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ANALYSIS OF KENIKIR LEAVES ON HEMOGLOBIN LEVELS IN MALE WISTAR RATS EXPOSED CIGARETTE SMOKE

(Analisis Pemberian Daun Kenikir Terhadap Kadar Hemoglobin pada Tikus Wistar Jantan yang Dipapar Asap Rokok)

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ABSTRACT

Background: cigarette smoke is one of the source of free radicals. The influence of free radicals can be suppressed through antioxidant administration. The kenikir leaves contain phenolic as antioxidants. This study aims to determine the effect of kenikir leaves on hemoglobin levels of the blood of rats exposed to cigarette smoke.

Method: the samples were divided into 3 groups (the control group and the treatment group 1 and 2). While the treatment group 1 and 2 were given the exposure to cigarette smoke by giving kenikir leaves at a dose of 4.5 grams and 6.3 grams. Blood sampling was carried out on days 0, 7 and 14, measuring the blood sample parameters using the Hematology Analyzer Sysmex KX-21®. Data were analyzed with One Way Anova test, and posthoc the Duncan test.

Results: there was a significant effect between the control group against the treatment group 1 and 2 ($p < 0.05$). There were no significant differences between kenikir leaves groups with a dose of 4.5 grams and 6.3 in increasing hemoglobin levels ($p > 0.05$).

Conclusion: there was significant effect of kenikir leaf on hemoglobin levels exposed cigarette smoking at a dose of 4.5 gram and 6.3 gram.

Key words: cigarette smoke, kenikir leaves, hemoglobin levels

ABSTRAK

Latar belakang: Asap rokok merupakan salah satu sumber radikal bebas. Pengaruh radikal bebas dapat ditekan melalui pemberian antioksidan. Daun kenikir mengandung senyawa fenolik yang bersifat antioksidan. Penelitian ini bertujuan untuk mengetahui efek daun kenikir terhadap kadar hemoglobin darah tikus yang dipapar asap rokok.

Metode: Sampel dibagi dalam 3 kelompok, yaitu kelompok kontrol dan kelompok perlakuan 1 dan 2. Kelompok kontrol hanya diberi paparan asap rokok, sedangkan kelompok perlakuan 1 dan 2 diberi paparan asap rokok dengan pemberian daun kenikir dengan dosis 4,5 gram dan 6,3 gram/hari. Pengambilan darah dilakukan pada hari ke 0, 7 dan 14, parameter sampel darah diukur menggunakan Hematology Analyzer Sysmax KX-21®. Data dianalisis dengan Uji One Way Anova, dan uji lanjut Duncan.

Hasil: adanya pengaruh yang signifikan antara kelompok kontrol terhadap kelompok perlakuan 1 dan 2 ($p < 0,05$). tidak terdapat perbedaan secara nyata pada kelompok perlakuan 1 dan 2, daun kenikir dengan dosis 4,5 gram dan 6,3 gram tidak berbeda nyata dalam meningkatkan kadar hemoglobin ($p > 0.05$).

Kesimpulan: terdapat pengaruh pemberian daun kenikir terhadap kadar hemoglobin yang diberi paparan asap rokok dengan dosis 4,5 gram dan 6,3 gram.

Kata kunci: asap rokok, daun kenikir, kadar hemoglobin

INTRODUCTION

Air is the most important factor in life. However, in this modern era in line with developments in the physical development of cities, industrial centers, lifestyles, and transportation developments, air quality has changed (Ismiyati, 2014). In some communities, smoking is a habit that is difficult to break and is harmful to health. Smoking is not only dangerous for smokers but also those around them who are exposed to cigarette smoke. Cigarette burning will produce cigarette smoke which is divided into mainstream cigarette smoke and sidestream smoke (Nurjanah, et al., 2014).

According to the WHO Regional Office for Southeast Asia (2020), there are 60.8 million male smokers and 3.7 million female smokers over the age of 15 in Indonesia. Some people do it in a closed room so that the people around them become passive smokers. Cigarette smoke is not only harmful to active smokers but also passive smokers. Research by Nurjannah, et al. (2014) stated that secondhand smoke can inhale at least 75% of the harmful substances in cigarette smoke.

Contain chemicals that are generally toxic, carcinogenic and addictive so that it is bad for health. Smoking can increase the activity of the hematological system which is characterized by an increase in the number of erythrocytes, leukocytes, platelets, and hemoglobin in the blood (Besime, et al., 2014). The smoke of cigarette can increase free radicals in the body (Susanti and Wirjatmadi, 2016).

