

Evaluation of Acute Toxicity of Ethanol Extract of Pirdot Leaf (*Saurauia vulcani* Korth.) in Rats

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ABSTRACT. Traditionally pirdot leaves are used to treat various diseases. The purpose of this study was to determine the potential for acute toxicity of ethanol extract of pirdot leaf (*Saurauia vulcani* Korth.) with value LD₅₀ and hematological Parameters in rats. The acute toxicity of ethanol extract of pirdot leaf was evaluated by OECD guidelines. The number of animals used in this research were 15 female rats. The control group was given Na CMC 0.5%, the treatment groups were given ethanol extract of pirdot leaves with dose of 2000 and 5000 mg/kg bw. The results showed that ethanol extract of pirdot leaves with dose of 2000 and 5000 mg/kg bw did not show any toxicity signs. There was no mortality was observe. The ethanol extract of pirdot leaves did not cause any changes in hematological parameters, these include red blood cells (RBC), hemoglobin, hematocrit, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), platelet, white blood cells (WBC), neutrophils, lymphocytes, monocytes, eosinophils, and basophils levels as compared to normal control (P>0.05). It was estimated that LD₅₀ of ethanol extract of pirdot leaves was higher than 5000 mg/kg bw and the extract were practically non-toxic. The ethanol extract of pirdot leaves did not cause any toxic effect on hematological parameters.

Keywords:. *Saurauia vulcani* Korth , Acute toxicity, LD₅₀, Hematological parameters,

Abstrak. Secara tradisional daun pirdot digunakan untuk mengobati berbagai penyakit. Tujuan dari penelitian ini adalah untuk menentukan potensi toksisitas akut ekstrak etanol daun pirdot (*Saurauia vulcani* Korth.), nilai LD₅₀ dan Parameter hematologis pada tikus. Toksisitas akut ekstrak etanol daun pirdot dievaluasi dengan pedoman OECD. Jumlah hewan yang digunakan dalam penelitian ini adalah 15 tikus betina. Kelompok kontrol diberi Na CMC 0,5%, kelompok perlakuan diberi ekstrak etanol daun pirdot dengan dosis 2000 dan 5000 mg / kg bb. Hasil penelitian menunjukkan bahwa ekstrak etanol daun pirdot dengan dosis 2000 dan 5000 mg / kg bb tidak menunjukkan tanda-tanda toksisitas. Tidak ada kematian yang diamati. Ekstrak etanol daun pirdot tidak menyebabkan perubahan dalam parameter hematologi, ini termasuk sel darah merah (RBC), hemoglobin, hematokrit, volume corpuscular rata-rata (MCV), rata-rata hemoglobin sel darah hitam (MCH), konsentrasi hemoglobin corpuskuler rata-rata (MCHC), tingkat platelet, sel darah putih (WBC), neutrofil, limfosit, monosit, eosinofil, dan basofil dibandingkan dengan kontrol normal (P> 0,05). Diperkirakan bahwa nilai LD₅₀ dari ekstrak etanol daun pirdot lebih tinggi dari 5000 mg / kg bb dan EEDP praktis tidak beracun. Ekstrak etanol daun pirdot tidak menyebabkan efek toksik pada parameter hematologi.

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1. Introduction

Indonesia is a tropical country with plant potential that has been traditionally used as traditional medicine so it has the prospect of developing traditional medicines for the benefit of national health [1]. One of the medicinal plants is *Saurauia vulcani* Korth. Known as pirdot (Toba), cepcepan (Karo) and sopsopan (Simalungun) [2], [3]. Pirdot plant (*Saurauia vulcani* Korth.) Is one of the natural ingredients as an alternative medicine that has been widely used by the Simalungun, Toba and Karo people to treat various diseases. Traditionally used for diabetes mellitus.

Secondary metabolites such as saponins and polyphenols can have adverse effects such as causing poisoning when overused. Polyphenols 0.5% -1% show hepatotoxic and nephrotoxic effects⁴ and saponins can cause serious damage to the liver and kidneys⁵. Unexpected adverse effects of these secondary metabolites can occur due to increased Reactive Oxygen Species (ROS) in the body that will trigger the release of acrolein, malondialdehyde and 4-hydroxy-2-nonenal, causing disruption of normal protein function which has an impact on changes in cell activity [4], [5], [6].

