Advances in Health Sciences Research, volume 40
2nd International Conference on Contemporary Science and Clinical Pharmacy 2021 (ICCSCP 2021)

Subacute Toxicity of Water Fraction of Africa Leaves (*Vernonia amygdalina Del.*) on Blood Parameters in Male White Mice

Dian Ayu Juwita[1], Helmi Arifin[1], Muhammad Yasin Abdullah[1], Dita Permatasari[1*]

[1]*Faculty of Pharmacy, Universitas Andalas, Limau Manis, Pauh District, Padang City, West Sumatera, 25163, Indonesia*
[*]*Corresponding author. Email: ditapermatasari@phar.unand.ac.id*

ABSTRACT
African leaves (*Vernonia amygdalina Del.*) is one of the plants used as traditional medicine. Although natural, traditional medicinal ingredients are not necessarily safe for use in humans. In this study, a subacute toxicity test of Afrian leaves water fraction was carried out on male white mice. This study aims to determine the effect of dosage variations and duration of administration of water fraction of African leaves (*Vernonia amygdalina Del.*) on blood parameters. Parameters observed were erythrocytes, leukocytes, haemoglobin and hematocrit. The experimental animals used were 36 male white mice which were divided into four groups. The first group as a negative control was given 0.5% Na CMC suspension, while three more groups were given test preparations at a dose of 10 mg/kg BW, 20 mg/kg BW and 40 mg/kg BW orally once a day for 7, 14 and 21 days. Blood parameter tests were carried out on days 8, 15 and 22. Data were analyzed by two-way Analysis of Variance (ANOVA). The results showed that variations in dosage (10 mg/kg BW, 20 mg/kg BW and 40 mg/kg BW) and duration of administration (7, 14 and 21 days) of the African leaves water fraction did not affect on blood parameters ($p>0.05$).

Keywords: *African leaves, Vernonia amygdalina Del., water fraction, subacute toxicity, blood parameters*

1. INTRODUCTION

Aaccording to the World Health Organization (WHO), the world community tends to return to nature to maintain and improve their health status [1]. Natural medicine or traditional medicine contains many types of active compounds with different levels. The use of traditional medicine is generally considered safer than the use of modern medicine. It is because traditional medicine has relatively fewer side effects than modern medicine [2].

One of the widely seen plants in Indonesia is the African plant (*Vernonia amygdalina Del.*), which is widely used for traditional medicine such as malaria treatment, anti-parasitic laxative, antihypertensive [3]. The phytochemical tests of African leaves (*Vernonia amygdalina Del.*) included saponins, flavonoids, glycosides, alkaloids, tannins, quinones and polyphenols. These compounds are influential ingredients and provide benefits in traditional medicine [4]. This statement is not necessarily true because in principle, drugs must be in the correct dose. A toxicity test can be done to see the side after using traditional medicines [5].

Toxicity is a condition that indicates the presence of a toxic effect or poison that can cause harmful effects if given. Toxicity testing uses animals whose purpose is to extrapolate animal data to humans, extrapolating from the highest dose to the lowest dose [6]. Tests are divided into acute, subacute, subchronic and chronic in vivo toxicity tests which are urgently needed for herbal preparations as preclinical tests [6,7]. Research discussing the acute toxicity of Afika leaves extract (*Vernonia amygdalina Del.*) showed an LD50 of 5.1523 g/kg when given orally. These results showed that the extract was not toxic [8]. Toxicity can occur in blood components where it greatly affects the shape, concentration, volume, and other matters related to blood components leading to several abnormalities in the blood, including anemia, polycythemia, leukopenia, leukemia, haemophilia, and thrombocytopenia [9]. Another search result was a subacute toxicity test using an infusion from African leaves (*Vernonia amygdalina Del.*) against the Kidney Histopathology of Balb/C Mice Strain. This study illustrates no effect of African leaf infusion on the kidney histopathology of BALB/c strain mice. Still, increasing the dose can reduce congestion and hemorrhagic in the kidney [10].

Indonesia has a very abundant availability of natural ingredients and people often use them as traditional medicine. It is necessary to know the level of safety in terms of dose, frequency and duration of administration. From the literature review that has been carried out by the authors so far, it can be seen that the discussion on safety testing related to African leaves (*Vernonia amygdalina Del.*) on blood parameters is still relatively small. This research is needed to be supporting data as a condition to be used as a phytopharmaceutical preparation.