Hemoglobin has an important role in binding, transporting and sending oxygen to all body tissues that need it (Susanti and Wirjatmadi, 2016). If cigarette smoke is inhaled into the respiratory system, it will enter the blood circulation system which can cause Reactive Oxygen Species (ROS) so that

it can cause oxidative stress on erythrocytes. In erythrocytes contained hemoglobin (Hb) which is a structure consisting of heme and globin. Hemoglobin can bind O₂ and CO₂ so that hemoglobin is a very important component in maintaining the integrity of the body's circulatory system (Harlev, et al., 2015).

A country in Southeast Asia has various types of plants that are rich in benefits, almost 90% of herbal plants are found in countries in the Asian region (Salim and Munadi, 2017). This is because kenikir plants are only consumed by the community as cooked vegetables and raw vegetables or fresh vegetables. In East Java and Central Java, it is often used as an ointment vegetable, while in West Java the leaves and buds of young kenikir plants are used as medicine to reduce bad breath and vegetables. Kenikir leaves (*Cosmos caudatus* Kunth.) Have many health benefits including inflammatory activity because the content of flavonoids can improve blood circulation and strengthen bones and can increase appetite (Bunawan, et al., 2014).

Kenikir plants (*Cosmos caudatus* Kunth.) are usually only considered as wild plants that often grow on houses and roadsides. This is the basis of studies to obtain data on the benefits and scientific influence of kenikir plants (*Cosmos caudatus* Kunth.) on the hemoglobin level in rats that have been exposed to cigarette smoke.

The health of the human body can be seen by blood hematological status of hemoglobin. The purpose of this research is to be expected with the giving of kenikir leaves can know and improve the health of rats that Wistar smoked through hemoglobin level.

METHOD

This study was true experimental study with a pre-post test design with random sampling. The research was conducted at the Center for Food and Nutrition Study Laboratory (PSPG) Universitas Gadjah Mada, Yogyakarta in December 2019 - January 2020.

Material used in this study are kenikir leaves and clove cigarette. Kenikir leaves are obtained from the Yogyakarta traditional market, then the leaves are separated from the stem, and extract kenikir leaves with aquadest before given to subject. Clove cigarette smoke with a tar content of 39 mg and nicotine 2.5 mg per cigarette as a testing material.

Subjects were 6-8 week-old wistar rats weighing 150-200 grams. The rats acclimatized for 1 week were randomized and divided into 3 groups (K- as control, P1 and P2) each consisting of 10 rat. Each rats given standard feed. For group P1 and P2 given kenikir leaves (4.5 and 6.3) grams/kg BW.

Research Stage

The dosage of Kenikir leaves given to animals was determined based on daily consumption of fruits and vegetables, which is 400-600 grams per person per day and two-thirds of the recommended amount of consumption is the portion of vegetables (Kementerian Kesehatan RI, 2016). This dose was converted to the dose for rats determined at 70 kg human body weight and 200-gram rat. Based on the calculation of dose conversion, the value of dose conversion for humans to the rat is 0.018. So that the consumption of vegetables for humans 250 grams per day, for a rat to 250×0.018 grams per day or 4.5 grams per day, and vegetable consumption for humans 350 grams per day, for a rat to 350×0.018 grams per day or 6.3 grams per day. The finer kenikir leaves in the blender were given

to each treatment group P1 and P2 as much as 10 ml, feeding of a rat using the sonde method for 3 weeks (Aiba, *et al.*, 2016). Standard feed and drinking water given by *ad libitum* method. Standard feed including choline, vitamin mix, methionine, mineral mix, sucrose, casein, and soybean oil.

Giving exposure cigarette smoke to Wistar male rats was utilizing each group of rats moved into three different tubes covered with wire around them and given a little air ventilation. The cigarettes used were clove cigarettes with a tar content of 39 mg and nicotine 2.5 mg. Exposure to cigarette smoke is carried out for 15 minutes every day (Permatasari and Purbosari, 2015). Kenikir leaves extract given 1 hour after exposure to cigarette smoke.

Blood was drawn on the morning of every week, that is, on days 0, 7, 14 for 3 weeks, rat blood was drawn through retro-orbital sinuses using capillary hematocrit, collected into the microtube. Determination of the hematology profile using the Sysmex KX-21® Hematology Analyzer. The results obtained were hemoglobin profile in categorical data with ratio scale.

