Effects of Rambutan Peel Extract to the Number of Erythrocytes and Haemoglobin in Rats Exposed to Cigarette Smoke

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Submission date: 20-Jun-2023 10:46AM (UTC+0700)
Submission ID: 2119467180
File name: Effect_of_rambutan_peel_extract_to_the_number.docx (63.88K)
Word count: 2962
Character count: 15376

Effects of Rambutan Peel Extract to The Number of Erythrocytes and Haemoglobin in Rats Exposed to Cigarette Smoke

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Abstract. Cigarette smoke is one of the exogenous free radicals sources. When it is inhaled, its activity may damage the structure of erythrocyte membrane function. The impacts of free radicals can be reduced through the provision of antioxidants. Rambutan fruit peel contains the phenolic compound in the form of polyphenols that are antioxidants. The purpose of this study is to determine the effect of rambutan fruit peel extracts to the number of erythrocytes and haemoglobin in rats exposed to cigarette smoke. This design used Post Test Control Group Design. A sample of 25 rats was divided into five groups, each group consisting of 5 rats. The positive control group (K+) were given a standard food and drinking water. The negative control group (K) by three cigarettes, the treatment group (KP1, KP2, KP3) by three cigarettes and skin extract of rambutan each treatment group with a dose 15 mg/kg, 30 mg/kg and 45 mg/kg for 30 days. Data on the number of erythrocytes and haemoglobin in rat blood was analysed with LSD and to determine the optimum dosage was analysed by using regression test. Research results shown that the content of rambutan fruit peel extract may increase the number of erythrocytes and haemoglobin of blood. Conclusions from this research are the rambutan fruit peel extract at a dose of 45 mg/kg body weight can increase and maintain the number of erythrocytes and haemoglobin in the blood of rat exposed to cigarette smoke.

1. Introduction

In some people, smoking is a habit that is hard to remove and harmful to health. Smoking is not only harmful to smokers but also for people who are exposed to cigarette smoke surrounding. Combustion will generate smoke cigarettes are divided into cigarette smoke (mainstream smoke) containing 25 % levels of hazardous materials and smoke the side (sidestream smoke) containing 75 % levels of hazardous substances [1].

The Cigarette smoke, both primary and side smoke when inhaled into the respiratory system can get into the blood circulation system, causing Reactive Oxygen Species (ROS) which cause oxidative stress [2], especially in erythrocytes. In the erythrocytes containing haemoglobin (Hb). If the content of cigarette smoke, especially carbon monoxide reacts with haemoglobin, it forms carbon mono oxyhemoglobin (HbCO). The affinity of haemoglobin for oxygen is much lower than its affinity for carbon monoxide so that the carbon monoxide replaces oxygen in haemoglobin and decreases the oxygen-carrying capacity of the blood as. HbCO relatively slow decomposition causes the cells work is hampered in its function of carrying oxygen throughout the body. Conditions such as these can result in serious, even fatal, as it can lead to poisoning [3].

Erythrocyte membranes composed of carbohydrates, proteins, oligosaccharides, and lipids. Phospholipids are one of the lipids that were the most numerous and is susceptible to oxidative stress [4]. If free radicals are not stopped, can damage all types of cellular macromolecules, including

carbohydrates, proteins, lipids and nucleic acids [5]. Free radical attack on membrane lipids can lead to the formation of lipid peroxidation.). Lipid peroxides of erythrocyte membranes may result in loss of membrane fluidity and increase the erythrocyte membrane fragility which in turn resulted in erythrocytes will be easily broken or hemolysis. Lysis of erythrocyte membranes causing haemoglobin into plasma, thus dwindling. It resulted in levels of haemoglobin contained in erythrocytes low. As a result, the cells will be starved of oxygen [6].

When the erythrocyte membrane damage continues, it is likely to cause anaemia. Effects of free radicals from tobacco smoke against erythrocytes and haemoglobin can be reduced through the provision of antioxidants. Antioxidants play a role in preventing the oxidative stress caused by exposure to free radicals. Antioxidants can be obtained from the results of the chemical compounds of various plant secondary metabolites. One type of skin of the fruit is efficacious as a drug and has antioxidant activity that is rambutan (Nephelium lappaceum L).

