



Hematological Profile of Iron Overload in Rats Administered with Fruit Extract of Mahkota Dewa (*Phaleria macrocarpa*)

Gambaran Hematologi Tikus Model Besi Berlebih yang Diberi Ekstrak Buah Mahkota Dewa (Phaleria macrocarpa)

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ABSTRACT

Background: Repeated blood transfusions lead to an accumulation of iron that exceeds the body's iron storage capacity. Free iron is able to catalyze the formation of hydroxyl radicals that cause oxidative damage and cell death. The use of iron chelating medicines for iron chelation therapy is limited by side effects. Mangiferin is a natural bioactive compound with iron chelating and antioxidant activity. Mahkota dewa is a plant native to Indonesia that contains mangiferin. **Objectives:** The aim of this study was to evaluate the effect of mahkota dewa fruit extract administration on hematological profile of iron overload rats. **Material and Methods:** Thirty rats were divided into 6 groups: normal rats (N), iron overload rats (IO), iron overload rats given deferiprone (D), mangiferin (M), Mahkota dewa fruit extract at a dose of 100 mg/kg BW (PM1), and 200 mg/kg BW (PM2). Rats were given injection of iron sucrose 15 mg every 3-4 days for 8 weeks. At week 8, rats were sacrificed. Hematological analysis was performed in this study. **Results:** Iron overload condition caused by iron sucrose injection did not cause significantly changes in the haematological profile. Likewise, the administration of mahkota dewa fruit extract at a dose of 100 or 200 mg/kg BW did not cause changes in the hematological profile. **Conclusions:** The administration of mahkota dewa fruit extract did not alter hematological profile of iron overload rats.



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ABSTRAK

Latar Belakang: Transfusi darah berulang menyebabkan akumulasi zat besi yang melebihi kapasitas penyimpanan zat besi tubuh. Besi bebas mampu mengkatalisis pembentukan radikal hidroksil yang menyebabkan kerusakan oksidatif dan kematian sel. Terapi kelasi besi menggunakan obat kelasi besi terhambat oleh efek sampingnya. Mangiferin adalah senyawa bioaktif alami dengan aktivitas pengkelat besi dan antioksidan. Mahkota dewa merupakan tanaman asli Indonesia yang mengandung mangiferin. Tujuan: Penelitian ini bertujuan untuk mengevaluasi pengaruh pemberian ekstrak buah mahkota dewa pada gambaran hematologi tikus model besi berlebih. Bahan dan Metode: Tiga puluh tikus dibagi ke dalam 6 kelompok: tikus normal (N), tikus model besi berlebih (IO), tikus model besi berlebih yang diberi deferiprone (D), mangiferin (M), ekstrak buah mahkota dewa dosis 100 (PM1), dan 200 mg/kg BB (PM2). Tikus diberi injeksi besi sukrosa 15 mg setiap 3-4 hari selama 8 minggu. Pada minggu ke-8, tikus dikorbankan. Analisis hematologi dilakukan dalam penelitian ini. Hasil: Kondisi besi berlebih yang disebabkan oleh injeksi besi sukrosa tidak menyebabkan perubahan signifikan secara statistik pada gambaran hematologi. Demikian pula pemberian ekstrak buah mahkota dewa baik dosis 100 mg/kgBB maupun 200 mg/kgBB tidak menyebabkan perubahan pada gambaran hematologi. Kesimpulan: Pemberian ekstrak buah mahkota dewa tidak menyebabkan perubahan pada gambaran hematologi tikus model besi berlebih.