2. Material And Methods

2.1 Material

The materials used in this study include plant materials and chemicals. Plant material used is the leaves of Pirdot (*Saurauia vulcani* Korth). The chemicals that will be used are 0.9% NaCl, 0.5% CMC Na, 95% ethanol, and distilled water.

2.2 Sample Preparation and Extraction

Fresh Pirdot (*Saurauia vulcani* Korth.) leaves obtained from the village of Sipangan Bolon, Girsang District, North Tapanuli Regency, Sumatera Utara Province. The plant identification was confirmed by Bogoriense Herbarium, LIPI, Jakarta Indonesia. The cleaned leaves are dried and blended until they turn into powder. An amount of 500 g the dried of pirdot leaves were extracted with maceration method using 5 L ethanol until discoloration. Then the ethanol macerate was evaporated at $\pm 40^{\circ}\text{C}$ in a rotary vacuum evaporator and thickened by heating in a water bath at $\pm 40^{\circ}\text{C}$.

2.3 Animals

All procedures were evaluated by Animal Research Ethics Committees (AREC) Faculty of Mathematics and Natural Science, Biological Department, University of Sumatera Utara. Fifty

Animals used were male wistar rats weighing 150-200 g, 6-8 weeks old. Before the experiment begins, the animals were acclimatized in the experimental room for 7-14 days with room temperature and conditions 12 hours of light and 12 hours of darkness. The rats were fed on a standard pellet diet and provided access to water ad libitum.

2.4 Phytochemical Screening

Phytochemical screening was conducted using a standard method for detection of alkaloids, flavonoids, tannins, glycosides, saponins and steroids/triterpenoids [7].

2.5 Acute Toxicity Study

Acute toxicity test was performed as per OECD guideline 423 for testing of chemicals (2001) [5]. Healthy young adult rats, non-pregnant female rats weighing about 150-200 g were administered only once orally at a single doses of 2000 mg/kg bw and 5000 mg/kg bw of ethanol extract of pirdot leaves, whereas the control group only received Na CMC 0.5%. All rats were then allowed free access to food and water and observed for 24 h, with special care given to first 4 h and once daily for 14 days for any signs of acute toxicity.

The visual observations of mortality, various changes in physical appearance, behaviour (salivation, lethargy), and any injury or illness were conducted once daily for 14 days. On the 15th day, all animals were anesthetized by intraperitoneal injection of ketamine. Blood samples were collected by cardiac puncture into EDTA containing tubes for haematological analysis.

2.6 Statistical Analysis

Data were analyzed using SPSS version 25.0 with Kolmogorov-Smirnov normality test, one-way analysis of variance (ANOVA) and Kruskal Wallis to see differences with significance ($p > 0.05$) between test groups.

3. Result and Discussion

3.1 Phytochemical Screening

Table 1. Phytochemical Constituent of ethanol extract *Saurauia vulcani* Korth

No	Screening	Dried Samples	EEDP
1	Alkaloids	-	-
2	Flavonoids	+	+
3	Glikocyde	+	+
4	Saponins	+	+
5	Tanins	+	+
6	Steroids/triterpenoids	+	+

Notes : (+) positive : contains of phytochemical compound
 (-) negative : not contains of phytochemical compound

Phytochemical screening on leaf *Saurauia vulcani* Korth showed the presence of glycosides, terpenoids, saponins, tannins and flavonoids [7]. The toxicity of saponin has been reported which indicated saponin as powerful haemolytic [8]. Flavonoids are polyphenol compounds that act as antioxidants [9].

