

## **Number of Erythrocytes in Male Mice (*Mus musculus*) Exposed to Cigarette Smoke After Treatment with Ethanol Extract of Red Pomegranate Peel (*Punica ganatu*)**

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### **ABSTRACT**

Plant extracts have the potential to serve as natural therapeutic agents that can counteract the negative effects of various toxins, including those found in cigarette smoke. One such plant with this potential is the red pomegranate (*Punica granatum*). This study aims to determine the effect of administering Red Pomegranate (*Punica ganatum*) peel extract on the number of erythrocytes and hemoglobin levels in white mice exposed to cigarette smoke. This type of research is experimental laboratory post test only group design. Mice were divided into 4 treatment groups, namely: P0, control group, test animals treated with distilled water; treatment groups P1, P2 and P3, which were treated with neem leaf ethanol extract at a dose of 10 each; 20; 40 mg/head/day and mice were exposed to cigarette smoke. The research results showed that the higher the extract given, the higher the HB and erythrocyte levels. From this research it can be concluded that administration of Red Pomegranate rind extract can increase the number of erythrocytes and hemoglobin levels but it is not significant ( $p > 0.05$ ). The contribution of this research provides important data on the interaction between toxins (in this case, cigarette smoke) and protective agents (red pomegranate peel ethanol extract). This information is valuable in the fields of toxicology and pharmacology for understanding cellular protection mechanisms and potential new treatments.

**Keywords:** *Erythrocytes, Hemoglobin, Extracts*



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### **INTRODUCTION**

Smoking is an activity that is dangerous to health. Cigarettes are processed from tobacco (*Nicotiana tabacum*) which is dried then burned, and the smoke is inhaled. Globally, it is estimated that more than 6 million deaths are caused by smoking, including around 600 thousand deaths from passive smoking. Most of the deaths that occur are premature deaths due to the bad effects of smoking on health. According to Riskesdas in 2013, smoking is practiced by 36.3% of the Indonesian population aged over 15 years. Cigarettes contain many dangerous substances such as tar, arsenic and nitrosamines which are carcinogenic. Also nicotine can cause problems with the cardiovascular system. Apart from the ingredients above, several cigarette ingredients are known to cause oxidative stress such as carbon monoxide gas and acetaldehyde.

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Cigarettes contain toxic ingredients and have an addictive effect. For smokers, not smoking will cause unpleasant symptoms, such as feeling drunk, tired, shaking in the hands, dizziness (Rahmi, 2018; Saktyowati, 2017). The ingredients in cigarettes include tar (Triyono et al., 2020). Nicotine, free radicals, lead (Pb), and carbon monoxide (CO) (Amalia, 2019) are some of the most dangerous chemicals in cigarette smoke. (Kahar et al., 2022) Carbon monoxide binds very easily to hemoglobin compared to oxygen or carbon dioxide. Carbon monoxide binding to hemoglobin can create a risk of hypoxia (Ramadhanti et al., 2019).

Smoking has many health impacts. The impact that smoking can have has acute and chronic effects (Malenica et al., 2017). Increasing cigarette consumption has an impact on increasing the burden of disease caused by smoking and increasing the death rate due to smoking. In 2030, it is estimated that the death rate for smokers in the world will reach 10 million people and 70% of them will come from developing countries (A. Rahim et al., 2019). Cigarettes are one of the main risk factors for several chronic diseases that can result in death (Prihatiningsih et al., 2020).

Hematological parameters are parameters related to blood and blood-forming organs (Gorelik et al., 2020). Jalil et al., (2022) stated that hematological parameters, such as hematocrit, hemoglobin, and the number of erythrocytes and white blood cells, can be used as indicators of toxicity and have wide potential applications in monitoring environmental conditions. Hematological examination is also a method that can be used to detect some changes in health status, which may not be visible in the physical examination but affect the animal's fitness (Yusuf et al., 2022). Ahmed et al., (2020) stated that hematological parameters are good indicators of animal physiological status because blood acts as a pathological reflector of the status of animals exposed to toxins and other conditions.

Parts of the Red Pomegranate tree such as the fruit, skin and roots have an astringent taste. This astringent taste is a sign that this part of the plant contains polyphenolic compounds (Osakabe et al., 2024). The polyphenol content in Red Pomegranate peel extract which functions as an antioxidant reaches 26% of all the chemical content contained in it (Fraschetti et al., 2023). However, the public does not know the benefits of Red Pomegranate peel, so the use of Red Pomegranate peel is still minimal. Based on the description above, the author is interested in examining the effect of giving Red Pomegranate (*Punica ganatum*) peel extract on the number of erythrocytes and hemoglobin levels in white mice.

## **METHODS**

The research carried out was experimental in nature and was carried out at the Biomedical Laboratory, Faculty of Medicine, Andalas University. This study used 24 adult male mice aged 2.5 months, with a weight ranging from 25-30 g. Before being treated with the test material, mice were acclimated to laboratory conditions for 2 weeks. Maintenance is carried out by placing mice in rearing cages at a density of 4-5 per cage. Food and water for the test animals were provided ad libitum. Lighting, temperature and humidity of the rearing cage were recorded every day.

