

THE EFFECT OF WHOLE SEED (*Barringtonia racemosa*) WATER EXTRACT ON ERYTHROCYTE, HEMOGLOBIN AND HEMATOCRIT COUNT OF WHITE RAT (*Rattus norvegicus*) EXPOSED TO CIGARETTE SMOKE

Dasrul¹, Yayang Nuri Al Aliya², Amalia Sutriana³, Nuzul Asmillia^{4*}, and Razali Daud⁴

¹Laboratory of Reproduction Fakultas Kedokteran Hewan Universitas Syiah Kuala, Banda Aceh, Indonesia

²Program Studi Pendidikan Dokter Hewan Fakultas Kedokteran Hewan Universitas Syiah Kuala, Banda Aceh, Indonesia

³Laboratory of Pharmacology Fakultas Kedokteran Hewan Universitas Syiah Kuala, Banda Aceh, Indonesia

⁴Laboratory of clinic Fakultas Kedokteran Hewan Universitas Syiah Kuala, Banda Aceh, Indonesia

*Corresponding author: nuzulasmilia@unsyiah.ac.id

ABSTRACT

This study aims to determine the effect of *Barringtonia racemosa* seed extract on the number of erythrocytes, hemoglobin levels, and the percentage of hematocrit in white rats (*Rattus norvegicus*) exposed to cigarette smoke. A total of 25 white rats Wistar strain, aged 3-4 months, male, weighing 180-200 g, were randomly divided into 5 treatment groups, each group consisting of 5 rats. Positive control group (KP) without exposure to cigarette smoke and without administration of *Barringtonia racemosa* seed extract, negative control group (KN) exposed to cigarette smoke without administration of *Barringtonia racemosa* seed extract. Treatment groups P1, P2, P3 were exposed to cigarette smoke and given *Barringtonia racemosa* seeds with doses of 50, 100, and 150 mg/kg BW/day, respectively. The extract was administered orally for 30 days. Blood collection in all groups was carried out via the orbital vein using a hematocrit pipette after the rats had been sedated with 0.2 mL of ketamine. Erythrocyte, hemoglobin, and hematocrit values were calculated using a hematology analyzer. Data were analyzed using one-way pattern analysis of variance (ANOVA). The results showed that giving *Barringtonia racemosa* seeds at doses of 50, 100 and 150 mg/kg/day had a significant effect ($P < 0.05$) in increasing the values of erythrocytes, hemoglobin, and hematocrit of white rats exposed to cigarette smoke compared to the control group which was not given the extract. In conclusion, the administration of *Barringtonia racemosa* seed extract can increase the number of erythrocytes, hemoglobin levels, and the percentage of hematocrit exposed to cigarette smoke.

Key words: *Barringtonia racemosa*, cigarette smoke, white rats

ABSTRAK

Penelitian ini bertujuan mengetahui pengaruh pemberian ekstrak biji putat air (*Barringtonia racemosa*) terhadap jumlah eritrosit, kadar hemoglobin, dan persentase hematokrit tikus putih (*Rattus norvegicus*) yang dipapar asap rokok. Sebanyak 25 ekor tikus putih strain Wistar, usia 3-4 bulan, jantan dengan bobot 180-200 g, secara acak dibagi menjadi 5 kelompok perlakuan, masing-masing kelompok terdiri atas 5 ekor tikus. Kelompok kontrol positif (KP) tanpa dipaparkan asap rokok dan tanpa pemberian ekstrak putat air, kelompok kontrol negatif (KN) dipaparkan asap rokok tanpa pemberian ekstrak putat air, kelompok perlakuan P1, P2, P3 dipaparkan asap rokok dan diberi biji putat air dengan dosis berturut-turut 50, 100, dan 150 mg/kg bobot badan/hari. Ekstrak diberikan secara oral selama 30 hari. Pengambilan darah pada semua kelompok dilakukan melalui vena orbitalis dengan menggunakan pipet hematokrit setelah tikus disedasi dengan 0,2 ml ketamin. Nilai eritrosit, hemoglobin, dan hematokrit dihitung dengan menggunakan hematology analyzer. Data dianalisis dengan menggunakan analisis varians (ANOVA) pola searah. Hasil penelitian menunjukkan bahwa pemberian biji putat air pada dosis 50, 100, dan 150 mg/kg bobot badan/hari berpengaruh nyata ($P < 0,05$) dalam meningkatkan nilai eritrosit, hemoglobin, dan hematokrit tikus putih yang dipaparkan asap rokok dibandingkan dengan kelompok yang tidak diberikan ekstrak. Kesimpulan pemberian ekstrak putat air dapat meningkatkan jumlah eritrosit, kadar hemoglobin, dan presentase hematokrit yang dipaparkan asap rokok.

