blood urea levels significantly after a dose of 400mg/kgBW, even being toxic to the kidneys and liver. at a dose of 600 mg/kgBW which was characterized by a significant increase in urea and GGT.

CONFLICT OF INTEREST

We have no conflict of interest related to this work.

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