Original research article

Effects of Sub-acute Ethanol Extract Toxicity of Karamunting (*Rhodomyrtus tomentosa*) Leaves on Hematological Profile in Female White Rats

Tia Wida Ekaputri[1], Hana Nurul Qur'ani[2,*], Citra Maharani[1], Anggelia Puspasari[1], Budi Justitia[3], Esa Indah Ayudia[4]

[1]*Department of Biochemistry and Medical Biology, Faculty of Medicine and Health Sciences, Universitas Jambi*

[2]*Bachelor of Medical Education Program, Faculty of Medicine and Health Sciences, Universitas Jambi*

[3]*Department of Anatomy, Faculty of Medicine and Health Sciences, Universitas Jambi*

[4]*Department of Physiology, Faculty of Medicine and Health Sciences, Universitas Jambi*

Correspondence: **hananurulq29@gmail.com**

ABSTRACT

Background: Karamunting leaves (*Rhodomyrtus tomentosa*) are a medicinal plant widely used in Indonesia for their bioactive compounds, which exhibit anti-inflammatory, antibacterial, antioxidant, antidyslipidemic, and anti-hyperglycemic properties. However, the safe dosage for human use remains undetermined. This study aims to assess the sub-acute toxicity of ethanol extracts of karamunting leaves on the hematological profile of female white rats. **Methods:** This study used an experimental laboratory design with a post-test control group. Karamunting leaves were extracted by maceration using 70% ethanol as the solvent. The hematological profiles measured included erythrocytes, leukocytes, platelets, and hematocrit levels. Female white rats of the Wistar were randomly divided into six groups. The negative control group received only a 0.5% Na CMC suspension, while the treatment groups received ethanol extracts of karamunting leaves at doses of 200 mg/kg BW, 400 mg/kg BW, 800 mg/kg BW, 1600 mg/kg BW, and 3200 mg/kg BW. Blood samples were collected after 14 days of treatment. **Results:** The hematological profiles indicated no significant differences among the groups. However, the treatment group that received extracts at a dose of 1600 mg/kg BW showed a poor hematological profile. **Conclusion:** Generally, the ethanol extract of karamunting leaves did not affect the hematological profiles of the test animals.

Keywords: *Rhodomyrtus tomentosa*; hematological profile; sub-acute toxicity

INTRODUCTION

In recent years, people worldwide have become increasingly interested in using herbal medicine as a source of medication. The reasons why herbal medicines are often chosen by the public for treatment include minimal side effects compared to synthetic drugs and positive experiences with the use of herbal remedies.[1] Karamunting (*Rhodomyrtus tomentosa*) is one of the medicinal plants that has been used empirically in South and Southeast Asia, including in Indonesia, such as in Kalimantan and Sumatera. The leaves of the karamunting plant are believed to treat diarrhea, dysentery,

gynecological issues, and wound healing. The Malay community in Jambi uses karamunting leaves to treat diabetes, hypertension, and hypercholesterolemia.[2,3] The leaves of the karamunting plant are the most researched part for their bioactive compound content. Karamunting leaves contain various bioactive compounds, including floroglucinol, flavonoids, tannins, terpenoids, and anthraquinone glycosides, which have benefits such as antioxidant, anti-inflammatory, antibacterial, and anticancer properties.[4]

To further explore the potential benefits of karamunting as an antidyslipidemic and antihyperglycemic agent related to its antioxidant activity, toxicity testing is needed to determine a safe dosage for human use. Toxicity testing aims to detect any toxic effects of a substance on biological systems, providing data on dosage and specific responses to the tested preparation.[5] Previous research using a maximum dose of 800 mg/kg BW found no significant effects on the hematological profile of the rats.[6] Based on this background, this study was conducted to examine the sub-acute toxicity effects of ethanol extract from karamunting leaves on the hematological profile of female white rats.

METHODS

This research was a laboratory experimental study with a post-test control group design. Karamunting leaves were extracted using maceration and evaporation techniques with 70% ethanol as the solvent. The extracted product was then weighed for individual oral administration to rats according to the specified doses. The stock extract was stored in a tightly sealed dark glass bottle wrapped in aluminum foil.

The research subjects were female Wistar strain white rats (*Rattus norvegicus*), aged 2.5–3 months, weighing 175–220 grams, and non-pregnant. A total of 18 rats that met the inclusion and exclusion criteria were first acclimatized in the Animal Laboratory of FKIK, Universitas Jambi, for two weeks to adapt to the environment. The rats were then randomly divided into six groups: one negative control group, which received only a 0.5% Na CMC suspension without karamunting leaf ethanol extract, and five treatment groups, each receiving karamunting leaf ethanol extract at doses of 200 mg/kg BW, 400 mg/kg BW, 800 mg/kg BW, 1600 mg/kg BW, and 3200 mg/kg BW. All liquid volumes administered to the rats were 3 ml. The treatments were given orally using a gastric tube every morning for two weeks.