### **Making Extracts**

Pomegranate skins are collected from several trees around the house. The leaves obtained were washed thoroughly with running water and then dried in an oven at 40 oC for 10 days. Dry leaves can be determined by weighing them for 3 consecutive days. If the weight is constant for 3 consecutive days then the leaves are considered dry. The dried leaves are made into flour by crushing them with a blender and then sifting them. The leaf meal is then extracted using 70% ethanol. The final result of ethanol extraction of pomegranate peel. The ethanol extract of pomegranate peel is made into a treatment preparation according to a predetermined concentration, namely 10, 20, 40 mg/head/day.

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**How to Treat**

Male mice were weighed before treatment was carried out. Mice with uniform body weights were selected as test animals, placed in rearing cages at a density of 3 mice per cage. Mice were divided into 4 treatment groups, namely: P0, control group, test animals treated with distilled water; treatment groups P1, P2 and P3, which were treated with neem leaf ethanol extract at a dose of 10; 20; 40 mg/ day. Treatment was given orally using a gavage needle, with a volume of 0.3 mL/test animal, every morning, for 21 consecutive days. The body weight of the test animals was measured once every week, while food and drink consumption was measured every day. Before coding, mice were exposed to cigarette smoke for 1 minute.

The weight of the test animals was measured before the test animals were sacrificed under anesthesia using chloroform. Blood is isolated directly from the heart, using a 1 mL syringe. The blood that has been successfully collected is then placed in a tube containing EDTA, then centrifuged at 3000 rpm. Blood plasma is separated and placed in Eppendorf tubes for analysis of Hb and erythrocyte levels. Analysis of Hb levels and erythrocyte counts was carried out at the Pramita Laboratory. The effect of pomegranate peel ethanol extract on Hb and erythrocyte levels was determined by comparing the Hb and erythrocyte levels of test animals in the control group and the treatment group.

**RESULTS AND DISCUSSION**

*Results*

*Table 1. total hemoglobin levels and erythrocyte counts before and after treatment*

<b>Perlakuan</b>	<b>HB (gr/dL)</b>	<b>Eritrosit (gr/dL)</b>
<b>P0</b>	12,4	7,50 (x10 <sup>6</sup> )
<b>P1</b>	11,2	5,25 (x10 <sup>6</sup> )
<b>P2</b>	11,4	5,70 (x10 <sup>6</sup> )
<b>P3</b>	11,7	6,20 (x10 <sup>6</sup> )

*Discussion*

This study only looked at the hemoglobin levels and erythrocyte counts of test animals after exposure to pomegranate peel extract. The results showed that the hemoglobin levels and erythrocyte counts of the test animals were influenced by exposure to pomegranate peel extract for 21 days.

Shows that administration of Red Pomegranate peel extract at a dose of 40 mg/Kg BW of mice/day can increase the number of erythrocytes and hemoglobin levels compared to the control group but not significantly. This shows that administration of Red Pomegranate rind extract provides a protective effect so that the number of erythrocytes and hemoglobin levels in P2 and P3 can approach the number of erythrocytes and hemoglobin levels in the control group, but the effect is less significant. The occurrence of oxidative stress in the mice's body is likely to form further free radicals, namely in the erythrocyte membrane. If reactive free radicals are not stopped, they will damage the erythrocyte cell membrane and lipid peroxidation will occur. The presence of lipid peroxidation of cell membranes makes it easier for erythrocyte cells to undergo hemolysis which causes hemoglobin to be released, so that the amount of hemoglobin decreases.

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Antioxidants are chemical compounds that are able to stop reactive free radicals by donating hydrogen electrons to free radicals to become stable free radicals that are non-damaging. In this study, the antioxidant used was red pomegranate peel to prevent oxidative stress exposure to cigarette smoke. In Indonesia this plant is used to treat stomach aches due to worms, chronic diarrhea, bleeding, sore throat, ear inflammation, vaginal discharge, and stomach pain (Fadhilah et al., 2023). According to de Oliveira et al., (2020), the compounds contained in Red Pomegranate skin include ellagic acid and punicalagin, both of which are polyphenolic compounds. Meanwhile, according to Gebicki & Nauser (2021), polyphenol compounds as antioxidant compounds are able to donate hydrogen atoms to free radicals to neutralize their radical properties. Research Liema et al., (2023) also proved that Red Pomegranate peel extract acts as an exogenous antioxidant which is able to neutralize free radicals in white mice. In (Shetty, 2018) research, it has been proven that giving 50 mg/Kg BW of mice/day Red Pomegranate rind extract to white mice can reduce ileal oxidative stress.

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## **CONCLUSION**

From this research it was concluded that giving skin extract Red Pomegranate (*Punica ganatum*) dose of 50 mg/Kg/day can be obtained increased the number of erythrocytes and hemoglobin levels but was not significant statistically ( $p>0.05$ ). The next researchers are recommended to conduct further studies with various doses of red pomegranate peel extract to determine a dose that might provide a more statistically significant effect. This experiment can help identify the optimal dose that effectively increases the number of erythrocytes and hemoglobin levels.

## **CONFLICT OF INTEREST**

There are no conflicts of interest related to the publication of this article.

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