Rambutan is a fruit commonly eaten in Indonesia, easily obtained, as well as widely known. Rambutan is one of the fruits that all the parts (bark, leaves, seeds and roots) can be useful as a drug [7]. Currently, rambutan peel is still a waste. His peel was red still not fully utilised. Rambutan peel is known to have antioxidant activity containing phenolic compounds, alkaloids, steroids and terpenoids [8], flavonoids [9] and anthocyanins with the highest content of phenolic compounds [10]. Rambutan fruit peel contains phenolic compounds in the form of polyphenols with the main component of acid, ellagic, geraniin and origin [8]. These polyphenols act to protect body cells from damage by free radicals by binding with free radicals [11].

Based on the research results test the antioxidant activity quantitatively showed that the methanol extract of the peel of rambutan IC50 value of 0.412 mg / mL; whereas the IC50 value of vitamin C of 1.776603 mg / mL. The results of research ethanol extract of rambutan showing the IC50 value of 4.29 mg/mL and vitamin E IC50 value of 8.48 mg / mL. Therefore, the extract of rambutan fruit peel has a stronger antioxidant activity than the compound of vitamin C and vitamin E. Based on the above research efforts are needed to determine whether the rambutan peel extract can have an effect on the number of erythrocytes and blood haemoglobin levels as a result of being exposed to cigarette smoke.

2. Materials and methods

This study is an experimental research design with The Post Test Only Control Group Design. The population of rats (Rattus norvegicus) Wistar, samples of 25 male rats were aged 2-3 months with a body weight of 180-200 grams. The samples were divided into five groups. K+ (positive control), K- (negative control), KP1, KP2 and KP3 (treatment group). Rats were taken at random and divided into five groups, the cages for five rats. Rats are adapted for one week, given the standard feed and drink ad libitum. KP, KP2 and KP3 treated rambutan fruit peel extract at a dose of 15 mg /kg, 30 mg /kg, and 45 mg /kg orally at 10.00 hrs for 30 days, 1x per day. Then the negative control group, KP1, KP2 and KP3 treated three cigarettes at 08.00, 12.00 and 14.00 pm for 30 days. Blood sampling performed on day 6, 12, 18, 24 and 30 through orbital sinus eye with a hematocrit of 1 ml and collected in Eppendorf tubes containing anticoagulant that has ethylenediaminetetraacetic acid (EDTA), then measure the blood sample with Hematology Analyzer BC 2600. Measurement data on day 30 were statistically analysed using One-Way ANOVA if the results are significantly different then tested further with LSD. The optimum dose of rambutan fruit peel extracts was analysed using regression test.

3. Results

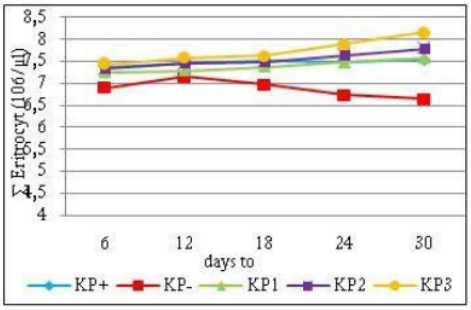

Figure 1. Number of Red Blood Cells (Erythrocytes)

Based on the graph in Figure 1 shows that the negative control showed the lowest average result than the number of erythrocytes in normal conditions ranging 6.64-7.15×106/ml of blood, when compared to the positive control group, KP1, KP2 and KP3. The results showed the highest number in the KP3, ranging 7.42-8.16×106/ml of blood.

The number of erythrocytes in normal rats ranged 7.2-9.6×106/ml. In the treatment group KP1, KP2 and KP3 by rambutan fruit peel extract increased when compared to the negative control group and positive control group.

