BioLink : Jurnal Biologi Lingkungan, Industri dan Kesehatan, Vol. 11 (1) August (2024)
ISSN: 2356- 458X (print) ISSN: 2597-5269 (online)
DOI: 10.31289/biolink.v11i1.12058

BioLink
Jurnal Biologi Lingkungan, Industri, Kesehatan

Available online http://ojs.uma.ac.id/index.php/biolink

TOXICITY OF ETHANOL EXTRACT OF PELAWAN LEAVES (TRISTANIOPSIS OBOVATA Benn.) ON THE HEMATOLOGICAL PROFILE OF WHITE RATS (RATTUS NORVEGICUS L.)

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Submitted: 31-05-2024; Reviewed: 04-06-2024; Accepted: 12-08-2024

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Abstract

Tristaniopsis obovata Benn. is one of the herbal plants that contains phenolics flavonoids, saponins, tannins and steroids/triterpenoids. The secondary metabolites are known to have antioxidant activity and toxicity that can affect hematological profiles. This study aims to determine the toxicity effects of ethanol extract of T. obovata Benn. leaves on LD50 value, erythrocyte morphology, and hematological profile in white rats. The study used a Complete Randomized Design (CRD) with 4 treatments and 4 repeats. The treatment consisted of P0 (control), P1 (dose of 300 mg/kg body weight) (P1), P2 (dose of 2000 mg/kg body weight), and P3 (dose of 5000 mg/kg body weight). LD50 calculation using AOT 425 StatPgm software and hematological profile analysis using ANOVA. The results showed LD50 value of T. obovata Benn. leaf extract. was 1.750 mg/kg BW as mild toxic. The morphology of P2 erythrocytes shows abnormal form anisocytosis and P3 form Burr cells and macrocytosis. The administration of extract in all treatments was not significant from the number of erythrocytes, leukocytes and hemoglobin comcentration of white rats.

Keywords*: Eryhrocytes; Hemoglobin; LD50; Leucocytes; Tristaniopsis.*

How to Cite*: Yusfiati, Marwan, A.D.S., & Husadha, A. (2024). Toxicity of Ethanol Extract of Pelawan Leaves (Tristaniopsis obovata Benn.) on The Hematological Profile of White Rats (Rattus norvegicus L.). BioLink: Jurnal Biologi Lingkungan, Industri, Kesehatan, Vol.11 (1): 32-40*

INTRODUCTION

Tristaniopsis obovata Benn. is an endemic plant that grows in peat areas such as Bangka Belitung, Riau and Kalimantan. This plant has potential as a herbal medicine (Januar *et al.,* 2020). Research by Verotta *et al.,* (2001) showed the leaves of *Tristaniopsis* genus are more widely used, which are proven contain higher levels of active phenolic compounds than the stems. Budiana *et al.,* (2020) reported that the ethanol extract of *T. obovata* leaves contains flavonoids, saponins, steroids/triterpenoids.

In vitro test shows that the ethanol extract of pelawan leaves has LC50 value of 4.82 µg/mL (Yusfiati *et al.,* 2020). High antioxidants level in secondary metabolites of a plant can affect the hematological profile by reducing free radicals which can increase erythropoiesis and reduce leukocytes (Dasopang *et* al., 2021; Syafira *et al.,* 2022; Atmaja *et al.,* 2023).

Hematology examination is a method to determine the condition and components of blood, which can be used for toxicity testing and disease diagnosis (Ruza, 2022). A good hematological profile indicates a good physiological condition of the body, while an abnormal profile can cause problems and its function (Guyton *et al.,* 2011). A test to determine the safety of a compound in medicinal plants on the hematological profile is acute toxicity using the LD50 method (Melisa *et al.,* 2022).

This study aims to determine the acute toxicity of ethanol extract of *T. obovata* Benn leaves. on the hematological profile of white rats which includes the lethal dose 50 (LD50) value, erythrocyte morphology, erythrocyte count, leukocyte count, and hemoglobin levels.

