

## **EFFECT OF MARIGOLD LEAF ON HEMOGLOBIN LEVELS ON WISTAR RAT EXPOSED CIGARETTE SMOKING**

***(Pengaruh Pemberian Daun Kenikir Terhadap Kadar Hemoglobin Pada Tikus  
Wistar 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 marigold leaves contain phenolic as antioxidants. This study aims to determine the effect of marigold 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 marigold 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 marigold leaves groups with a dose of 4.5 grams and 6.3 in increasing hemoglobin levels ( $p < 1.00$ ). Conclusion: there was significant effect of marigold leaf on hemoglobin levels exposed cigarette smoking at a dose of 4.5 gram and 6.3 gram.

Key words: cigarette smoke, marigold 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 ( $p < 1.00$ ). 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).

Indonesian people's smoking behavior tends to increase from 34.2% in 2007 to 36.3% in 2013 (Restuti, 2018). Death rates from diseases caused by smoking continue to increase. In 2030, it is estimated that the death rate of smokers in the world will reach 10 million people, and 70% of them come from developing countries. (Kemenkes, 2015).

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). Increased free radicals will increase the immune response, for example activation of inflammatory cells. This process will produce excess ROS (reactive oxygen species), which are the main oxidants in the body (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 marigold 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 marigold plants are used as medicine to reduce bad breath and vegetables. Marigold 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).

Marigold 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 marigold 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 marigold 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.

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 each consisting of 10 rat.

Animals were grouped into 3 groups, each of which consisted of 10 heads, namely: group I (negative control) only exposed cigarette smoke. Group II and III were given additional marigold leaves (4.5 and 6.3) grams/kg BW.

### Research Stage

Feeding the treatment lasted for 3 weeks and only once as much as 4.5 and 6.3 gr/day for each male rat, and drinking water *ad libitum*. Giving marigold leaves 1 hour after exposure to cigarette smoke. The giving of marigold leaves using the sonde method was carried out since day 2 (Permatasari and Purbosari, 2015).

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

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			$\Delta_1$ (mean±SD)	$\Delta_2$ (mean±SD)	<i>p-value</i> <sub>1</sub>	<i>p-value</i> <sub>2</sub>
	I	II	III				
K-	12,8	11,3	9,8	-1,2 ± 1,4 <sup>a</sup>	-2,7 ± 1,6 <sup>a</sup>		
P1	11,6	13,6	15,4	1,6 ± 1,3 <sup>b</sup>	3,3 ± 1,8 <sup>b</sup>	0,000	0,000
P2	11,8	13,9	15,6	1,7 ± 1,5 <sup>b</sup>	3,3 ± 1,3 <sup>b</sup>		

Note: K = negative control group, P1 = treatment group giving 4.5-gram marigold leaves, P2 = treatment group giving marigold leaves 6,3 gram. 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 marigold leaves in 2 groups with different doses of hemoglobin levels as presented in Table 5 proves the effect of giving marigold 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 groups 1 and 2 ( $p > 0.05$ ), which means marigold leaves with a dose of 4.5 grams and 6.3 grams have almost the same effect in increasing hemoglobin levels.

Low hemoglobin levels in the control group occur because one of the contents of the dominant cigarette smoke is carbon monoxide (Inayatillah,

2014). Carbon monoxide is a poisonous gas that is colorless and odorless. Carbon monoxide can reduce oxygen delivery and utilization in body tissues (Batubara, *et al.*, 2013). Carbon monoxide harms health because CO can shift oxygen (O<sub>2</sub>) which is bound to hemoglobin and binds hemoglobin to carboxyhemoglobin (Heryanita, *et al.*, 2018). This is because CO affinity for Hb is approximately 210 times stronger than the O<sub>2</sub> affinity for Hb. This reaction causes a reduction in the capacity of the blood to channel O<sub>2</sub> to the body's tissues. This means that if there is CO in the body even with small levels. It can drastically reduce the ability of hemoglobin to carry oxygen. Low doses of CO gas cause effects or disorders in patients with lung, heart, brain, and vital organs (Lovita, *et al.*, 2014).

Increased hemoglobin levels due to marigold leaves contain polyphenols which act as donors of hydrogen atoms (H<sup>+</sup>) 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 marigold 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 marigold 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 marigold leaves affect the increase in hemoglobin levels in the normal range of blood of male Wistar rats exposed to cigarette smoke by giving marigold 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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