Kata kunci: Mahkota dewa; *Phaleria macrocarpa*; Mangiferin; Besi berlebih; Gambaran hematologi

INTRODUCTION

Iron overload is a condition in which too much iron accumulates in the body. Iron overload can result from excessive iron absorption in the small intestine mucosa and repeated red blood cell transfusions in patients with chronic anemia, such as beta thalassemia major. Until recently, the modality for treating iron overload, especially in patients with transfusion-dependent thalassemia, was long-term iron chelation therapy using iron chelating drugs (Kontoghiorghe & Kontoghiorghes, 2016). Iron chelation therapy is not optimal in many countries, particularly in developing countries. This is due to the expensive cost of iron chelating drugs and their intolerable side effects, which can lead to poor adherence to therapy (Kontoghiorghe & Kontoghiorghes, 2016). The limitations of currently available iron chelator drugs highlight the need for alternative pharmacological interventions using natural iron chelators. One of the bioactive compounds that has iron chelating activity is mangiferin. Mangiferin has iron chelating activity due to the presence of catechol groups.

Phaleria macrocarpa (Scheff.) Boerl, popularly known as mahkota dewa, is a popular medicinal plant in Indonesia. Its fruit contains high mangiferin levels (Aini Hashim et al., 2017). The fruit of mahkota dewa is green when unripe and becomes red when ripe. Fruit appears in about 10-12 months and takes about 2 months to ripen (Altaf et al., 2013). Mahkota dewa is a medicinal plant that is used in the treatment and management of cancer, diabetes mellitus, liver disease, and hypertension based on traditional uses, but until now there has been no scientific report on its use in iron overload condition.

In the development of natural products, it is necessary to prove safety at the pre-clinical level before proving efficacy. The study of hematological parameters can be evidence that supports the safety of the extract in experimental animals because it can describe physiological or pathological changes. If the

extract shows a toxic effect on the body, its components such as red blood cells, hemoglobin, white blood cells, and platelets will undergo alterations. Measurements of the red blood cell count, hematocrit, and hemoglobin can be used to determine anemia, which can be caused by a decrease in the number of erythrocytes, or a decrease in the size of red blood cells (MCV), the amount of hemoglobin per erythrocyte (MCH), the concentration of hemoglobin per total erythrocyte (MCHC). In addition, changes in leukocyte count can be an indicator of immunotoxicity. The purpose of this study was to evaluate the effect of mahkota dewa fruit extract administration on hematological profile of iron overload rats.

MATERIAL AND METHODS

Materials

Study drugs for experimental animals are deferiprone, ethanol extract of mahkota dewa fruit, mangiferin powder, and food grade CMC powder. The dried fruit of mahkota dewa (*Phaleria macrocarpa*) was purchased from Flozindo, Purwokerto. Deferiprone (Ferriprox) was purchased from Apotex Inc. Iron sucrose (Venofer) was purchased from Vifor SA. Mangiferin powder derived from *Mangifera indica* L leaf extract with 98% mangiferin content was purchased from Henan Senyuan Biological Technology Co., Ltd. Sprague-Dawley male rats, 4-5 weeks old of age, weighing between 100-150 g were obtained from PPPOMN Badan POM. Experimental animals were treated for \pm 1 month until they reached a body weight between 200-250 g. Experimental animals care, acclimatization, and intervention were carried out at IMERI's Animal Research Facilities during September 2020 – January 2021. Hematology parameters were analyzed using a hematology analyzer "SK8800 Vet automatic hematology analyzer".

Methods

Extraction

Ethanol extract of mahkota dewa fruit was made at Balitro Bogor. Dried mahkota dewa fruit was extracted by maceration using 70% ethanol as a solvent. To obtain a concentrated extract, the ethanolic extract was concentrated for 6 hours using a rotary evaporator. The solvent used in this study was 70% ethanol because prior research has shown that the content of mangiferin can be dissolved well in both water and ethanol solvents (Aini Hashim et al., 2017; Tayana et al., 2019)