RESEARCH METHODS

Research that was carried out from September-Desember 2023. The research was carried out in Zoologi Laboratory at Univeristy of Riau. The experiment used 16 healthy male white rats (*Rattus norvegicus* L.) Wistar strain, weight 200–250 g and aged 3-4 months, 2 L of 70% ethanol solution, 200 g of *T. obovata* Benn. leaf., HCL 0.1 N, Hayem's reagent, Turk's reagent, Giemsa staining solution, Wright's solution, distilled water, filter paper, 1 cc syringe, and EDTA tube.

Leaf Extract Preparation

T. obovata Benn. leaves washed with water, air dried and ground into powder to obtain somplisia powder. 200 g of simplicia powder was macerated with 2 L of 70% ethanol for 72 hours. The macerate was filtered and the solution was evaporated with a rotary evaporator to obtain thick extract.

Experimental Animals Preparation

The research was carried out experimentally using a completely randomized design (4 x 4), which consisted of 4 treatments, P0 (control), P1 (dose 300 mg/kg BW), P2 (dose 2000 mg/kg BW), and P3 (dose 5000 mg/kg BW) with 4 replications of each treatment. Rats were acclimatized for 14 days. The feed provided was CitraFeed pellets and drinking water ad libitum. Administration of *T. obovata* Benn leaf extract. carried out orally for 14 days (Elzar, 2019).

Blood Collection

Blood samples were taken on the 15th day. If death occurs within 24 hours, blood was taken directly from the heart using a 1 cc syringe and collected in an EDTA tube.

Peripheral Blood Smear

Blood was taken with a pipette and placed on a glass object, then spread at angle of 25-30°. The preparations were stained with Wright's and Giemsa's solutions, then observed under a microscope with 100x magnification.

Erythrocytes and Leukocytes Counts

The number of erythrocytes was counted using an erythrocyte pipette and diluted with Hayem's reagent, while the number of leukocytes was counted using a leukocyte pipette and diluted with Turk's reagent. Calculations using improved Neubauer.

Hemoglobin Determination

Hemoglobin concentration were measured using Sahli method with 0.1 N HCL solution and distilled water. Color change was indicated when the colour change according the standard color of the hemoglobinometer.

Data Analysis

Hematological profile data were analyzed using ANOVA, while LD50 values were tested using AOT 425 StatPgm software.

RESULTS AND DISCUSSION

Toxic Symptoms and Determination Of LD50 Value

Toxic symptoms observed in rats after the administration can be seen in Tables 1 and 2. At P2 and P3, rats showed losing appetite, slightly closed eyes, shortness of breath, and convulsions which led to death. The LD50 value of the ethanol extract of *T. obovata* Benn. leaves is 1.750 mg/kg BW, which is categorized as mild toxic.

One of the compounds contained in the ethanol extract of *T. obovata* Benn. leaves as flavonoids. Flanonoids can influence the activity of the respiratory system of the animals. Flavonoids can

attack the nervous system in the respiratory system, which will slowly weaken the animal and cause death. The death of experimental animals can also caused by the damage of central nerve cells, causing symptoms in the form of seizures and leading to death (Kurniawan *et al.,*2021).

Table 1. Toxic symptoms of ethanol extract leaves of *T. obovata* Benn. in white rats.

Treatment	Number of experimental animals	Number of Death animals	Total % of deaths
P0 (Control)	4	0	0
P1 (Dose 300 mg/kg BW)	4	0	0
P2 (Dose 2000 mg/kg BW)	4	2	50
P3 (Dose 5000 mg/kg BW)	4	3	75

Table 2. Acute toxicity test of ethanol extract and *T. obovata* Benn. in white rats.

Treatment	Toxic Symptoms
P0 (Control)	Normal behavior
P1 (Dose 300 mg/kg BW)	Normal behavior
P2 (Dose 2000 mg/kg BW)	Losing appetite, slightly closed eyes, decreased respiratory activity (shortness of breath) and seizures
P3 (Dose 5000 mg/kg BW)	Losing appetite, slightly closed eyes, decreased respiratory activity (shortness of breath) and seizures

Erythrocyte Morphology

Results of erythrocyte morphology at P2 and P3 on day 1 after administration can be seen in Figure 1. The morphology of erythrocytes at P2 and P3 shows normal erythrocyte morphology with an oval or round shape, does not have a nucleus, but a disc structure has not yet been formed with both surfaces having a biconcave (concave) shape as reticulocytes. Presumably, the central part of the cells that fades is an internal transition phase maturation of reticulocytes into mature erythrocytes (Wei *et al.,* 2023).