The hematological parameters to be measured include erythrocytes, leukocytes, platelets, and hematocrit. Blood samples were collected from the retroorbital sinus of the rats using a microhematocrit, then transferred into tubes containing EDTA and sent to the Health Laboratory of Jambi City. The data from the study were analyzed using the Statistical Package for the Social Sciences

(SPSS) version 25. If the data were normally distributed and homogeneous, bivariate statistical tests would be conducted to determine the means among groups using one-way ANOVA.

RESULTS

The normal range of hematological values for this study was referenced from the journal "Toxicology Research and Application", which provided values specific to female Wistar strain rats. According to this guideline, the normal values used in this research are as follows: erythrocytes: 6.4 – 8.9 x 10^6/mm³, leukocytes: 1.4 – 7.7 x 10^3/mm³, platelets: 750 – 1600 x 10^3/mm³, and hematocrit: 37 – 49%.[7]

In **Table 1**, the average levels of hematological profiles (erythrocytes, leukocytes, platelets, and hematocrit) for each treatment group are listed. The average levels of erythrocytes, platelets, and hematocrit in all groups were within the normal range, except for the average leukocyte levels, which were above the normal range across all groups.

The hematological profiles of all groups were analyzed for differences using a One-Way ANOVA test, yielding p-values of 0.625 for erythrocyte levels, 0.190 for leukocyte levels, 0.079 for platelet levels, and 0.060 for hematocrit levels. Thus, there were no significant differences in hematological profiles between the treatment groups.

Table 1. Average and significance testing of hematological profile levels in female white rats administered ethanol extract of karamunting leaves (*Rhodomyrtus tomentosa*)

Treatment Group	Mean Hematological Profile ± SD			
	Erythrocytes (10^6/mm³)	Leukocytes (10^3/mm³)	Platelets (10^3/mm³)	Hematocrit (%)
Control	8.0 ± 0.9	**9.2 ± 2.0**	757.3 ± 290.1	46.7 ± 4.2
200 mg/kg BW	7.5 ± 0.3	**9.7 ± 1.3**	1048.3 ± 150.0	39.6 ± 0.8
400 mg/kg BW	7.9 ± 0.5	**10.5 ± 3.0**	890.3 ± 196.2	40.4 ± 0.8
800 mg/kg BW	7.3 ± 0.3	**11.3 ± 0.8**	915.0 ± 139.9	38.6 ± 1.5
1600 mg/kg BW	7.6 ± 0.7	**14.3 ± 0.5**	1265.0 ± 156.0	39.7 ± 3.3
3200 mg/kg BW	7.4 ± 0.4	**10.0 ± 4.4**	922.3 ± 126.4	39.9 ± 3.3
p-value	0.625	0.190	0.079	0.060

Note: Bold values indicate mean values that fall outside the normal range according to the guidelines

DISCUSSION

Toxicity testing is conducted to assess the extent to which a substance or compound can produce harmful or toxic effects on biological systems. The data obtained will be used to identify a safe dosage for the tested formulation and to understand potential side effects and the degree of risk posed by the substance or compound if applied to humans. One type of toxicity test is the subacute toxicity test, which aims to assess the impact of a drug or compound, along with any side effects, after repeated administration over a period of 7–90 days. The results of the toxicity test help to determine whether the drug or compound under study can cause bodily damage or injury, whether such injury is reversible or irreversible, and at what dose side effects may begin to appear.

Based on research by Situmorang et al., erythrocytes, leukocytes, and platelets parameters were affected by the dosage of Karamunting administered (200-3200 mg/kg body weight).[8] These findings differ from the present study, which found no significant difference between all dose groups (200-3200 mg/kg body weight) and the control group in the hematological profile of rats following the administration of ethanol extract from Karamunting leaves (*Rhodomyrtus tomentosa*).

Erythrocytes play an essential role in the blood as oxygen carriers from the lungs to tissues. Low erythrocyte levels indicate anemia, while high erythrocyte levels indicate polycythemia. Erythrocyte levels may decrease in anemic conditions, leading to reduced kidney function and hemolysis. Another factor affecting erythrocyte levels is environmental temperature; erythrocytes increase at lower temperatures and decrease at higher temperatures. Leukocytes function within the body's defense system, causing their levels to fluctuate depending on the presence of foreign substances entering the body. Elevated leukocyte levels can result from wounds, bleeding, toxins, necrosis, or stress. Additionally, in this study, high leukocyte levels may have been caused by contamination from microorganisms present in the rats' food or cages. Platelets function in the blood clotting process and help repair damaged blood vessels to prevent blood loss. They circulate in the bloodstream for a relatively short duration, around 8-10 days. Platelets can become damaged if they remain outside the body for more than 24 hours or are stored at inappropriate temperatures.[9]

