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Research Article

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

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ABSTRACT

Background: *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 antidiabetic, anthelmintic, and antioxidant. However, the toxicity of the plant extract is rarely explored. This work was to investigate toxicity of the leaf ethanolic 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 ethanolic extract of *P. fel-terrae* to have weakly toxicity on *A. salina* with the LC_{50} of 768.07 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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INTRODUCTION

Picria fel-terrae belongs to family *Linderniaceae* is also known as Pugun tano by Indonesian people [1]. This plant is an annual herb that grows up to 1 m with leaves oppositely arranged, egg-shaped with blunt teeth margins, and covered with short soft hair. Its stems laxly branched, erect, or prostrate, and have rooting at the nodes. Conventionally, the leaves of plant have been used for the treatment of abdominal pain, cough, helminthiasis, and asthma [2].

The leaves of *P. fel-terrae* contain glycosides [3,4], flavonoids [5], saponins [6], and terpenoids [7]. Ethanolic extract of the plant has pharmacological activities such as anthelmintic [8,9], antidiabetes [10,11], anticancer [12-14], cardioprotective [15], diuretic [16], and inhibition of muscarinic receptor [17]. Nevertheless, according to the best of our knowledge, there is only few information regarding to the plant extract toxicity. This work was conducted to study the acute toxicity of the leaves ethanol extract of *P. fel-terrae*.

METHODS

Plant materials

P. fel-terrae was collected from Dairi district, North Sumatra Province, Indonesia. The plant specimen was identified and deposited by Herbarium Medanense, Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Sumatera Utara, Medan, Indonesia.

Chemicals

Chemicals used in this work, namely, 96% ethanol, sodium carboxymethyl cellulose, tween-80, hematoxylin, and eosin, were obtained from Merck, Germany.

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. 578/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 (LC_{50}) 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 (LC_{50}) 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 LC_{50} was 500–1000 µg/ml, moderate toxic when the LC_{50} was 100–500 µg/ml and designed as strong toxic when the LC_{50} ranged from 0 to 100 µg/ml [23,24]. The LC_{50} value above 1000 µg/ml was classified as non-toxic [25]. In this study, the leaves ethanolic extract of *P. fel-terrae* exhibits the LC_{50} 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

Table 1: Median lethal concentration (LC_{50}) of the leaves ethanol extract of *P. fel-terrae* on nauplii (*A. salina*)

No.	Experiment	Regression equation	LC_{50} (µg/ml)
1.	I	y=2.4573x+1.9301	661.01
2.	II	y=2.2729x+1.5444	757.39
3.	III	y=2.3149x+1.8228	885.81
		Mean	768.07
		Standard of error	65.11

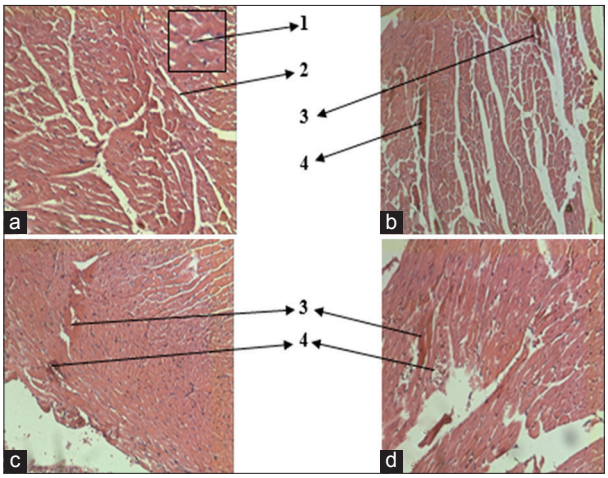

Fig. 1: Histopathological changes of animal heart during treatment with the leaves ethanol extract of *Picria fel-terrae* (a) control; (b) plant extract at 1000 mg/kg bw; (c) plant extract at 2000 mg/kg bw; (d) plant extract at 5000 mg/kg bw; (1) cell nucleus; (2) blood vein; (3) hyperemia; (4) bleeding

