Acute toxicity test of kenikir leaf (Cosmos caudatus H.B.K) ethanolic extract on Wistar white male rats with fixed dose procedure method and its effect on histopathology of pancreatic cells

Abstract

In this study, acute toxicity of kenikir leaf (Cosmos caudatus HBK) ethanolic extract was conducted on Wistar white male rats with fixed dose procedure. The extraction method used was maceration using 70% ethanol. A dose of 2000 mg/kgBW was determined as the starting dose based on the preliminary test result. The rats used in the main test were divided into the normal group and the 2000 mg/kgBW dose group, each used five animals in each group. The results of the test showed that there were no deaths or toxic symptoms both of the normal group and the 2000 mg/kgBW dose group. The conducted preliminary test results a dose of 2000 mg/kgBW as the starting dose. Next, main test is done toward two groups of rats: Normal group and 2000 mg/kgBW dose group each consist of five animals. The main test results in neither deaths nor toxic symptoms from those groups. The range of toxic doses of kenikir leaf ethanolic extract that can cause acute toxicity is >2000 mg/kgBW, and hence, this experiment classified in the practically nontoxic category. Statistical analysis on effect to macroscopic organs of the liver, heart, and kidney showed no significance (P > 0.05) from kenikir leaf ethanolic extract. Average levels of biochemical parameters from the 2000 mg/kgBW group and the normal group were in the normal range. Ethanolic extract at a dose of 333 mg/kg BW does not cause severe pancreatic damage, and hence, it is considered safe for use as an antidiabetic.

Key words: Acute toxicity, Cosmos caudatus HBK, fixed dose procedure, kenikir leaf

INTRODUCTION

Knowledge about the efficacy and safety of medicinal plants in Indonesia is usually only based on empirical experience which is usually passed down from the generation to generation and has not been scientifically tested. For this reason, research on traditional medicine is needed, so that later the drug can be used safely and effectively.[1]

Kenikir (Cosmos caudatus) are plants that are often found in the surrounding environment, as well as plants that are already familiar and have been widely consumed as vegetables. Kenikir leaf (C. caudatus) is used as a cytotoxic...
with $IC_{50}$ was 344.91 µg/ml, as antioxidants with $IC50$ was 31.97 ± 1.42 µg/ml, and as antidiabetic that reduction in plasma blood glucose. Kenikir leaf contains phenolic acids, flavonoids, carotenoids, sesquiterpene lactone, vitamins, and phenylpropanoids. The safety of using kenikir leaf as natural medicinal preparations must be supported by the scientific research. One of the studies that must be done is toxicity testing.

The method used in this toxicity test is the fixed dose procedure. Fixed dose procedure is an alternative method in toxicity testing. This method is used because the number of test animals used in determining the final parameters is not more than conventional methods, so it does not conflict with animal welfare.

Based on this, it is necessary to do an acute toxicity test to determine the potential toxicity of ethanolic extract kenikir leaf against white male wistar rats, so as to provide basic information for consideration in the use of these plants as medicinal ingredients. The biochemical parameters observed in this toxicity test were Serum Glutamic Pyruvic Transaminase (SGPT), Serum Glutamic Oxaloacetic Transaminase (SGOT), creatinine, and ureum.

**MATERIAL AND METHODS**

**Extraction**

Ethanolic extract of kenikir leaf was carried out by the maceration method using 70% ethanol. The 1 kg of simplicia powder is put into a dark glass jar and macerated for 3 days. Solvent replacement was carried out two times and then remaceration for 1 day. Thereafter, the filtrate was evaporated under reduced pressure at 70°C.

**Animal ethical clearance**

This research had approval for the methodology and concerned ethical issues by Animal Research Ethics Committees, RSMH-FK UNSRI – Indonesia (No. 555/kepkrsmhfkunsri/2019).

