

Effect of Single-Bulb Garlic (*Allium sativum* Var. Solo Garlic) Extract on The Hematological Profile In E-Cigarette-Induced Male Sprague Dawley Rats

Maya Nurwartanti Yunita^{1*}, Julia Calvina Fauzi², Zahra Defitra Rahmania², Bella Safinda², Tridiganita Intan Sholikhah³, Bodhi Agustono⁴, M. Gandul Atik Yuliani⁵, Shekhar Chhetri⁶, Reina Puspita Rahmani⁷

Maya Nurwartanti Yunita^{1*}, Julia Calvina Fauzi², Zahra Defitra Rahmania², Bella Safinda², Tridiganita Intan Sholikhah³, Bodhi Agustono⁴, M. Gandul Atik Yuliani⁵, Shekhar Chhetri⁶, Reina Puspita Rahmani⁷

¹Veterinary Pathology Division, School of Health and Life Sciences, Universitas Airlangga, Surabaya 60115, Indonesia

²Veterinary Medicine, School of Health and Life Sciences, Universitas Airlangga, Indonesia.

³Clinic Veterinary Medicine Division, School of Health and Life Sciences, Universitas Airlangga, Surabaya 60115, INDONESIA.

⁴Animal Husbandry Division, School of Health and Life Sciences, Universitas Airlangga, Surabaya 60115, INDONESIA.

⁵Basic Veterinary Medicine Division, Faculty of Veterinary Medicine, Universitas Airlangga, Surabaya 60115, INDONESIA.

⁶Department of Animal Science, Royal University of Bhutan, BHUTAN.

⁷Department of Microbiology, Faculty of Veterinary Medicine, Wijaya Kusuma University, Surabaya, INDONESIA.

Correspondence

Maya Nurwartanti Yunita

Veterinary Pathology Division, School of Health and Life Sciences, Universitas Airlangga, Surabaya 60115, INDONESIA.

E-mail: mayanurwartanti@fkh.unair.ac.id

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ABSTRACT

Garlic and its derivative preparations have long been used in traditional medicine for good health. Garlic is known to act as an antioxidant, antitumor, antibacterial, and antifungal. The antioxidant compound in single-bulb garlic is considered to be better than the antioxidant content in other species of garlic. This research aimed to study the effect of hematological profile in oral administration of single-bulb garlic extract in e-cigarette-induced male *Sprague Dawley* rats. The method used in this study was randomized post-test only control group design. Twenty-four rats were randomly distributed into six groups. The group consisted of C (-) as normal rats' control; C (+) only exposed to e-cigarette smoke; T1 exposed to e-cigarette smoke and treated with vitamin C; T2 exposed to e-cigarette and treated with single-bulb garlic extract 75 mg/kg BW; T3 exposed to e-cigarette and treated with single-bulb garlic extract 100 mg/kg BW; and T4 exposed to e-cigarette and treated with single-bulb garlic extract 125 mg/kg BW. Exposure to e-cigarette smoke is conducted by evaporating the liquid into a gas chamber with a nicotine dose of 3.6 mg/ml. All treatments were given for 14 days. On the 15th day, the rats were sacrificed. Blood samples were collected by the cardiac puncture method and were examined using a hematology analyzer. Data obtained were white blood count (WBC), platelets, hemoglobin, hematocrit, and erythrocytes that were analyzed with one-way ANOVA. The result of this study is that garlic extract may boost immunity in the body. However, higher doses can be toxic to erythrocytes.

Key words: Antioxidant, E-cigarette, Good health, Hematology profile

INTRODUCTION

Electric cigarettes (e-cigarettes) are a popular substitute for tobacco cigarettes in many countries, including Indonesia. E-cigarettes have been distributed globally as a potential approach to help quit smoking and as an alternative for safer smoking.¹ A mouthpiece, a refillable cartridge, a lithium battery, and a heating atomizer comprise an e-cigarette. A power button on many devices allows the user to activate the heating element during inhalation, producing the flavored vapor. The battery provides energy for e-cigarette devices.² Other than conventional tobacco cigarettes, smokers don't need to burn the tobacco. The atomizer will produce heat that will transform the liquid into an aerosol so it can be smoked.³ The nicotine in e-cigarettes can be vaporized in the form of propylene glycol or glycerin. Propylene glycol is a liquid with characteristics clear, odorless, and has a low vapor pressure. Glycerin acts as a solvent with a sweet taste.⁴ A toxic acrolein compound is produced when glycerin is heated at a temperature above 100° Celcius.⁵ Nicotine in e-cigarettes liquid will give smokers relieving sensation.⁶