Table 1. LSD test results on the number of erythrocytes (106/ml). The letters different in the same column shows the difference in each group with a level of accuracy of $p < 0.05$

Group	Treatment	Σ Erythrocyte (Mean ± SD)
K+	Control l	7.53 ± 0.200^{b}
K-	3 cigarettes	6.64 ± 0.339^{a}
KP 1	Rambutan fruit peel extracts 15 mg/kgBB + 3 cigarettes	7.58 ± 0.516^{b}
KP 2	Rambutan fruit peel extracts 30 mg/kgBB + 3 cigarettes	7.79 ± 0.347^{b}
KP 3	Rambutan fruit peel extracts 45 mg/kgBB + 3 cigarettes	8.16 ± 0.082^{c}

However, from the graph, the average number of erythrocytes in Figure 1, that group KP1, KP2 and KP3 has increased the number of erythrocytes up to 30 days. It suggests that the increase in the number of erythrocytes in line with the increase in the concentration of rambutan fruit skin extracts in the treatment group. Results of linear regression data on the number of erythrocytes showing the relationship between the dose of rambutan fruit peel extracts and the number of erythrocytes with the linear regression model are $Y = 7.26 + (0.29) X$, with R2 values of 0.320 = 32 %.

Based on the graph on figure 2. showed that the average haemoglobin levels from day 6 to day 30 in the positive control group, KP1, KP2 and KP3 have elevated levels of haemoglobin. When compared to the negative control group decreased haemoglobin levels. On day 30, the average haemoglobin levels higher KP3 group compared with positive control group, the negative control group, KP1 and KP2. The negative control group had an average of the lowest haemoglobin levels below normal levels of haemoglobin in conditions ranging from 10.88 g/dL of blood. Haemoglobin levels when compared with that measured in the positive control group and the group KP1, KP2, KP3 shows the results tend to be constant and within the normal range. Normal haemoglobin level in rat between 11.1 to 18 g/dl.

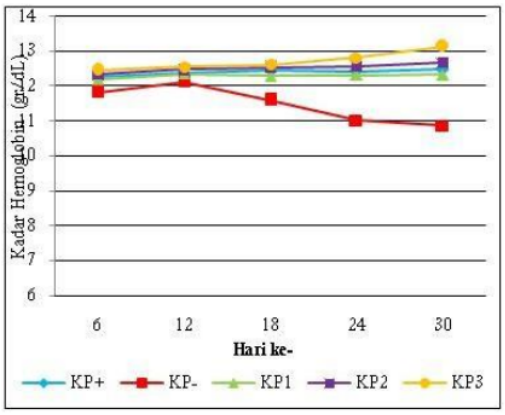

Figure 2. Average Levels of Haemoglobin (g/dl)

The statistical test by using One Way ANOVA shows that there are significant differences in the negative control group with a significance level of 0.000 less than 0.05 (p <5%) of the positive control group, KP1, KP2 and KP3. It is because in each group KP1, KP2, KP3 given rambutan fruit peel extracts with different doses, while the negative control without rambutan peel extracts only exposure to cigarette smoke. In the group KP1, KP2 and KP3 no significant difference where it is shown that the concentration of the rambutan fruit peel extracts has a similar effect in increasing haemoglobin levels within the normal range (Table 2). However, from the mean haemoglobin level diagram in Figure 2 an increase of up to 30 days. It suggests that the increase in haemoglobin levels with increased concentrations of rambutan fruit peel extracts in the treatment group. Accordance with the results of the linear regression test that the greater the dose of rambutan fruit peel extract is given, then it can increase haemoglobin levels. Results of linear regression test haemoglobin levels of data showing the relationship between dose and rambutan fruit peel extract with haemoglobin levels. The linear regression model are Y = 11.92 + (0.40) X, with R2 values of 0.297 = 29.7%.

Table 2. LSD test results on the Levels of haemoglobin (gr/dL). The letters different in the same column shows the difference in each group with a level of accuracy of $p < 0.05$.

Group	Treatment	Hemoglobin Levels (Mean ± SD)
K+	Control	12.48 ± 0.506^{b}
K-	3 cigarettes	10.88 ± 0.725^{a}
KP 1	Rambutan fruit peel extracts 15 mg/kgBB + 3 cigarettes	12.34 ± 0.114^{b}
KP 2	Rambutan fruit peel extracts 30 mg/kgBB + 3 cigarettes	12.68 ± 0.496^{b}
KP 3	Rambutan fruit peel extracts 45 mg/kgBB + 3 cigarettes	13.14 ± 0.826^{b}

4. Discussion

Based on the chart pattern and the overall value obtained from this study looks in line with the decrease and increase in the number of erythrocytes and haemoglobin concentration of blood. The decline in the number of erythrocytes and haemoglobin levels in the negative control group occurred because one of the contents of the dominant cigarette smoke is carbon monoxide (CO) Carbon monoxide is a toxic gas that is colourless and odourless. Carbon monoxide can cause a reduction in the delivery and utilisation of oxygen in body tissue [1].