Kata kunci: *Barringtonia racemosa*, asap rokok, tikus putih

INTRODUCTION

Cigarettes are one of the biggest contributors to the cause of death which is difficult to prevent in society (Kemkes 2015). Smoking is the activity of burning substances, generally tobacco, to inhale the smoke. Burning cigarettes will release the active substances contained such as nicotine, tar and carbon monoxide so that these active substances can be sucked into the lungs, and then will dissolve into the bloodstream. Many studies have shown that smoking causes abnormalities in blood vessels and is a risk factor for coronary heart disease, cerebrovascular disease, and peripheral blood vessels (Novo 2002; Stephanie 2003).

Cigarette smoke is a source of free radicals for the body (Tendra 2003), one of which is hydroxyl radicals (Allen and Tressini 2000). Hydroxyl radicals are very reactive oxygen radicals and can damage cells by oxidizing or reducing electrons from/to other cells around them (Halliwell and Gutteridge 1999). Previous

research has reported that smoking can affect blood components such as erythrocytes, platelets, and hemoglobin (Asif *et al.* 2013). Erythrocytes are red blood cells which contain hemoglobin, a structure consisting of heme and globin. Hemoglobin has the ability to bind O₂ and CO₂ so that hemoglobin is an important component in maintaining the integrity of the body's circulatory system. The lysis of the erythrocyte membrane causes hemoglobin to be released into the plasma, thus the body's cells will lack oxygen (Ahumibe and Braide 2009). Carbon monoxide also has a strong tendency to bind to hemoglobin in erythrocytes, this bond is 210-300 stronger than that of hemoglobin with oxygen (Shah *et al.* 2012). As a result, carbon monoxide causes hemoglobin desaturation, reduces the supply of oxygen throughout the body, interferes with the release of oxygen, and accelerates the thickening of blood vessel walls (Stephanie 2003). In order to minimize the effects of free radicals or reactive oxygen compounds from

cigarette smoke on erythrocytes, hemoglobin and hematocrit, the administration of antioxidants could be a potential solution. Antioxidants can be obtained from synthetic chemical compounds and secondary metabolites from various fruits, vegetables and plants.

Barringtonia racemosa is a type of plant where all parts of the plant such as skin, leaves, seeds, and roots can function as anti-fungal, antibacterial, anti-diabetic (Musman *et al.* 2017). Several studies have succeeded in isolating antioxidant compounds contained in the leaves, seeds, fruits, roots and stems of these plants such as polyphenols, flavonoids and triterpenoids (Hussin *et al.* 2008; Behbahani *et al.* 2011; Dalila *et al.* 2015; Nurul *et al.* 2015). Musman *et al.* (2017) proved that giving ethyl acetate extract of *Barringtonia racemosa* seeds at a dose of 100 to 200 mg/kg could significantly reduce the blood malondialdehyde (MDA) of rats with diabetes. However, reports on the use of *Barringtonia racemosa* seed extract as a prevention of damage from cigarette smoke exposure to hematopoiesis are still limited. Therefore, this study aims to examine the effect of *Barringtonia racemosa* seed extract on the number of erythrocytes, hemoglobin levels and hematocrit values of white rats (*Rattus norvegicus*) exposed to cigarette smoke.

MATERIALS AND METHODS

Preparation of *Barringtonia racemosa* Seed Extract

The preparation of *Barringtonia racemosa* seed extract refers to previous research conducted by Musman *et al.* (2017). A total of 1500 g of *Barringtonia racemosa* fruit were obtained from community plantations in Darussalam District, Aceh Besar. Seeds are selected in fresh conditions and do not emit sap. Furthermore, the *Barringtonia racemosa* fruit washed and peeled to get the seeds. Then the white seeds are cut into pieces and air-dried for 2-3 days, then crushed using a blender. The seed powder obtained was macerated using 80% ethyl acetate solvent for 3x24 hours until a clear macerate was obtained, then the macerate was evaporated using a vacuum rotary evaporator until a concentrated extract was obtained and weighed.

