ACUTE TOXICITY STUDY OF THE LEAVES ETHANOLIC EXTRACT OF PICRIA FEL-TERRAE LOUR.

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INTRODUCTION

Picria fel-terrae belongs to family Linderniaceae is also known as Pugun tano by Indonesian people. The ethanolic extract of plant leaves has several potential pharmacological activities including antiarthritic, antihelminthic, and antioxidant. However, the toxicity of the plant extract is rarely explored. This work was to investigate toxicity of the leaf ethanol extract of P fel-terrae on Artemia salina and male mice.

METHODS

Acute toxicity of the plant extract was studied by in vitro and in vivo methods. In vitro study was carried out by exposing nauplii to the plant extract at concentrations of 10, 100, 200, 500, and 1000 µg/ml for 48 h. In vivo study was performed on male mice that divided into four groups. Groups I, II, III, and IV were treated with sodium carboxymethyl cellulose 0.5%, the ethanolic extract of plant leaves at doses of 1000, 2000, and 5000 mg/kg bw, respectively. The animal toxic symptoms were observed every day for 14 days. On day 15, the blood of mice was collected to measure alanine aminotransferase, aspartate aminotransferase, and creatinine levels. The effects of plant extract on vital animal organs such as heart, liver, and kidney were also studied. Statistical analysis of data was performed using analysis of variance and followed by Tukey post hoc.

RESULTS

The results showed that the leaf ethanol extract of P. fel-terrae have weakly toxicity on A. salina with the LC50 of 76807 µg/ml. At in vivo studies, the toxic symptoms of mice were not identified during experiment with all doses of the plant extract for 14 days. In addition, aspartate aminotransferase and creatinine levels were no significantly different between control and all treatment groups (p>0.05). However, alanine aminotransferase level changed when mice were exposed by the plant extract at the doses of 2.000 and 5.000 mg/kg bw. Although the mice were not dead during experiment, the animal organs such as heart, liver, and kidney were histologically changed.

CONCLUSION

This study suggests that the ethanolic extract of P. fel-terrae leaves has weakly toxicity on A. salina and causes histological changes on male mice organs at the high doses.

Keywords: Picria fel-terrae, Linderniaceae, Acute toxicity, Indonesia.

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Preparation of plant extract
Extraction method from Patilaya et al. (2017) was adopted in this study. Briefly, the leaves powder of P. fel-terrae was percolated in 96% ethanol at room temperature and then dried to obtain the crude extract. The extract was kept in dark bottle at 4°C until used for experiment [9].

Ethical clearance
This study was approved by the Ethical Committee of the Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Sumatera Utara, Medan, Indonesia (No. 576/KEPH-FMIPA/2017).

In vitro acute toxicity testing
In vitro toxicity of the extract of plant leaves was determined using brine shrimp lethality test (BSLT) method. The eggs of Artemia salina were hatched in a vessel containing original seawater. The vessel was facilitated with good aeration and kept under the lamp for 48 h at room temperature. 1 ml the seawater containing 10 hatched nauplii were transferred into tube to yield a series of concentration of the plant extract of 10, 100, 200, 500, and 1000 ppm. The vehicle was used as negative control. In each case, three replicates of each concentration were assayed. The tubes were kept under the lamp for 24 h at room temperature. After 24 h, the tubes were observed and the dead nauplii from each tube were counted and percentage of death calculated. Median lethal concentration (LC50) of the plant extract was obtained using probability unit (probit) analysis [18].

In vivo acute toxicity testing
In vivo toxicity of the plant extract was studied on male mice. The healthy animal was acclimated at room temperature for 8 days and divided into four groups where each group consisted of five mice. The groups were separately treated with control, 1000, 2000, and...
5000 mg/kg bw respectively. Animal death was observed for 24 h, while the toxic symptoms such as tremor, diarrhea, salivation, and body moving were observed for 14 days. On day 15, the mice were sacrificed and followed by the blood collection to measure alanine aminotransferase, aspartate aminotransferase, and creatinine levels. The effects of plant extract on vital animal organs such as heart, liver, kidney, pancreas, spleen, lung, and testis were histologically also observed under light microscopy [19,20].

RESULTS

In vitro acute toxicity study

The results of brine shrimp lethality assay of ethanolic extract of P. fel-terrae leaves are shown in Table 1. The plant extract has median lethal concentration (LC₅₀) of 768.07 µg/ml.

In vivo acute toxicity study

Toxic symptoms and animal organ weights

The results showed that the toxic symptoms such as tremor, diarrhea, salivation, normal body moving, and animal death were not identified during experiment.