2. MATERIAL AND METHODS

2.1 Experimental Animals Treatment

The number of 36 males white mice aged 2-3 months and the bodyweight of ±20-30 g was divided into four groups (nine mice per group). The first group as a negative control was given 0.5% Na CMC suspension, while three more groups were given test preparations at a dose of 10 mg/kg BW, 20 mg/kg BW and 40 mg/kg BW orally once a day for 7, 14 and 21 days. The nine mice in each group were divided into three groups consisting of groups A, B, and C. Tests were carried out on days 8 (group A), 15 (group B) and 22 (group C). The blood sample of mice was taken to measure the number of erythrocytes, leukocytes, haemoglobin and hematocrit respectively on the day 8, 15 and 22.

2.2 Plants Materials

The African leaves (*Vernonia amygdalina Del.*) were collected in the Padang area. The leaves were identified by *Herbarium Universitas Andalas (ANDA),* Department of Biology, Faculty of Mathematics and Natural Sciences Universitas Andalas. The sample was prepared into simplicia powder and the following process was making African leaf extract. After that, the extract will be processed to make African leaves fraction.

2.3 Preparation of 0.5% NaCMC Suspension

50 mg of Na CMC was weighed and sprinkled over 1 mL of hot water in a hot mortar and left for 15 minutes. Then crushed until homogeneous, then added aquadest to a volume of 10 mL.

2.4 Preparation of African Leaves Water Fraction Suspension

Na CMC has weighed as much as 50 mg. Sprinkled over one mL of hot water in a hot mortar and left for 15 minutes. Then grind until homogeneous, add the water fraction that has been weighed according to the planned dose and then grind homogeneously. Then aquadest was added to a volume of 10 mL.

2.5 Blood Parameters Testing

2.5.1 Erythrocytes

Blood samples through several specific treatments for erythrocytes and observed under a microscope with a magnification of 40x. Then, count the number of erythrocytes in five fields consisting of 16 small fields, at the four corners of the large field plus the one in the middle. The formula for calculating the number of erythrocytes:

Σ Erythrocytes = Σ Erythrocytes in 5 fields x 10,000

2.5.2 Leukocytes

Blood samples through several specific treatments for leukocytes and observed under a microscope with a magnification of 40x. Then, count the number of leukocytes in the four large corners arranged in 16 medium squares. The formula for calculating the number of leukocytes:

Σ Leukocytes = Σ Leukocytes in 4 fields x 50

2.5.3 Haemoglobin

Blood was collected using microtubes that had been added with EDTA. Then 5 ml of Drabkin's solution was put into a test tube. After that, the solution was mixed by shaking the tube slowly until the solution was homogeneous and left for three minutes at room temperature. Then read using a micro lab 300 photometers with a wavelength of 540 nm.

2.5.4 Hematocrit

Take blood approximately of the pipette's length with a capillary pipette, after which the capillary hole at the end containing the sample is closed with wax. Then place the capillary pipette into the centrifuge with the plugged end pointing outward. To balance the rotation, place the other capillary pipette across from it. Close the centrifuge tightly, adjust the rotation speed and the required time. Then measure the height of the erythrocyte deposits by the hematocrit percentage using a microhematocrit reading scale.

2.6 Ethical Consideration

Approval of study conduction was obtained from the ethics committee of the Faculty of Medicine, Universitas Andalas, Padang – Indonesia (The registration number are 396/UN.16.2/KEP-FK/2021).

2.7 Statistical Analysis

All data in each parameter were analyzed using two-way ANOVA and significance was taken at $p < 0.05$.

3. RESULTS AND DISCUSSION

In this study, selected mice with similar ages and weight were used to reduce deviations from the study results. These animals were acclimatized for seven days to determine whether the test animals were included in the test criteria. The test animals used must also be healthy and during the acclimatization process, there was no change in body weight of more than 10%.

Table 1 showed a fluctuation trend in the mean of the observed blood parameters. This trend could occur due to factors originating from test animals that experience stress due to treatment during the study and differences in the physiological conditions of each test animal. Measurement of erythrocyte parameters showed that the average number of erythrocytes was almost the same for each group of experimental animals. The number of erythrocytes is within the normal range for the number of erythrocytes in mice, which is between 3.5 million/µl – 15.2 million/µl [11].