Animal Treatments

The protocol was approved by the Health Research Ethics Committee, Faculty of Medicine, Universitas Indonesia-Cipto Mangunkusumo Hospital no. KET-656/UN2.F1/ETIK/PPM.00.02/2020. The number of experimental animals involved in this study was determined using Frederer's formula for 6 treatment groups and each group was added 1 extra animal to anticipate the loss of the experimental unit. Therefore, the number of rats used in each treatment group was 5 rats. Sprague Dawley rats were

allocated to 6 experimental groups as follows: a group without iron sucrose injection as a normal control (N), an iron overload group (IO), and four treatment groups. Deferiprone dose was calculated based on extrapolation from recommended dose of deferiprone in humans, which is 75 mg/kg BW/day, while mangiferin dose was selected based on previous studies (Estuningtyas, Setiabudy, et al., 2019). Determination of the dose of mahkota dewa fruit extract 100 and 200 mg/kgBW was based on the study of subchronic toxicity in Sprague Dawley rats conducted by sulistiyani (2004). The study found that ethanolic extract of mahkota dewa administration at doses of 100 mg/kg BW and 200 mg/kg BW once a day for 28 days did not cause death in experimental animals, and histological investigation revealed mild, reversible alterations (Sulistiyani, et al., 2004). The iron overload and treatment groups received an intraperitoneal injection of 0.75 mL of iron sucrose containing 15 mg of elemental iron every 3-4 days for 8 weeks, for a total dose of 225 mg/rat. After being injected with iron sucrose for 3 weeks to induce iron overload condition (Estuningtyas, Wahyuni, et al., 2019), the treatment group received either deferiprone 462.5 mg/kg BW (D), mangiferin 50 mg/kg BW (M), mahkota dewa fruit extract 100 mg/kg (PM1) and 200 mg/kg BW (PM2) orally once a day for 28 days. At the end of the study, all rats were anesthetized (ketamine-xylazine) and euthanized exsanguination via cardiac puncture. For hematological examination, the blood sample was collected in tube containing anticoagulant lithium heparin to maintain its liquidity.

Data Analysis

Statistical data were expressed as mean±standard deviation of the number of animals used in each group. All data were analyzed using IBM SPSS program. The significant difference between the mean values was determined by one-way ANOVA test and followed with Tukey test. For non-parametric analysis, Kruskal Wallis and Mann-Whitney tests were used. p-values below 0.05 were considered to have significant difference.

RESULTS AND DISCUSSION

Several parenteral iron formulations including iron sucrose, iron dextran, and iron polymaltose have been used to produce iron overload conditions in experimental animals (Estuningtyas, Setiabudy, et al., 2019; Lê et al., 2011; Toblli et al., 2015) The accumulation of iron in experimental animals that received parenteral iron injection mainly occurred in reticuloendothelial cells because parenteral iron first entered from the veins to the liver, then macrophages released iron from carbohydrate ligands (Koskenkorva-Frank et al., 2013). In this study, no significant differences were observed in all hematological parameters between the normal group and the negative control group. This is because iron sucrose injection that performed in experimental animals were not in iron deficient. It is assumed that iron was not transported to the bone marrow for the formation of red blood cells. The iron-polysaccharide

complex is taken up by macrophages and broken down so that iron is released, then stored in the form of ferritin (Lê et al., 2011) or excreted in the urine and feces in higher amounts than in experimental animals that were not injected with iron.

The results of the statistical test of this study showed that mangiferin and mahkota dewa fruit extract administration to iron overload rats did not show significant side effects on hemoglobin levels and erythrocyte index (MCV, MCH, and MCHC) when compared to normal and negative control groups. In this study, MCV and RDW values in the deferiprone group were significantly different from the normal and negative control groups. When MCV and RDW values in the deferiprone group were compared with the mangiferin and mahkota dewa fruit extract groups, statistical analysis showed significant differences (Table 1).