E-cigarette smoking is as dangerous as conventional tobacco cigarette smoking. E-cigarette smoking activates enzymes that metabolize carcinogens and oxidative stress, which play important roles in the pathogenesis of chronic, inflammatory, and degenerative diseases such as chronic obstructive pulmonary disease (COPD) and cancer.^{7,8}

Exposure to secondhand smoke is not limited to humans. Regardless, many smokers have pets in their homes. An experimental study on rat's fetus and guinea pigs shows that nicotine (which is given *via* intravenous route) is related to brain development and inhibits melatonin synthesis. Hence, prolonged exposure to secondhand smoke can be as harmful to animals as it is to humans. Exposure to secondhand smoke increases the incidence of diseases in pets including nasal/oral cavity cancer, dermatitis, and respiratory diseases. Moreover, exposure to secondhand smoke is proven to influence rats (*Rattus norvegicus*) hematological profile.⁹

For centuries, garlic and its derived preparation have been used in traditional medicine to treat diseases such as asthma, hypertension, constipation, diarrhea, fever, antitumor, antibacterial, and antifungal.^{10,11} The antioxidant contained in single-bulb garlic is better than the antioxidant in other varieties of garlic.¹² Single-bulb garlic contains compounds such as alliin, alliinase, allicin, S-allyl cysteine (SAC), diallyl disulfide (DADS), diallyl trisulphide (DATS) dan methyl allyl trisulphide which is able to protect body tissues from any damage and disturbance.¹³ Allicin is a well-known compound of garlic. Allicin is produced from the reaction between a non-protein amino acid (allicin) and an amino acid enzyme (alliinase) that occurs when garlic is crushed (Wedhasari, 2014).

This research aims to study the effect of single-bulb garlic extract on the hematological profile in e-cigarette-induced male *Sprague Dawley* rats.

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MATERIALS AND METHODS

Experimental design

The experimental animal used in this study is the male *Sprague Dawley* rat (*Rattus norvegicus*) which distributed into 6 different experimental groups. Each group consists of 4 rats as repetition. The rats were obtained from UD Tiput Abadi Jaya Peternakan Hewan Uji, Yogyakarta. The hematological profile examined were platelets, hemoglobin, hematocrit, erythrocytes, and white blood count (WBC). This research has met the ethical requirements for research using experimental animals and has received an ethical clearance certificate from the ethical committee of the Faculty of Dentistry, Universitas Airlangga, Surabaya with certificate number 049/HRECC.FODM/II/2022.

Tools and materials

Tools used in this research are cages, gas chambers, food and drink container, tuberculin sputit, e-cigarettes mods type, aquarium aerators, sawdust, tissue, and oral gavage needle. The materials used are single-bulb garlic extract, 60 mL e-liquid containing 3 mg/mL nicotine using the same brand and flavor, vitamin C (IPI), aquadest, 10% buffered formalin, commercial feed and drinking water, gloves, ketamine, and xylazine.

Single-bulb garlic extraction

Single-bulb garlic (*Allium sativum* Var. *solo garlic*) as much as 2000 grams were peeled, washed, and cut into small pieces. After that, the garlic was dried using an oven at the temperature of 50°C for 24 hours. Dried garlic then blended until it forms the unprocessed natural ingredient, and weigh it. Dissolve the unprocessed natural ingredient using ethanol 96% as a solvent with a ratio of 1:3.¹⁴

Extraction is done by maceration method using a shaker water bath with a speed of 120 rpm for 24 hours.¹⁵ Then, refine the solution using Buchner as a filter until it forms filtrate. After that, solidified the filtrate using rotary vacuum evaporator to obtain a single-bulb garlic condensed extract concentration of 100%.