3.2 Observation Sign of Toxicity

Observations made every day for 14 days for the existence of tremors, diarrhea, salivation, weakness, walk backwards and walk with stomach. The observation of clinical signs were performed based on OECD guidelines. The observation sign of toxicity showed no toxic symptoms were found in the control group and the test group up to a dose of 5000 mg / kg bw during the 14 day observation. According to OECD criteria under its Globally Harmonised Classification System (GHS) for chemical substances and mixtures with LD₅₀ was higher than 5000 mg/kg bw and the extract were practically non-toxic [10].

Mortality Rats

The last of observation there was no mortality was recorded and also no signs of observable toxicity was detected during the experimental period. Result of mortality shown in table 2. Treatment of single dose of ethanol extract *Saurauia vulcani* Korth up to a dose of 5000 mg / kg bw did not cause mortality in rats of all groups until the 14th day so LD₅₀ of ethanol extract of *Saurauia vulcani* Korth was higher than 5000 mg/kg bw and the extract were practically non-toxic.

Table 2. Total of rats mortality after giving ethanol extract *Saurauia vulcani* Korth for 14 days

Treatment	Total of rats	Total of Mortality
CMC Na 0,5 %	5	0
EEDP 2000 mg/kg bw	5	0
EEDP 5000 mg/kg bw	5	0

3.3 Observation of Hematological Parameters

Blood sampling was performed at the end of the 15th day treatment. Table 3 shows results of observations of rats blood hematology parameters. From the present study it was seen that there was no significant difference ($p > 0.05$) in the haematological parameters in the administrated of ethanol extract of *Saurauia vulcani* Korth leaves on treated group compared to control group. Hence it can be concluded that the ethanol extract of *Saurauia vulcani* Korth leaf did not affect the hematology value of the animal test.

Hematological results in animals do not show consistent treatment effects. Hematopoietic system is the most sensitive target for toxic substances and it is the important index of physiological and pathological status and hence hematological investigation was carried out. No abnormality was observed in hematopoietic function indices for ethanol extract of *Saurauia vulcani* Korth leaf treated groups compared with control groups indicating the extract is safe [11], [12]

Table 3. Results of observations of rat blood hematology parameters (Mean \pm SEM)

Hematology parameters (Mean \pm SD)	Group		
	CMC Na 0,5 %	EEDP 2000 mg/kg bw	EEDP 5000 mg/kg bw
WBC	7.93 \pm 0.94	8.06 \pm 0.86	8.77 \pm 1.19
RBC	7.55 \pm 0.62	7.80 \pm 0.70	7.65 \pm 0.90
Platelets	862.82 \pm 87.45	833.88 \pm 78.81	859.48 \pm 47.14
Hemoglobin	16.15 \pm 2.26	15.62 \pm 1.58	15.76 \pm 1.59
Hematocrit	49.68 \pm 6.37	51.24 \pm 7.18	49.52 \pm 5.19
MCH	18.45 \pm 0.93	18.36 \pm 1.27	17.61 \pm 1.42
MCV	55.80 \pm 2.58	55.40 \pm 2.24	55.80 \pm 2.84
MCHC	29.88 \pm 0.86	31.88 \pm 4.78	29.27 \pm 7.12
Eosinophils	2.87 \pm 0.74	3.25 \pm 0.67	2.96 \pm 0.33
Monocytes	1.84 \pm 0.69	2.62 \pm 0.80	2.10 \pm 0.58
Basophils	0.33 \pm 0.09	0.35 \pm 0.09	0.37 \pm 0.07
Limfocytes	63.78 \pm 10.74	64.52 \pm 15.99	65.18 \pm 13.92
Neutrophils	30.18 \pm 6.94	29.58 \pm 4.39	28.88 5.15

4. CONCLUSION

The ethanol extract of *Saurauia vulcani* Korth leaf did not induce a toxic effect at the dose of 2,000 mg/kg bw and 5000 mg/kg bw compared to the normal group. No mortality or any signs of toxicity was observed after oral administration in acute toxicity study up to a dose of 5000 mg/kg of ethanol extract of *Saurauia vulcani* Korth in rats. LD₅₀ of ethanol extract of *Saurauia vulcani* Korth was higher than 5000 mg/kg bw and the extract were practically non-toxic. In addition, the hematological parameters were still in normal range.

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