**Preliminary test**

The starting dose in the preliminary test can be chosen from the level of fixed dose: 5, 50, 300, and 2000 mg/kgBW as the dose expected to cause toxic effects. Observation is routinely carried out in the first 4 h after administering the dose for 24 h. The observation interval is at least 24 h at each dose.

**Main test**

The starting dose was derived from the preliminary test results. If death occurs at the starting dose, the dose is reduced; if there are no death or toxicity symptoms, then the main test is followed by increasing the dose. Observation is routinely carried out in the first 30 min after the administration of the test preparation and periodically every 4 h for the first 24 h then once a day for 14 days. Observations made include the presence or absence of death or toxic symptoms exhibited by test animals.

**Observation**

The things that must be observed in the observation period are number of animals that experience toxic symptoms, such as changes in animal behavior and number of animals that died during the test, and also, observations of macroscopic organs in the form of changes in shape, color, and weight of organs (especially the liver, kidneys, and heart). In addition, measurements of biochemical parameters were also carried out in the form of SGOT, SGPT, creatinine, and urea in the blood of test animals, and histopathological observations were made on pancreatic organs.

**Determination of biochemical parameter levels**

The determination of SGOT and SGPT levels was carried out using a Clinical Chemistry Analyzer at 340 nm wavelength. The determination of creatinine levels was performed using a Clinical Chemistry Analyzer at a wavelength of 550 nm. Determination of urea levels was performed using a Clinical Chemistry Analyzer at a wavelength of 578 nm. Urea levels were measured using three working reagents.

**Data analysis**

Analysis of research data is processed by the SPSS data processing application. The results of the study were statistically analyzed by the normality test. For data on organ weights, SGOT, SGPT, creatinine, and urea levels, an independent t-test was performed.

**RESULTS AND DISCUSSION**

**Extraction**

The result of ethanolid extract yield was 16.37%. The results indicate that the extraction yields increased with the same polarity with solvent, reduced particle size, and increased of temperature.

**Phytochemical screening**

Phytochemical tests include flavonoids, alklaoids, saponins, tannins, phenolics, steroids, and triterpenoids. Positive results are indicated by the presence of a color change reaction, the formation of cediment, and the formation of foam in the test solution.

Phytochemical screening results in Table 1 show that the extract of kenikir leaf contains flavonoids, saponins, tannins, phenolics, and steroids.

**Preliminary test**

The animal used in this study was Wistar white male rats. Ethical approval was published by the Ethics Committee Mohammad Hoesin Central General Hospital and Faculty of Medicine Sriwijaya University. The result of preliminary test
is showed in Table 2. Based on the results in Table 2 shows that there are no toxic symptoms such as backward walk, stomach walk, tremor, diarrhea, salivation, and weakness. The main test adopted the dose of 2000 mg/kgBW because there were no deaths and toxic symptoms up to that dose.[9]

Main test
The chosen starting dose for the main test is derived from the results obtained in the Table 2, and the result of main test is showed in Table 3.

From Table 3, it shows that after 14 days’ period of observation, there were no deaths or toxic symptoms in the test animals. This showed that the preparation test did not have an acute toxicity effect. Detectable test animals are all test animals that survive to the end of the main test. Before being detroped, the test animals are weighed first and then sacrificed. Observations were made on macroscopic liver, kidneys, and heart of test animals which had been detroped.

The observations of liver, kidney, and heart showed that there were no differences in the shape and color of the liver, kidneys, and heart in the normal group rat and the rat group dose of 2000 mg/kgBW. The results of observations on the liver showed that macroscopic from the liver of the normal group and the dose group 2000 mg/kgBW were dark red, smooth surface, and supple consistency. A normal liver has a flat, smooth surface, and dark red, whereas an abnormal liver has a mottled surface, cysts, and changes in color.[9] This shows that the liver of the normal group and the 2000 mg/kgBW dose group is macroscopically normal.