Experimental design

Experimental animals were adapted to the laboratory environment for 7 days. Each cage is inhabited by 4 rats. Feed and water are provided ad libitum for 14 days. Exposure to e-cigarettes smoke was given for 14 days. E-cigarettes used were the third generation of e-cigarettes mods type with berry cheese doughnut flavored e-liquid (Jack). Treatment to experimental animal:

C (-) : The negative control group without any treatment.

C (+) : The first positive control group was given e-cigarette smoke exposure with a dose of 3 mg/ml.

T1 : The second positive control group was given e-cigarette smoke exposure with a dose of 3 mg/ml and vitamin C with a dose of 3,6 mg/day.

T2 : The first treatment group was given e-cigarette smoke exposure with a dose of 3 mg/ml and single-bulb garlic extract with a dose of 75 mg/kg BW.

T3 : The second treatment group was given e-cigarette smoke exposure with a dose of 3 mg/ml and single-bulb garlic extract with a dose of 100 mg/kg BW.

T4 : The third treatment group was given e-cigarette smoke exposure with a dose of 3 mg/ml and single-bulb garlic extract with a dose of 125 mg/kg BW.

Exposure to e-cigarette smoke and single-bulb garlic extract was given to experimental animals for 14 days and conducted at the SIKIA Experimental Animal Laboratory, Universitas Airlangga, Banyuwangi.

Blood sample collection

On the 15th day, the experimental animals were weighed and body weight was recorded. Anesthesia was given to the rats through intramuscular injection using ketamine HCL with a dose of 8 mg/kg BW and xylazine with a dose of 1 mg/kg BW. The blood sample was collected by the Intracardiac Puncture method. Blood samples collected were stored in an EDTA tube to be stored in a cool box.

Hematological profile examination

Blood samples in the EDTA tube which were stored in the cool box shipped from the Experimental Animal Laboratory SIKIA Universitas Airlangga, Banyuwangi to the Clinical Pathology Laboratory Faculty of Veterinary Medicine Universitas Airlangga, Surabaya for examining the hematological profile. Hematological profiles were examined using the hematology analyzer. The variables examined were platelets, hemoglobin, hematocrit, erythrocytes, and white blood count (WBC).

RESULTS AND DISCUSSION

Effect of e-cigarettes exposure and single-bulb garlic extract treatment on leukocyte count

This study shows that the fourth treatment (T4) group has the lowest leukocyte count mean. It can be happened since antioxidant administration may lower leukocyte count after the experimental animal is exposed to the free radical.¹⁶ Meanwhile, the T1 has the highest leukocyte count. The high level of leukocytes is caused by antioxidant activity from the oral administration of vitamin C. Treatment of the T1 group was significantly different from the T4 group.

E-cigarette smoke exposure may lower leukocyte count which can be a sign of immune system activation and shows that inflammation occurs.¹⁷ Free radical can cause oxidative stress in the body so the activation of leukocytes, such as monocyte and lymphocyte, occur. The presence of monocyte and lymphocyte indicate a response from inflammation. Leukocytes are released into the bloodstream as a response to inflammation.¹⁸ Cytokine will be released by lymphocytes and monocyte. An increase in cytokine will affect hematopoiesis so leukocyte count increases (Barrett *et al.*, 2015). The increase in leukocyte count indicates an immune system boost by single-bulb garlic extract. This shows that garlic extract contains bioactive agents that can increase leukocyte production.¹⁹⁻²²

Effect of e-cigarettes exposure and single-bulb garlic extract treatment on types of leukocyte

The lymphocyte means after e-cigarette smoke exposure in the C (+) control group was lower than the C (-) control group. It demonstrates that e-cigarette smoke exposure may reduce lymphocyte count inside the body. Decreasing lymphocyte count is affected by decreasing in leukocyte production, decreasing in leukocyte release from bone marrow, and increasing in leukocyte migration to infected organs or tissues. Decreased activity of myeloid precursors in the spinal cord related to decreased production and retention of leukocytes from the spinal cord.^{23,24} Lymphocyte levels in the T1 and T2 treatment groups decreased compared to C (+). This shows that oral administration of single-bulb garlic extract can reduce inflammation so that lymphocyte levels decrease.²⁵ According to Sharma's *et al* (2010)²⁶ research, garlic supplementation significantly increased leukocyte counts. The same results were observed when garlic was added to fish food, namely a significant increase in the number of leukocytes.