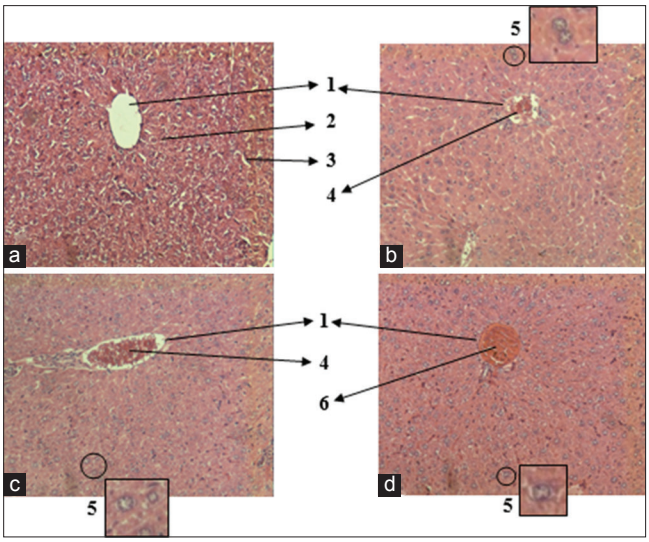

Fig. 2: Histopathological changes of animal lever during treatment with the leaves ethanol extract of *Picria fel-terrae* (a) control; (b) plant extract dose of 1000 mg/kg bw; (c) plant extract dose of 2000 mg/kg bw; (d) plant extract dose of 5000 mg/kg bw; (1) central vein; (2) hepatocyte; (3) sinusoid; (4) infiltration; (5) hydropic change; (6) bleeding

Table 2: The ALT, AST, and creatinine level of mice during treatment

Treatments	Biochemical parameters		
	ALT level (IU/L)	AST level (IU/L)	Creatinine level (mg/dl)
Control	159.33±18.05	195.33±46.60	0.22±0.04
EEDPT 1000 mg/kg bw	179.33±10.27*	262.66±41.45*	0.32±0.01*
EEDPT 2000 mg/kg bw	210.67±5.78*	297.66±20.22*	0.32±0.08*
EEDPT 5000 mg/kg bw	247.33±12.91*	328.33±31.47*	0.49±0.07*

*Statistically significant different with control (p<0.05). ALT: Alanine transaminase, AST: Aspartate transaminase

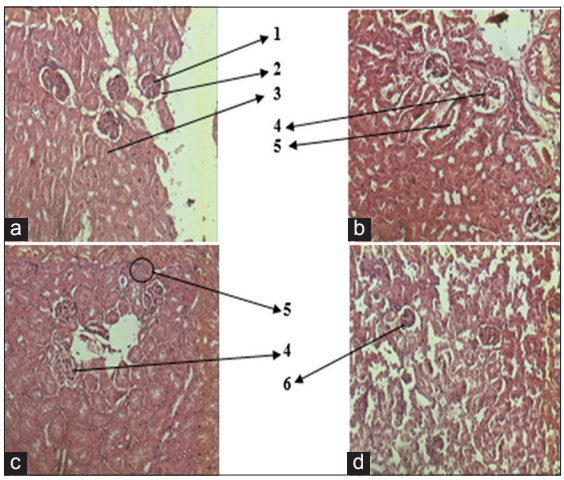

Fig. 3: Histopathological changes of animal kidney during treatment with the leaves ethanol extract of *Picria fel-terrae* (a) control; (b) plant extract at 1000 mg/kg bw; (c) plant extract at 2000 mg/kg bw; (d) plant extract at 5000 mg/kg bw; (1) glomerulus; (2) Bowman's capsule; (3) proximal tubular; (4) degeneration of glomerulus; (5) degeneration of proximal tubular; (6) glomerulus shrinkage

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 LD_{50} between 5 and 340 mg/kg bw in mice after oral administration [31]. To the best of our knowledge, this is the 1st 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.

CONCLUSSION

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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