Data was presented in the form of quantitative analysis. The data was obtained using the One Way ANOVA Test, to determine the differences in each treatment, and further tests with Duncan's test of 95% confidence level or $= 0.05$. This study was approved by the ethics commission of the Faculty of Medicine, Muhammadiyah University, Surakarta, with ethics number 2650/A.1

RESULT AND DISCUSSION

Hemoglobin

Table 1. Average Hemoglobin Levels for 3 Weeks

Group	Hemoglobin (g/dL)			Δ_1 (mean \pm SD)	Δ_2 (mean \pm SD)	<i>p-value</i> ₁	<i>p-value</i> ₂
	I	II	III				
K-	12,8	11,3	9,8	-1,2 \pm 1,4 ^a	-2,7 \pm 1,6 ^a		
P1	11,6	13,6	15,4	1,6 \pm 1,3 ^b	3,3 \pm 1,8 ^b	0,001	0,001
P2	11,8	13,9	15,6	1,7 \pm 1,5 ^b	3,3 \pm 1,3 ^b		

Note: K = negative control group, P1 = treatment group giving 4.5-gram kenikir leaves, P2 = treatment group giving kenikir leaves 6.3 gram. Δ_1 = (P1 - (K-)), Δ_2 = (P2 - (K-)). The results of the data use the One Way Anova test, (a & b) = Notification of Duncan's test results, (a) significant differences, (b) no significant difference.

Giving of kenikir leaves in 2 groups with different doses of hemoglobin levels as presented in Table 1 proves the effect of giving kenikir leaves to male Wistar rats that have been exposed to cigarette smoke ($p < 0.05$). The treated rat showed consecutive increases in hemoglobin levels for 3 weeks. To determine the location of the differences in each of these groups, Duncan's continued testing was done at a 95% confidence level. Duncan's further test results showed a significant difference between the negative control group with treatment groups 1 and 2, but there was no significant difference between treatment P1 and P2 ($p > 0.05$), which means kenikir leaves with a dose of 4.5 grams and 6.3 grams have almost the same effect in increasing hemoglobin levels.

In this study, there was a decrease in hemoglobin levels in the blood. This is not in accordance with several studies which declare that exposure to cigarette smoke causes an increase in hemoglobin levels (Wassem and Alfi, 2020) (Khan, *et al.*, 2014) (Shah, *et al.*, 2012). The hemoglobin level shown in Table 1 shows that it is decreased in control mice. Rats experience stress due to moving from the cage to the nursery every morning, which causes a decrease in hemoglobin levels. Several trials have

decreased hemoglobin levels due to acute psychological stress (Austin, *et al.*, 2011) (Stankiewicz, *et al.*, 2014) (Wei, *et al.* 2008).

Increased hemoglobin levels due to kenikir leaves contain polyphenols which act as donors of hydrogen atoms (H^+) to stable free radicals that are not destructive, so that erythrocyte lipid membranes can be protected from free radicals and hemoglobin is not free into the plasma. One of a group of polyphenol compounds found in kenikir leaves which acts as an antioxidant and flavonoid (Dewi, *et al.*, 2018). Flavonoids are lipophilic so they can bind to erythrocyte cell membranes and function as protectors against free radicals. Flavonoids contained in kenikir leaves will undergo a process of digestion and absorption by the digestive walls which are then circulated through the blood. Flavonoids that are in the bloodstream will stimulate the kidneys to release a hormone called erythropoietin. Erythropoietin is a glycoprotein hormone found in the blood, then the erythropoietin hormone circulating in the blood vessels stimulates the bone marrow to increase red blood cell formation, namely erythropoiesis (Murningsih and Fathoni, 2016).

Bone marrow primordial stem cells will form new hemocytoblasts

continuously. Hemocytoblasts first form erythroblast basophils which begin to synthesize hemoglobin. The synthesis of heme takes place inside the mitochondria and occurs gradually (Wahyuni dan Purwaningsih, 2016). Erythroblast then becomes a poly chromatophilic erythroblast, after which the cell nucleus shrinks, whereas hemoglobin is formed in greater numbers and the cell becomes normoblast. After the normoblast cytoplasm is filled with hemoglobin, the nucleus becomes very small and is removed. At the same time, the endoplasmic reticulum is re-adopted.

Cells at this stage are called reticulocytes because they still contain small amounts of basophilic endoplasmic reticulum which intersperse between hemoglobin in the cytoplasm. While cells in this reticulocyte stage, they enter the blood capillaries with diapedesis (slipping through the pore membrane). The endoplasmic reticulum remaining in reticulocytes continues to produce hemoglobin for one to two days, but at the end of that time, the reticulum disappears altogether and eventually becomes erythrocytes and divides by mitosis (Wijayanti, et al., 2016).

CONCLUSION

Based on the data and the results of research that has been done, it can be concluded that giving kenikir leaves affect the increase in hemoglobin levels in the normal range of blood of male Wistar rats exposed to cigarette smoke

by giving kenikir leaves with a dose of 4.5 grams and 6.3 grams.

Cigarettes can cause a decrease in hemoglobin levels that cause anemia, therefore it is expected that cigarette consumers will stop this bad habit because it causes a lot of harm to health.

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