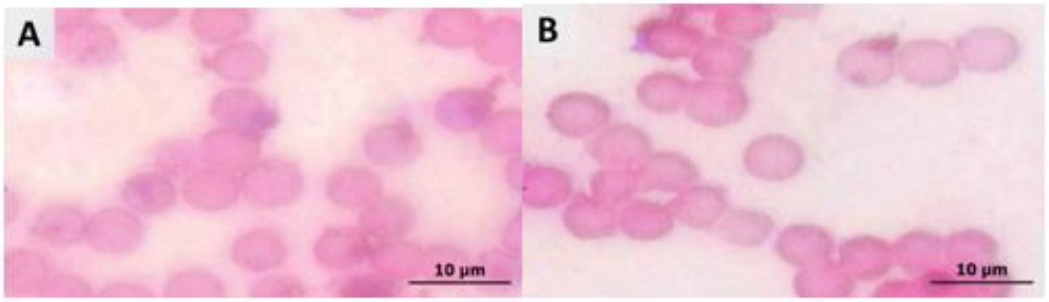

Figure 1. Erythrocyte morphology. (A) P2 (dose 2000 mg/kg BW); (B).P3 (dose 5000 mg/kg BW) after aministration the extract on day 1. Bar. 10 µm.

Another factor is the long storage period of blood samples. Based on Situmorang *et al.,* (2023), factors that can influence erythrocyte morphology are sample storage time, sample processing, and sample examination location.

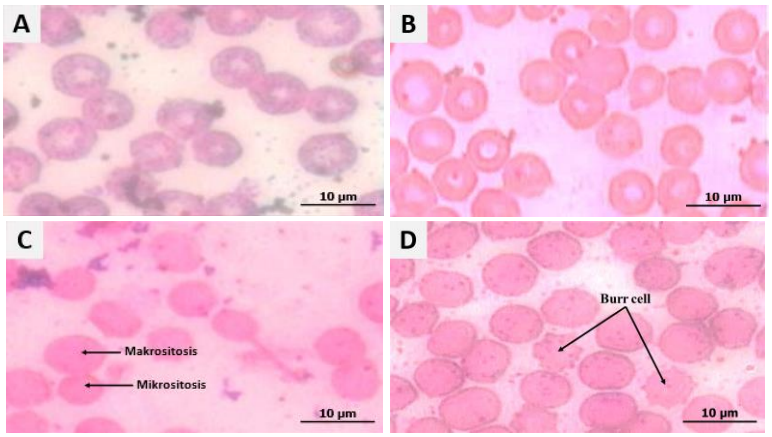

Figure 2. Erythrocyte morphology. (A) Control; (B). P1 (dose 300 mg/kg BW); (C). P2 (dose 2000 mg/kg BW); (D). P3 (dose 5000 mg/kg BW) for 14 days. Bar. 10 µm.

The morphology of erythrocytes at P2 and P3 given the extract for 14 days (Figure 2) showed abnormalities such as anisocytosis and Burr cells. It indicates there is damage to the liver and kidneys of rats which may be caused by lack of vitamin B12 and iron deficiency. Anisocytosis is an abnormality in the size of erythrocytes with differences in cell size in the form of macrocytosis and microcytosis.

Creanated cells or known as burr cells are characterized by short blunt protrusions composed of 10-30 spines with relatively the same size, shape and distance, and a star-like shape. Burr cells often occur due to changes in the external environment (Ridwan *et al.,* 2021). It is similar to research by Subiyantara *et al.,* (2020) of sweet potato extract at dose 5000 mg/kg BW showed abnormalities in erythrocyte morphology in the form of burr cells.

Erythrocyte Count

The number of erythrocytes at P1, P2, and P3 was below the average number of normal erythrocytes, indicating that the rats were anemia (Table 3). The normal erythrocytes number in healthy male white rats is 6.1-8.5×106/mm^3 (Vigneshwar *et al.,* 2021).