Animal Acclimatization

In this study, 25 male Wistar rats aged 3-4 months with a body weight of 180-200 g were used as experimental animals. Mice were obtained from UPT Animal Experiment, Faculty of Veterinary Medicine, Universitas Syiah Kuala. During adaptation, all white rats were given standard feed (T79-4). The activity and body weight of the experimental animals were also monitored, so that the experimental animals can move actively and no body weight was less than 200 g during or after the adaptation.

Exposure to Cigarette Smoke and Administration of *Barringtonia racemosa* Seed Extract

At the end of the adaptation period, each rat was weighed to find out the weight according to the

inclusion criteria that had been set. Then, the rats were randomly allotted into 5 groups. Group 1 as positive control (KP), white rats did not exposed to cigarette smoke and without administration of *Barringtonia racemosa* seed extract, while in group 2 as negative control (KN), the rats exposed to cigarette smoke and without administration of *Barringtonia racemosa* seed extract. In group 3 (P1), 4 (P2), and 5 (P3), the rats exposed to cigarette smoke and received 50, 100, 150 mg/kg BW/day of *Barringtonia racemosa* seed extract, respectively. The extract was administered orally once a day for 30 days using a gastric tube. The rats in group KN, P1, P2, and P3 were exposed to cigarette smoke four times a day for 30 minutes in a modified smoking chamber. The treatment of exposure to cigarette smoke was carried out for 30 days.

Blood Collection

Sampling of rat blood was carried out through the orbital sinus using a hematocrit pipette. The blood was put into a vacutainer tube that had been filled with ethylenediaminetetraacetic acid (EDTA) as an anticoagulant to prevent blood clots.

Measurement of Erythrocytes, Hemoglobin, and Hematocrit

Blood was taken as much as ± 1 mL/rat and put into each vacutainer tube which was already filled with anticoagulant. The total erythrocytes, hemoglobin, and hematocrit analyses was carried using a Mindray YSTE880V hematology analyzer.

Analysis Data

Data on the number of erythrocytes, hemoglobin levels and the percentage of hematocrit obtained from the results of this study were statistically analyzed using the Analysis of Variance (ANOVA) and followed by Duncan's test to determine differences between treatments.

RESULTS AND DISCUSSION

The value of erythrocytes, hemoglobin levels and blood hematocrit of rats in this study is presented in Table 1. Based on the erythrocyte values obtained in Table 1, it can be seen that the average erythrocyte value of Wistar rat in the KP group was $7.17 \pm 1.16 \times 10^6/\mu\text{L}$, then decreased in the KN group, which was $5.06 \pm 1.33 \times 10^6/\mu\text{L}$, then increased again in groups P1, P2 and P3 with values were $7.33 \pm 1.18 \times 10^6/\mu\text{L}$; $7.21 \pm 1.08 \times 10^6/\mu\text{L}$ and $6.73 \pm 0.93 \times 10^6/\mu\text{L}$, respectively. In general, the normal value of blood erythrocytes in white rats ranges from 7-11.7 ($10^6/\mu\text{L}$) (Douglas and Wardrop 2010). Statistical test results showed that the average number of erythrocytes obtained in the treatment group was given *Barringtonia racemosa* seed extract at doses of 50 mg/kg BW/day (P1), 100 mg/kg BW/day (P2) and 150 mg/kg BW/day (P3), significantly different ($P < 0.05$) compared to the KN group but not significantly different ($P > 0.05$) compared to the positive control

group (KP). This showed that the concentration of *Barringtonia racemosa* seed extract given has an effect on increasing the number of erythrocytes and the value is within the normal range of white rat erythrocyte values.