Pancreatic histopathology observations
The sample used in the pancreatic histopathological observation was namely the normal group, the dose group 333 mg/kgBW, and the dose group 2000 mg/kgBW. The dosage of 333 mg/kgBW is ED50 from the ethanol extract of kenikir leaves as antidiabetic.

Microscopic observations showed that the condition of the pancreas in mice in the normal group was healthy and undamaged [Figure 1a]. This is proven by the degree of damage from the pancreatic rats in the normal group is 0 (no damage to the island of Langerhans). The degree of pancreatic damage in mice in the dose group 333 mg/kgBW was 2 (damage ¼ Langerhans Island). This shows that the condition of the pancreas in rats in the group dose 333 mg/kgBW [Figure 1b] was protective activity. In Figure 1c, it can be seen that the condition of the pancreas of rats in the dose group of 2000 mg/kgBW appears to be relatively severe damage as indicated by the empty space of Langerhans Island reaching more than half.[10]

Based on the results of these microscopic observations, it can be seen that the administration of ethanol extract kenikir leaves at a dose of 333 mg/kgBW does not cause severe pancreatic damage so it is considered safe for use as antidiabetic.

Biochemical parameter level check
The results of measurements of the average levels of SGOT, SGPT, creatinine, and urea in the normal group and the dose group of 2000 mg/kgBW are seen in Table 4. Normal levels of SGPT for white mice are ≤ 134.57 U/L,[11] while the normal levels of SGOT are 45–90 U/L, creatinine are 0.3–0.8 mg/dL, and urea are 12–18 mg/dL.[12]

By comparison to the literature, the average levels of SGOT, SGPT, creatinine, and urea were measured from the 2000 mg/kgBW dose group and the normal group are concluded within the normal range, which can be seen in Table 4.

The increase in creatinine levels is not only caused by the administration of kenikir leaf ethanol extract. Protein levels in feed can affect the increase in creatinine levels. This is because creatinine is a nitrogenous amino acid (glycine, L-arginine, and S-adenosyl-L-methionine) which is involved in energy transfer in the form of phosphocreatine and metabolized to creatinine to be excreted by the kidney.[13,14]
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The results of the SGOT, SGPT, creatinine, and urea levels were processed and analyzed with the SPSS 21.0. The normality test results showed that the all data were normally distributed so that it could be followed by an independent t-test to compare 2 different treatment groups, namely the normal group and the 2000 mg/kgBW dose group. While the normality test results on SGPT level data indicate that SGPT level data are not normally distributed.

Independent t-test (Independent t-test) was carried out to statistically analyze the SGOT, creatinine, and urea levels of test animals. An independent t-test was performed to compare two groups with different treatments, namely the normal group and the 2000 mg/kgBW dose group. Statistical analysis showed that there was no significant difference ($P > 0.05$) in the levels of SGOT, creatinine, and urea between the normal group and the dose group of 2000 mg/kgBW.

In the results of the Mann–Whitney test, statistical analysis showed that there were no significant differences ($P > 0.05$) in SGPT levels between the normal group and the 2000 mg/kgBW dose group. By the results of statistical tests on SGOT, SGPT, creatinine, and urea levels, it can be concluded that there was no significant effect from administering ethanolic extract of kenikir leaves at a dose of 2000 mg/kg body weight on levels of SGOT, SGPT, creatinine, and urea in test animals.

**CONCLUSION**

Ethanolic extract of kenikir leaf at a dose 2000 mg/kgBW was chosen as starting dose for the main test. In this dose, the liver is macroscopically normal; the levels of SGOT, SGPT, creatinine, and urea are concluded within the normal range. However, the condition of the pancreas of rats in the dose group of 2000 mg/kgBW appears to be relatively severe damage. Hence, the administration of ethanolic extract of kenikir leaf at a dose of 333 mg/kgBW (ED50 from ethanol extract of kenikir leaf) considered safe for use as antidiabetic because did not cause severe pancreatic damage.

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**Conflicts of interest**

There are no conflicts of interest.

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