Table 3. Average number of erythrocytes in white rats after administration of ethanol leaf extract *T. obovata Benn.*

Treatment	Average number of eryrocytes (*×10⁶/mm³*)
P0 (Control)	6.3 ± 0.37
P1 (Dose 300 mg/kg BW)	4.1 ± 0.69
P2 (Dose 2000 mg/kg BW)	5.2 ± 1.10
P3 (Dose 5000 mg/kg BW)	5.4 ± 1.56

Data is an average±SD. The results showed that the data were not significantly different (P>0.05).

Anemia can caused by the compounds in the leaf of *T. obovata* Benn. and physiological factors, such as stress, housing environment and exposure to microorganisms. Research by Rahmawati *et al.,* (2023), extract of sambiloto can reduce number of erythrocytes, due to the presence of saponins. Saponins are active on erythrocyte membranes which can trigger hemolysis.

Leukocyte Count

The number of leukocytes increased at P0, P2, and P3, can be caused of pathogen infection and the flavonoid content in the extract which act as an immunostimulator (Table 4). The normal leukocyte count in male white rats is 3.7-5.8 103/mm^3 (Vigneshwar *et al.,* 2021).

Table 4. Average number of white rat leukocytes after administration of ethanol leaf extract *T. obovate Benn.*

Treatment	Average number of eryrocytes (×10⁶/mm³)
P0 (Control)	10.8 ± 1.17
P1 (Dose 300 mg/kg BW)	5.9 ± 0.99
P2 (Dose 2000 mg/kg BW)	9.7 ± 3.71
P3 (Dose 5000 mg/kg BW)	9.0 ± 2.82

Data is an average± SD. The results showed that the data were not significantly different (P>0.05).

Flavonoids compunds in *T. obovata* Benn leaf extract. Can acts as an immunostimulator which can increase the number of leukocytes to improve the immune system (Martinez *et al.,* 2019). Flavonoids can directly on cytokines produced by T helper (Th) cells by activating various types of immune cells such as macrophage cells which can phagocytose extracellular and intracellular antigens (Masihin *et al.,* 2021; Rosnizar *et al.,* 2021). Other factors that can cause an increase in the number of leukocytes are stress and inflammation (Giyartika & Keman, 2020).

Hemoglobin Concentration

Hemoglobin conentration in all treatments still in normal range, indicating good oxygen transport both in the rats body (Table 5). Normal

hemoglobin concentration in white rats in the ranged from 11.8-16.2 g/dL (Vigneshwar *et al.,* 2021).

Table 5. Average hemoglobin levels in white rats after administration of ethanol extract of leaves *T. obovata* Benn.

Treatment	The average number of hemoglobin levels ($\times 10^6/mm^3$)
P0 (Control)	6.3 ± 0.37
P1 (Dose 300 mg/kg BW)	4.1 ± 0.69
P2 (Dose 2000 mg/kg BW)	5.2 ± 1.10
P3 (Dose 5000 mg/kg BW)	5.4 ± 1.56

Data is an average±SD. The results showed that the data were not significantly different (P>0.05).

Normal hemoglobin concentration in this study can be caused by an increase in the need for oxygen (O_2) in the body's metabolism. Hemoglobin levels that are in normal limits indicate that O^2 transport to all body tissues is fulfilled, so the physiological processes in the body work optimally. This hemoglobin concentration is similar to the research of Meilani *et al.,* (2023), the talas leaf extract in anemic rats caused an increase in hemoglobin concentration in the blood. It is suspected the flavonoid compounds in talas leaf extract, which can increase hemoglobin levels. Flavonoids will form ferrylHb which prevents oxyHb from being oxidized to metHb which contains Fe3+ (ferric) iron, so hemoglobin continues to function in binding O_2 in form of oxyHb and the amount of hemoglobin formed increases.

CONCLUSION

The toxicity effects of ethanol extract of *T. obovata* Benn. leaves showed the LD50 value is 1.750 mg/kg BW, which was categorized as mild toxic. Administration of the extract did not have a significant effect on the number of erythrocytes, leukocytes and hemoglobin concentration in white rats.

ACKNOWLEDGEMENT

This research has been funded by research LPPM DIPA Funds for 2023 with contract number: 8282/UN19.51.3/AL.04/2023.

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