In the KN group, the levels of erythrocytes were lower compared to the other groups. The decreased number of erythrocytes in the KN group was probably caused by the effects of harmful ingredients in cigarette smoke such as carbon monoxide, nicotine, tar, and Polynuclear Aromatic Hydrogen (PAH) compounds, which can interfere with the process of cellular respiration in the mitochondria, which leads to an increase in the formation of reactive oxygen (Mohod *et al.* 2014). Cigarette smoke can trigger the formation of Reactive Oxygen Species (ROS) and induce oxidative stress by increasing pro-oxidants and reducing antioxidant protection. Cigarette smoke that is inhaled into the respiratory system, it will enter the blood circulation system so that it triggers the formation of ROS and causes oxidative stress in erythrocyte cells. These hydroxyl radicals can cause a chain reaction known as lipid peroxidation (Yanbaeva *et al.* 2007). As a result of this peroxidation reaction, the levels of essential fatty acids in the plasma membrane are reduced and the permeability of the membrane is disrupted so that ROS can more easily penetrate into cells and cause various damages, such as damaging DNA which can trigger cell death, including erythrocyte cells (Sundaryono 2011)). These results proved that exposure to cigarette smoke can reduce the number of white rats' erythrocytes.

The hemoglobin level of the male rats in the positive control group (KP) was 16.00 ± 4.12 g/dL and was within the normal range according to Douglas and Wardrop (2010), which ranged from 11.6-16.1 g/dL. In the KN group (exposed to cigarette smoke), there was a decrease in hemoglobin to 11.20 ± 4.04 g/dL. The decrease in blood hemoglobin levels in the KN group in this study was possibly caused by the effects of harmful compounds in cigarette smoke such as nicotine, tar, nitrosamines, carbon monoxide, phenols, carbonyls, chlorindoxins, and polynuclear aromatic hydrogen compounds, which act as free radicals (Fowles *et al.* 2000). In general, cigarette smoke enters the white rat's body through the respiratory tract and enters the lungs. In the lungs, the exchange of oxygen and carbon monoxide with carbon dioxide is carried by the blood and distributed throughout the body by hemoglobin. Hemoglobin is a complex protein consisting of protein, globin and heme pigment which

contains iron. Hemoglobin functions as a carrier of iron-rich oxygen in red blood cells and oxygen is carried from the lungs to the tissues (Hoffbrand 2006). When it reaches the alveoli, hemoglobin's affinity for oxygen is much lower than its affinity for CO, so it can reduce the capacity of blood as an oxygen carrier. Some of the incoming CO₂ dissolves in the fluid that moistens the thin epithelium of the alveoli. Then CO diffuses into the blood which is present in the capillaries in the alveolar walls. Most of the CO then binds to hemoglobin to form carboxyhemoglobin which is present in red blood cells. Simultaneously, some of the carbon dioxide in the blood diffuses into the alveoli where it can be exhaled. Blood circulation then carries CO to all body cells. In the long term, due to the strong affinity of CO for hemoglobin, it can cause oxidative stress, resulting in a decrease in the number of erythrocytes in the blood circulation due to hemolysis of erythrocyte cells and hemoglobin is released into the plasma so that it cannot carry out its functions properly, causing hemoglobin levels to decreased (Saputo and Junaidi 2015).

Based on Table 1 above, it can be seen that the percentage of hematocrit varies between treatment groups. The average hematocrit percentage of male Wistar rats in the KN group was $41.29 \pm 6.20\%$, then decreased in the KP group to $32.24 \pm 7.57\%$, then increased again in groups P1, P2, and P3 with values were $42.04 \pm 4.84\%$, $45.38 \pm 5.34\%$ and $40.80 \pm 3.87\%$, respectively. According to several previous researchers, the normal hematocrit percentage of white rats of the Wistar strain ranged from 45-47% (Smith and Mangkoewidjojo 1988), and 37.6%-51% (Douglas and Wardrop 2010).

These results proved that exposure to cigarette smoke can reduce the percentage of white rat blood hematocrit. Meanwhile, the administration of *Barringtonia racemosa* seed extract at a dose of 50-150 mg/kg BW/day was able to increase the percentage of blood hematocrit in white rats exposed to cigarette smoke. The decrease in the percentage of hematocrit in the KP group in this study was possibly due to the effect of the active ingredients in cigarette smoke such as nicotine, tar, nitrosamines, carbon monoxide, phenol, carbonyl, chlorindoxine, and PAH compounds, which act as free radicals. A decrease in the percentage of hematocrit in the KP group below this normal value may indicate anemia. This low hematocrit percentage can be caused by blood that was too dilute because the number of erythrocytes was low. A low hematocrit percentage can also be caused by an