Table 1: Effect of single-bulb garlic extract treatment on hematological profile in e-cigs induced Sprague Dawley rats.

Variable	C (-)	C (+)	T1	T2	T3	T4
WBC	10.6 ^{ab} ± 0.938	9.425 ^{ab} ± 0.727	11.1 ^b ± 0.316	9.675 ^{ab} ± 2.371	10.875 ^{ab} ± 0.478	9.05 ^a ± 0.341
Lymphocyte	69.5 ^b ± 3.872	68.25 ^b ± 3.095	62.25 ^a ± 4.425	61.5 ^a ± 4.509	68.375 ^b ± 0.478	71.45 ^b ± 0.341
Monocyte	12.25 ^a ± 4.031	12.25 ^a ± 1.707	17.75 ^b ± 3.862	11.5 ^a ± 1.29	8.375 ^a ± 0.478	8.45 ^a ± 0.341
Neutrophil	18.25 ^a ± 2.5	19.5 ^a ± 2.886	20 ^a ± 0.816	27.75 ^b ± 5.965	26.375 ^b ± 0.478	21.45 ^a ± 0.341
Thrombocyte	357.75 ^{ab} ± 132.026	299.25 ^a ± 76.743	293.5 ^a ± 59.963	430.25 ^b ± 101.932	283.375 ^a ± 0.478	318.45 ^{ab} ± 0.341
Hemoglobin	15.2 ^a ± 1.086	15 ^a ± 0.787	14.95 ^a ± 0.597	15.225 ^a ± 1.678	15.875 ^a ± 0.478	15.95 ^a ± 0.341
Hematocrit	43.4 ^a ± 1.23	43.375 ^a ± 1.417	42.125 ^a ± 1.304	42.425 ^a ± 1.35	43.575 ^a ± 0.478	43.65 ^a ± 0.341
Erythrocyte	7.6 ^a ± 1.013	8.675 ^{ab} ± 0.386	8.55 ^{ab} ± 1.22	8.675 ^{ab} ± 0.485	9.175 ^b ± 0.478	8.85 ^b ± 0.341

Oral administration of single-bulb garlic extract may increase neutrophils and monocytes level. Neutrophils act as a protection from various intracellular organisms by both phagocytic and non-phagocytic mechanisms. The increase in leukocytes level is in line with the increase in neutrophils level.²⁷ Garlic contains Sulphur compound which initiate immune boost. Increase in leukocyte level due to oral administration of single bulb garlic extract is known to inhibit neutrophil migration. Neutrophil migration inhibition increases neutrophil levels in the bloodstream.²⁸

The least monocyte mean can be seen in the T3 treatment group, whereas the highest monocyte mean can be seen in the T1 control group. Vitamin C may increase leukocyte, monocyte, and neutrophil count.²⁹ Oral administration of garlic extract is known to lower monocyte levels on colitis-induced rat and increase monocyte levels on the control group.²⁵

Effect of e-cigarettes exposure and single-bulb garlic extract treatment on hemoglobin

Based on this research result, the least hemoglobin mean can be found in the T1 group which was given 3,6 mg of vitamin C daily and exposed to e-cigarette smoke with 3 mg/ml of nicotine. The highest hemoglobin mean can be found in the T4 treatment group which was given 125 mg/kg BW of single bulb garlic extract and exposed to e-cigarette smoke with 3,6 mg/ml of nicotine. An increase in hemoglobin levels rate can be found in the T2, T3, and T4 treatment group whereas hemoglobin levels decreased in the C (+) control group which was only exposed to e-cigarette smoke and T1 group which was exposed to e-cigarette smoke and treated vitamin C. Data on the Table 1 shows that oral administration of single-bulb garlic elevates hemoglobin level. The result of this study goes along with a study conducted by Sharma *et al* (2010)²⁶ demonstrated that hemoglobin concentration was significantly increased by aqueous garlic extract at doses of 250 mg/kg body weight and 500 mg/kg body weight. Anti-platelet, procirculatory, anti-inflammatory properties, and anti-apoptotic effects of garlic are comparable to those published for the HO-CO pathway for heme degradation.³⁰ This condition may be attributable, at least in part, to the elevated level of the effective anti-oxidant bilirubin (and biliverdin), which is also produced by increased hemoglobin degradation. Therefore, chemoprevention mediated by modulation of cytoprotective in enzymes alone cannot explain the general beneficial effects of garlic.³¹