Effects on biochemical parameters

Biochemical parameters of the mice during experiment were described in Table 2. The results indicated that administration of the leaves ethanol extract of P. fel-terrae to the animal caused the increasing of alanine transaminase (ALT), aspartate transaminase (AST), and creatinine levels.

Histopathological study

In vivo acute toxicity effects of the plant extract on animal organ were described in Figs. 1-3. As shown in Fig. 1, hyperemia and bleeding are found on heart when the mice were treated by the plant extract at doses of 1000, 2000, and 5000 mg/kg bw.

Histopathological features of animal lever (Fig. 2) showed on the normal level have central vein at the lobes, sinusoids, hepatocytes with radial in form, and cell nucleus surrounded by cytoplasm. However, administration of the plant extract at the doses of 1000 and 2000 mg/kg bw induced histopathological changes of the animal lever such as hydropic changes of hepatocytes and infiltration of inflammatory cells to the central vein. Congestion of the central vein occurred when the plant extract dose was increased to 5000 mg/kg bw.

Fig. 3 describes histopathological of mice’s kidney. The normal kidney has glomerulus, Bowman's capsule, and proximal tubules. Histopathological changes as glomerulus and proximal tubules degenerations observed when the mice receive the plant extract at the dose of 1000 mg/kg bw. Besides these changes, infiltration of leukocytes in proximal tubules also occurred when the animal was exposed by the plant extract at 2000 mg/kg bw. The results also indicated that the plant extract at the dose of 5000 mg/kg bw caused proximal tubular damage and glomerulus shrinkage.

DISCUSSION

Meyer et al. have introduced a general toxicity method using the larvae of brine shrimp [18]. This method is very good because it can be easily employed, low cost requires small amounts of test material, and simple to perform [21]. In addition, the shrimp eggs can also be obtain from markets and can remain to be used for many years when stored in appropriate conditions [22]. The general toxicity activity was considered weakly toxic when the LC₅₀ was 500-1000 µg/ml moderate toxic when the LC₅₀ was 100-500 µg/ml and designed as strong toxic when the LC₅₀ ranged from 0 to 100 µg/ml [23,24]. The LC₅₀ value above 1000 µg/ml was classified as non-toxic [25]. In this study, the leaves ethanolic extract of P. fel-terrae exhibits the LC₅₀ of 768.07 µg/ml which indicates to have weakly toxicity on A. salina. According to Kumarasingha et al., the fraction from whole plant extract of P. fel-terrae at the concentration <0.015 mg/ml exhibited insignificantly cytotoxicity in human mammary epithelial cell line [26].

The animal study using mammals such as rabbit or mice can be performed to predict safety of plant extract in human because its metabolism system similar to the human [27]. In vivo acute toxicity study

![Fig. 1: Histopathological changes of animal heart during treatment with the leaves ethanol extract of Picria fel-terrae](image1)

(a) control; (b) plant extract at 1000 mg/kg bw; (c) plant extract at 2000 mg/kg bw; (d) plant extract at 5000 mg/kg bw; (1) cell nucleus; (2) blood vein; (3) hyperemia; (4) bleeding

![Fig. 2: Histopathological changes of animal lever during treatment with the leaves ethanol extract of Picria fel-terrae](image2)

(a) control; (b) plant extract dose of 1000 mg/kg bw; (c) plant extract dose of 2000 mg/kg bw; (d) plant extract dose of 5000 mg/kg bw; (1) central vein; (2) hepatocyte; (3) sinusoid; (4) infiltration; (5) hydropic change; (6) bleeding
of substances can be studied by observing the toxic symptoms of animal tested including the animal death, biochemical properties, and histopathological changes [28]. In this study, nevertheless, the animal death not found, but there are the increasing of ALT, AST, and creatinine levels and also histopathological changes of the mice's organs. The biochemical properties changes could be the indication of alteration or damage in some tissues, organs, and systems of mice [29]. According to Dalimunthe et al, the administration of ethanolic extract of P. fel-terrae leaves at the single doses of 2000 and 5000 mg/kg bw causes no toxic symptoms on albino mice [16]. Cucurbitacins, a chemical compound of the plant reported to have toxicity effects to animals and even human [30]. According to Gry et al. (2006), cucurbitacin derivatives have LD₅₀ between 5 and 340 mg/kg bw in mice after oral administration [31]. To the best of our knowledge, this is the first time that the effect of ethanol extract of P. fel-terrae leaves on histopathological features of experimental animal has been studied. The histopathological changes found in this study showed that the plant extract-induced damage to the heart, liver, and kidney of mice. However, further studies are needed to identify the mechanism of mice organs damages.

CONCLUSION

This study suggests that the leaves ethanolic extract of P. fel-terrae has weakly toxicity which causes histopathological changes on the vital organs of mice.

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