Table 1. The mean (\pm SD) of erythrocytes counts, hemoglobin levels, and hematocrit percentages of white rats (*Rattus norvegicus*) exposed to cigarette smoke after being given various doses of *Barringtonia racemosa* seed extract for 30 days

Group	Erythrocyte ($10^6/\mu\text{L}$)	Hemoglobin (g/dL)	Hematocrit (%)
Positive control (KP)	7.17 ± 1.16^b	16.00 ± 4.12^b	41.29 ± 6.20^b
Negative control (KN)	5.06 ± 1.33^a	11.20 ± 4.04^a	32.24 ± 7.57^a
Cigarette smoke and received 50 mg/kg BW/day (P1)	7.21 ± 1.08^b	16.16 ± 2.55^b	42.04 ± 4.84^b
Cigarette smoke and received 100 mg/kg BW/day (P2)	7.33 ± 1.18^b	17.34 ± 2.82^b	45.38 ± 5.34^b
Cigarette smoke and received 150 mg/kg BW/day (P3)	6.73 ± 0.93^b	15.70 ± 0.81^b	40.80 ± 3.87^b

^{a,b} Different superscripts within the same column indicate significant differences ($P < 0.05$)

old erythrocyte destruction process (Dharma *et al.* 2010 as cited in Andriyanto *et al.* 2010). The percentage of hematocrit depends on the total number of erythrocytes and the amount of oxygen needed for the body's metabolism. The number of erythrocytes also has a correlation with the measured hemoglobin level.

In this study it was also known that giving *Barringtonia racemosa* seed extract doses of 50, 100, and 150 mg/kg BW/day orally could increase the average value of erythrocytes, hemoglobin levels, and blood hematocrit values of rats exposed to cigarette smoke 4x30 minutes/day for 30 days. The increase in blood profile in groups P1, P2 and P3 which were treated with *Barringtonia racemosa* seed extract is strongly suspected due to the active ingredients they contain such as polyphenolic compounds, flavonoids, saponins, phenolics, triterpenoids and carotenoids which act as antioxidants (Behbahani *et al.* 2007, Hussin *et al.* 2008; Setyawati *et al.* 2013; Musman 2017). Sundaryono (2011) stated that active compounds from the polyphenol, flavonoid and triterpenoid groups function as antioxidants, which can increase erythropoiesis (the process of forming erythrocytes) in the bone marrow. Flavonoids act as donors of hydrogen atoms to free radicals so that they become stable free radicals that are not damaging in nature, so that the lipid membrane of the erythrocytes can be protected from free radicals and hemoglobin is not released into the plasma. Flavonoids are lipophilic so they are able to bind to the cell membrane of the erythrocytes and function as a protector against free radicals (Ahumibe and Braide 2009; Muchtadi and Sugiyono 2013).

Flavonoids contained in *Barringtonia racemosa* seed extract which are administered orally, will undergo a process of digestion and absorption by the digestive walls and then be circulated through the blood. Flavonoids that are in the blood circulation will stimulate the kidneys in plasma globulin cells to secrete a hormone called erythropoietin. Erythropoietin plays a role in the formation of hemoglobin (Utami and Fuad 2018).

Based on the values obtained from the entire study, an increase in the number of erythrocytes due to the administration of *Barringtonia racemosa* seed extract was in line with the increase in hemoglobin levels and the percentage of hematocrit in the blood of white rats. This is understandable because the values of erythrocytes, hemoglobin and hematocrit are interrelated series and run parallel if there is a hematological change. In line with the results of this study, several previous studies also reported an increase in erythrocytes and hemoglobin in experimental animals after being given essential oil of black cumin (Rakhmawati *et al.* 2020), marigold leave (Sari and Damayanti 2021), rambutan peel extract (Lisdiana and Dewi 2017). However, the results of this study are different from the results of a study conducted by Heryanita *et al.* (2018) which reported a decrease in the levels of erythrocytes,

hemoglobin and hematocrit in mice exposed to cigarette smoke and given red watermelon extract.

CONCLUSION

The administration of *Barringtonia racemosa* seed extract orally can increase the number of erythrocytes, hemoglobin levels and the percentage of blood hematocrit of white rats exposed to cigarette smoke 4x30 minutes per day for 30 days.

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