Table 1. The effect of dosage variations and duration of administration of water fraction of African leaves (*Vernonia amygdalina Del.*) on blood parameters

	Doses (mg/kg BW)	The averages of blood parameters on days ± SE			Averages ± SE	Test of Significance*
		7	14	21		
Erythrocytes (millions/µl)	Control	4.18 ± 0.24	4.33 ± 0.24	4.22 ± 0.24	4.24 ± 0.14	0.966
	10	4.21 ± 0.24	4.45 ± 0.24	4.39 ± 0.24	4.35 ± 0.14	
	20	4.54 ± 0.24	4.30 ± 0.24	4.51 ± 0.24	4.45 ± 0.14	
	40	4.54 ± 0.24	4.49 ± 0.24	4.49 ± 0.24	4.50 ± 0.14	
	Averages ± SE	4.37 ± 0.12	4.39 ± 0.12	4.40 ± 0.12		
Luekocytes (thousands/µl)	Control	9.15 ± 0.27	9.05 ± 0.27	9.20 ± 0.27	9.13 ± 0.16	0.998
	10	9.35 ± 0.27	9.25 ± 0.27	9.13 ± 0.27	9.24 ± 0.16	
	20	9.33 ± 0.27	9.28 ± 0.27	9.25 ± 0.27	9.29 ± 0.16	
	40	9.25 ± 0.27	9.35 ± 0.27	9.27 ± 0.27	9.29 ± 0.16	
	Averages ± SE	9.27 ± 0.13	9.23 ± 0.13	9.21 ± 0.13		
Haemoglobin (g/dl)	Control	12.50 ± 0.73	13.20 ± 0.73	12.67 ± 0.73	12.79 ± 0.42	0.111
	10	13.90 ± 0.73	12.87 ± 0.73	14.43 ± 0.73	13.73 ± 0.42	
	20	12.70 ± 0.73	13.23 ± 0.73	14.50 ± 0.73	13.48 ± 0.42	
	40	13.87 ± 0.73	14.50 ± 0.73	12.03 ± 0.73	13.47 ± 0.42	
	Averages ± SE	13.24 ± 0.36	13.45 ± 0.36	13.41 ± 0.36		
Hematocrit (%)	Control	26.00 ± 2.25	25.67 ± 2.25	25.00 ± 2.25	25.56 ± 1.30	0.951
	10	26.67 ± 2.25	29.00 ± 2.25	28.67 ± 2.25	28.11 ± 1.30	
	20	29.67 ± 2.25	28.33 ± 2.25	26.67 ± 2.25	28.22 ± 1.30	
	40	28.00 ± 2.25	27.67 ± 2.25	28.33 ± 2.25	28.00 ± 1.30	
	Averages ± SE	27.58 ± 1.12	27.67 ± 1.12	27.17 ± 1.12		

**two-way ANOVA test*

An increase in leukocyte cells can be an indicator of a disease caused by a foreign object. This condition can cause leukocytosis,where an uncontrolled rise in the number of leukocytes can cause anemia [12]. The study results showed that the number of leukocytes was within the normal range. It did not cause an indication of the emergence of various diseases due to the increase in the number of leukocytes beyond normal limits. The average of the leukocyte cell count parameters was within the normal range for the leukocyte cell count of male white mice, which ranged from 1.06–56.08 thousand/µl [11].

Haemoglobin is closely related to erythrocytes. It is a protein that has a red pigment in erythrocytes that acts as an oxygen carrier. The formation of haemoglobin occurs during the process of erythropoiesis. If erythropoiesis is disturbed, the production of red blood cells will decrease, resulting in an anemic condition characterized by haemoglobin levels that are less than the normal range of

haemoglobin levels [13]. The haemoglobin value obtained in this study is within the normal range so that it will not cause disease, one of which is anemia. The average haemoglobin value parameter is within the normal haemoglobin range, which is between 6.1-21.7 g/dl [11].

The hematocrit value is the ratio between red blood cells and overall blood volume. With increasing red blood cells and haemoglobin, the hematocrit value will also increase. Hematocrit levels are closely related to anemia [12]. A decrease in the hematocrit percentage below the normal range may indicate anemia. The low hematocrit percentage is caused by the destruction of old erythrocytes or a low number of erythrocytes. Suppose there is an increase in hematocrit levels from the normal range. It that case, caution must be taken because too high levels can cause the blood to become thick, triggering disruption of blood circulation and the supply of nutrients to the tissues that it passes through and resulting in tissue death. The average of the parameters of the hematocrit level is within the normal range of the hematocrit level which ranges from 16.7 to 69.8% [11].

Two-way ANOVA statistical test showed that the number of erythrocytes, leukocytes, haemoglobin and hematocrit were not significantly affected ($p>0.05$) by dose and duration of administration. Likewise, there is no interaction between the two. Based on the description above, the blood parameters observed in this study were not significantly affected by variations in dose and duration of administration to male white mice, so that the fraction of African leaf water (*Vernonia amygdalina Del.*) given did not cause toxic effects. Future research is expected to examine chronic, sub-chronic, and other specific toxicity tests of African leaves (*Vernonia amygdalina Del.*). These supporting data can be obtained as a requirement to be used as phytopharmaca preparations.