Table 1. The effect of iron overload and the administration of mahkota dewa fruit extract on hematological profile

Unit parameter	N	IO	D	M	PM1	PM2
Hemoglobin (g/L)	93,36±47,82	107,40±23,65	100,00±26,42	108,25±18,64	122,20±29,62	97,50±21,38
RBC (10 ⁶ /uL)	6,18±0,75	5,82±0,75	4,89±0,94	5,95±0,74	6,00±0,46	5,46±0,87
HCT (%)	38,40±4,64	35,10±5,64	36,50±7,37	34,93±4,10	35,76±3,36	33,13±5,77
MCV (fL)	62,20±0,82	60,20±2,11	74,72±2,84 ^{a,b}	59,45±0,90 ^c	59,64±1,30 ^c	60,70±1,25 ^c
MCH (pg)	19,14±1,48	18,24±1,59	20,16±2,03	18,28±1,22	18,26±1,00	17,70±1,28
MCHC (g/L)	308,80±23,52	678,80±531,41	270,20±21,10	308,25±18,45	307,00±13,32	292,00±16,47
RDW (%)	15,48±0,99	15,38±1,06	20,36±0,85 ^{a,b}	15,20±0,24 ^c	15,14±0,46 ^c	15,38±0,30 ^c
WBC (10 ³ /uL)	5,86±1,09	6,44±2,85	3,30±1,02	7,60±1,35	6,44±2,00	5,68±3,41
Granulosit (%)	40,54±9,26	36,14±7,21	31,14±4,48	41,45±11,16	43,28±5,80	35,83±2,83
Limfosit (%)	38,22±5,96	37,10±6,27	45,22±11,52	37,28±8,25	35,50±5,84	40,05±2,56
Mid (%)	21,24±5,39	26,76±4,72	23,64±9,02	21,28±3,38	21,22±3,91	24,13±3,59
Platelet (10 ³ /uL)	474,80±162,01	303,60±17,30	793,80±522,77	847,75±302,17	663,80±140,53	512,25±317,54

Data are presented as mean ± standard deviation; a) p<0.05 versus normal group, b) p<0.05 versus negative control group, c) p<0.05 versus deferiprone group after Kruskal-Wallis test followed by Mann-Whitney test; RBC: Red blood cells, HCT: hematocrit, Hb: hemoglobin, MCV: mean corpuscular volume, MCH: mean corpuscular hemoglobin, MCHC: mean corpuscular hemoglobin concentration, RDW: red cell distribution width, WBC: white blood cells.

Administration of 100 and 200 mg/kg BW of mahkota dewa fruit extract did not cause significant differences in the levels of white blood cells and the proportions of granulocytes, lymphocytes, and mids (basophils, eosinophils, and monocytes) compared to the normal and negative control groups. This shows that administration of 100 and 200 mg/kg BW mahkota dewa fruit extract did not cause changes in the function of innate immune system in experimental animal models of iron overload. In the deferiprone group, the levels of white blood cells and the proportion of granulocytes, lymphocytes, and mids were lower than the normal, negative control, and other treatment groups. This finding is in line with studies in human subjects which state that administration of deferiprone can cause side effects of neutropenia and agranulocytosis (Kontoghiorghe & Kontoghiorghes, 2016).

Based on our findings, we suggest iron injections are given more frequent to cause liver damage and enhance free radical generation in attempt to develop animal models of iron overload with organ damage for future study. Alternatively, parenteral iron other than sucrose can be used, for example iron dextran which has a high molecular weight and is more stable than iron sucrose so that it delivers iron from the iron-carbohydrate complex to transferrin in a controlled manner and is not immediately stored in the form of ferritin. Thus, it takes longer to be excreted in the urine or feces than iron sucrose. By taking into account the half life of the selected parenteral iron, it is also necessary to consider the timing of blood collection for the measurement of iron levels. Because parenteral iron may have been eliminated through urine and feces or it may have been retained in stable iron forms if blood samples were taken for longer than its half-life. We suggest that blood sampling should be carried out before iron is uptake by the reticuloendothelial system that is less than 24 hours after the last iron injection.

CONCLUSION

This study showed that administration of the ethanol extract of mahkota dewa fruit orally for 28 days did not affect the hematological profile of iron overload rats at doses of 100 and 200 mg/kg BW. Studies are needed to determine further toxic effects on organs.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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