The content of cigarette smoke, especially carbon monoxide reacts with haemoglobin to form carbonmonoxyhemoglobin (HbCO). The affinity of haemoglobin for oxygen is much lower than its affinity for carbon monoxide, so the carbon monoxide replaces oxygen in haemoglobin and decreases as the oxygen-carrying capacity of the blood. HbCO relatively slow decomposition causes the function

of erythrocytes to carry oxygen throughout the body is inhibited. Conditions such as these can result in serious, even fatal, as it can lead to poisoning [3].

Erythrocyte membranes composed of carbohydrates, proteins, oligosaccharides, and lipids. Phospholipids are one of the lipids that was the most numerous and is one of the lipid membranes are susceptible to oxidative stress. With the oxidative stress will cause lipid peroxidation [6]. Peroksida lipids in erythrocyte membranes are resulting in loss of membrane fluidity and increase the erythrocyte membrane fragility or friability which further lead to easily broken or erythrocyte hemolysis. Lysis of erythrocyte membranes causes free haemoglobin into plasma, thus dwindling. It resulted in levels of haemoglobin contained in erythrocytes low. As a result, the body's cells will be starved of oxygen [6].

Based on the results of the linear regression test showed that the magnitude of the dose of rambutan fruit peel extracts, it can increase the number of erythrocytes and haemoglobin levels. The increase in the number of erythrocytes and haemoglobin levels occurred in the group KP1, KP2 and KP3. Flavonoids are one of the active compounds from the group of polyphenolic compounds that can be found in the peel of rambutan as an antioxidant [12]. Flavonoids are the active compounds polyphenols that act as antioxidants, which can increase erythropoiesis (the process of formation of erythrocytes) in the bone marrow [13]. It is consistent with the statement that the flavonoid on rambutan fruit peel extract can increase the number of erythrocytes and haemoglobin levels. By way of flavonoids stimulate renal plasma globulin cells to secrete a hormone called erythropoietin. Erythropoietin is a glycoprotein hormone that is found in the blood, then the hormone erythropoietin circulating in the blood vessels that stimulates the bone marrow to increase red blood cell formation or erythropoiesis.

Polyphenol compounds are known to have various biological effects such as antioxidant activity through a mechanism as reducing agents, catcher of free radicals, chelating the metal and the formation of singlet oxygen absorbers as well as the electron donor [8]. Besides flavonoids also may prevent oxidative stress. Flavonoids are found in the peel extract of rambutan is thought to inhibit the occurrence of lipid peroxidation, so that free radicals can not develop into a free radical new way of donating one electron hydrogen atoms in unpaired electrons in free radicals so many free radicals become less and less reactive [14].

The relationship between flavonoids and reduction activity of free radicals (free radical scavenging) shows that there is a difference between flavonoid compound activity. The difference depends on the structure and substituents on the heterocyclic ring C and B. There are two major functional groups in flavonoids that determine the potential reduction of free radicals, namely: (a). Hydroxyl group 3', 4' (ortho-dihydroxy) in the Bring flavonoids, which has the properties as an electron donor and radical targets; (B). 2.3 conjugated double bonds with 4-oxo clusters (groups 1,4-Piron) on rings C and (c). the hydroxyl group in position 3 and five on the heterocyclic ring which acts on the electron delocalization.

5. Conclusion

Based on the result it was concluded that *C. dichotama* was a rare plant at Kendal Regency. The plant was not well known and commonly found. Its distribution is very limited because it is underutilized by local people. As a result, there are no significant cultivation activities. There are many types of research exploring the utility of *C. dichotoma* organs. Because *C. dichotoma* proved as a very beneficial and have a role as identity flora of Kendal Regency, it is necessary to planting it on a large scale. This activity requires the optimum breeding techniques. The generative reproduction is difficult to implement. Therefore it is recommended to develop a vegetative reproduction technique through cuttings, grafting and tissue culture.

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