Hematocrit is the ratio of the volume of blood containing erythrocytes to the total blood volume. Hematocrit levels depend on the number and size of erythrocytes, as well as other factors that affect erythrocytes. In this study, no significant differences in hematocrit values were observed in rats following the administration of ethanol extract from Karamunting leaves (200-3200 mg/kg body weight and the control group). This aligns with the findings of Situmorang et al., who also found no differences when

administering Karamunting doses of 100-300 mg/kg body weight in rats.[8,9]

The absence of significant hematological toxicity in rats after the administration of high doses of ethanol extract from Karamunting leaves (*Rhodomyrtus tomentosa*) can be attributed to several potential factors. Many herbal extracts, including Karamunting, are known to exhibit low toxicity at typical therapeutic doses, which may be why even high dosages in your study did not produce adverse effects on the hematological profile of the rats. This is consistent with the low toxicity profiles of several natural herbs, which have been recognized for their therapeutic potential without severe side effects.[10]

Certain herbal compounds may have inherent protective or reparative effects on biological systems, helping maintain homeostasis even when administered at higher doses. For instance, bioactive compounds like flavonoids or alkaloids, found in many plants, often support anti-inflammatory, antioxidant, or blood-modulating properties, which can mitigate potential toxic effects.[11] Additionally, herbal extracts often interact with biological systems in a more gentle and regulated manner compared to synthetic chemicals, which may help explain the lack of significant hematological changes at high doses.

CONCLUSION

There was no significant difference in the hematological toxicity effects of the ethanol extract of Karamunting leaves (Rhodomyrtus tomentosa) on the hematological profile of white rats, including erythrocyte, leukocyte, platelet, and hematocrit levels across treatment groups. However, a trend toward increased toxic effects was observed at the dose of 1600 mg/kg body weight.

RECOMMENDATIONS

Further studies are needed to assess hematological parameters both before and after treatment. Additionally, histopathological analysis of the liver and kidneys following the administration of ethanol extract from Karamunting leaves should be conducted to determine the extent of its toxic effects.

REFERENCES

1. Albayudi & Saleh, Z. Potensi Tumbuhan Obat Yang Digunakan Masyarakat Melayu Kota Jambi Di Hutan Kota Bagan Pete Kota Jambi. Bio-Lectura: Jurnal Pendidikan Biologi. 2020; 7 (1).
2. Vo, T. S. & Ngo, D. H. The Health Beneficial Properties of Rhodomyrtus tomentosa as Potential Functional Food. Biomolecules. 2019;9(2); 76.
3. Hz TW, Khallilah N, Enis RN. Pengaruh Ekstrak Etanol Daun Karamunting (*Rhodomyrtus Tomentosa*) Pada Kadar Kolesterol Total dan Glukosa Darah Tikus (*Rattus Norvegicus*) Jantan yang Diinduksi Diet Tinggi Lemak. Jambi Medical Journal: Jurnal Kedokteran dan Kesehatan. 2022;10(2):306-13.
4. Hui WH, Li MM, Luk K. Triterpenoids and steroids from Rhodomyrtus tomentosa. Phytochemistry 1975;14(3):833-834.
5. BPOM RI. Pedoman Uji Toksisitas Nonklinik Secara In Vivo. Jakarta: BPOM RI; 2022.

6. Nugraha, GA. Efek pemberian ekstrak etanol 70% daun karamunting (rhodomyrtus tomentosa (aiton) hassk) topikal terhadap gambaran histopatologi ketebalan serat kolagen penyembuhan luka insisi kulit tikus putih galur wistar. Naskah Publikasi Fakultas Kedokteran Universitas Tanjungpura Pontianak. 2016
7. De Kort M, Weber K, Wimmer B, et al. Historical control data for hematology parameters obtained from toxicity studies performed on different Wistar rat strains: Acceptable value ranges, definition of severity degrees, and 38 vehicle effects. Toxicology Research and Application. 2020;4. doi:10.1177/2397847320931484.
8. Situmorang PC, Ilyas S, Syahputra RA, Nugraha AP, Putri MSS, Rumahorbo CGP. Rhodomyrtus tomentosa (Aiton) Hassk. (haramonting) protects against allethrin-exposed pulmo damage in rats: mechanistic interleukins. Front Pharmacol. 2024;6(15)
9. Guyton A. C., Hall J. E. Buku Ajar Fisiologi Kedokteran. Edisi 13. Jakarta : EGC. P; 2016
10. Yang J, Yu B, Zheng J. Natural herbal extract roles and mechanisms in treating cerebral ischemia: A systematic review. Frontiers in Pharmacology. 2024;15(1424146).
11. Jităreanu A, Trifan A, Vieriu M, Caba IC, Mârțu I, Agoroaei L. Current trends in toxicity assessment of herbal medicines: A narrative review. Processes. 2022;11(1):83.