Effect of e-cigarettes exposure and single-bulb garlic extract treatment on thrombocyte

This research demonstrates that there is significant effect between the T2 treatment group and the C (+), T1, and T3 groups. Platelets are blood components which plays a role in the blood clotting process and cover damaged blood vessels.³² Nicotine in cigarettes will interfere with the sympathetic nervous system so the alpha adonoreceptors will stimulate the release of platelets. This causes an increase in the number of platelets in the blood. Nicotine can also activate platelets, causing platelets to agglomerate into the blood vessel walls.³³

Effect of e-cigarettes exposure and single-bulb garlic extract treatment on hematocrit

There is no significant effect amongst each group. The decrease in hematocrit can be seen in the C (+) and T1 groups whereas the increase in hematocrit can be seen in the T2, T3, and T4 treatment groups. The T4 treatment group has the highest hematocrit level of all. The percentage of red blood cells in blood (hematocrit) is a major determinant of arterial O2 content as well as a measure of blood capacity to carry O2.³⁴

Effect of e-cigarettes exposure and single-bulb garlic extract treatment on erythrocyte

There is a significant effect between the T3 and the T4 treatment groups. The T3 group has the highest erythrocyte mean amongst all. There is no significant effect between the C (+) and T1 groups and the T2 treatment group. Meanwhile, the erythrocyte levels decreasing in the T4 treatment group. It can be happened since garlic in higher dose decreasing the erythrocytes levels. The same thing can be seen in the research that were conducted by Banerjee and Maulic (2002)³⁵ that oral administration of garlic extract in higher dose may cause anemia on rats. Rats that were treated with 75 mg/kgBW and 100 mg/kgBW dose of single bulb garlic extract shows an increase in erythrocyte levels compared to the positive control groups. The higher dose that is given so the level of erythrocytes increases, the increase in the number of erythrocytes does not affect the condition of the hematocrit. Sharma *et al* (2010)²⁶ research revealed that garlic administration significantly increased the number of erythrocytes. The addition of garlic to fish feed produced the same results, a significant increase in the number of erythrocytes. In addition, garlic has the potential to protect lipoproteins and cell membranes from damage caused by free radical activity resulting from exposure to fat-soluble active compound components of toxic compounds.

In this study, the increase in the number of erythrocytes was accompanied by an enhancement in their shape. This is likely due to the antioxidant content of garlic extract's ability to regulate and neutralize free radicals resulting from the presence of lead in the blood. In accordance with Sunita's (2002) research, antioxidant compounds serve as the primary line of defence against the peroxidase process of polyunsaturated fatty acids found in cellular and subcellular membrane phospholipids. Antioxidant compounds provide hydrogen from the hydroxyl group (OH) in the ring structure to free radicals as their primary function. Garlic's antioxidants can enhance the assimilation of iron, which is necessary for erythrocyte maturation. In addition, it can affect erythropoiesis during the final stage of erythrocyte maturation.³⁶

CONCLUSION

Oral administration of single-bulb garlic extract doses of 75 mg/kg, 100 mg/kg, and 125 mg/kg demonstrated an effect on the hematological profile of e-cigarette-induced male *Sprague Dawley* rats which is an elevation on WBC, erythrocyte count, and hemoglobin content. Allicin, a compound that can be found in single-bulb garlic, is able to

act as an antioxidant. In addition, single-bulb garlic extract is able to boost the immune system in the rat's body.

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CONFLICTS OF INTEREST

The authors declare that they have no competing interests.

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