4. CONCLUSION

Variations in dose and duration of administration of the water fraction of African leaves (*Vernonia amygdalina Del.*) did not affect on the number of erythrocytes, leukocytes, haemoglobin, and hematocrit ($p>0.05$). These indicated that African leaves water fraction (*Vernonia amygdalina Del.*) did not give a toxic effect at doses of 10 mg/kg BW, 20 mg/kg BW, and 40 mg/kg BW in males white mice.

ACKNOWLEDGMENT

The authors would like to thank all those who were involved and contributed significantly to this research. However, the authors received no financial support for the research, authorship and/or publication of this article.

REFERENCES

[1]. Wijayaputri A, Tjahjadi E. Galeri Obat Tradisional dan SPA (Traditional Medicine Gallery and SPA). J STUPA. 2019;1(1):48–59.

[2]. RI MK. Peraturan Menteri Kesehatan Republik Indonesia Nomor 006 Tahun 2012 tentang Industri dan Usaha Obat Tradisional (Traditional Medicine Industry and Business). 2012.

[3]. Ijeh II, Ejike CECC. Current perspectives on the medicinal potentials of Vernonia amygdalina Del. J Med Plants Res. 2011;5(7):1051–61.

[4]. Kharimah NZ, Lukmayani Y, Livia S. Identifikasi Senyawa Flavonoid pada Ekstrak dan Fraksi Daun Afrika (Vernonia amygdalina Del.) (Identification Flavonoid Compound toward Extract and Fraction of Africa Leaves (Vernonia amygdalina Del.). In: Prosiding Farmasi. 2016. p. 703–9.

[5]. Bhowmik D, Chiranjib, Dubey P, Chandira M, Kumar KPS. Herbal Drug Toxicity and Safe Evaluation of Traditional Medicines. Sch Res Libr. 2009;1(2):32–56.

[6]. Hodgson E. A textbook of modern toxicology. Fourth Edi. Vol. 8, Trends in Pharmacological Sciences. John Wiley & Sons, Inc.; 2010. 670 p. https://doi.org/10.1016/0165-6147(87)90110-6

[7]. BPOM. Peraturan Kepala BPOM RI No HK.00.05.41.1384 tentang Kriteria Dan Tata Laksana Pendaftaran Obat Tradisional, Obat Herbal Terstandar dan Fitofarmaka (Criteria and Procedures for Registration of Traditional Medicines, Standardized Herbal Medicines and Phyto. Badan Pengawas Obat dan Makanan Republik Indonesia 2005 p. 1–16.

[8]. Adiukwu PC, Amon A, Nambatya G, Adzu B, Imanirampa L, Twinomujuni S, et al. Acute toxicity , antipyretic and antinociceptive study of the crude saponin from an edible vegetable:

Vernonia amygdalina leaf. Int J Biol Chem Sci. 2012;6(3):1019–28.

[9]. Hall JE. Guyton and Hall Textbook of Medical Physiology. Thirteenth. Elsevier; 2016. https://doi.org/10.1016/b978-0-12-800883-6.00072-0

[10]. Faradisa N, Marfu'ah N, Amal S. Uji Toksisitas Akut Infusa Daun Afrika (Vernonina amygdalina Del.) Terhadap Hispatologi Ginjal Mencit Galur BALB/C (Acute Toxicity Test of African Leaf Infusion (Vernonina amygdalina Del.) Against Kidney Hispathology of BALB/C Strain Mice). Pharm J Islam Pharm. 2018;2(1):1–8. https://doi.org/10.21111/pharmasipha.v2i1.2131

[11]. Brayton C, McBean NF, Watson J. Mouse Pathobiology & Phenotyping Short Course Lab Manual. In: 5th Editio. USA; 2019.

[12]. Arifin H, Oktavia S, Chania S. Efek Toksisitas Sub Akut Fraksinasi Air Ekstrak Etanol Daun Bandotan (Ageratum Conyzoides (L .) L .) Terhadap Beberapa Parameter Darah Mencit Putih Jantan (Effects of Sub-Acute Toxicity of Water Fractionation of Ethanol Extract of Bandotan Leaves (Agera. J Farm Higea. 2019;11(2):166–74.

[13]. Dzierzak E, Philipsen S. Erythropoiesis: Development and differentiation. Cold Spring Harb Perspect Med. 2013;3(4):1–16. https://doi.org/10.1101/cshperspect.a011601