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THE EFFECT OF PINEAPPLE (ANANAS COMOSUS (L) MERR) EXTRACT TO INFLAMMATORY INHIBITION AND ERYTHROCYTE SEDIMENTATION RATE (ESR) ON MALE WISTAR RAT (RATTUS NORVEGICUS)

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ABSTRACT

The aim of the study was to determine the effect of pineapple extract and optimal dosage on the decrease in inflammation and erythrocyte sedimentation rate in male Wistar rats. This research is an experimental study with a completely randomized design consisting of 5 treatments with giving of pineapple extract dose 3 g/200 g BB, 3.5 g/200 g BB and 4 g/200 g BB) orally with 5 replications. The results of the research data were analyzed using variance test and continued with Duncan's New Multiple Range Test at the level of α = 5%. The results showed that giving of pineapple extract significantly affected the decrease in inflammation with Fcount 4.202> Ftable 2.67. Duncan's New Multiple Range Test results at the level of α = 5% indicate that the optimal dose of pineapple extract that can affect the decrease in inflammation in Wistar rats is 3 g/200 g BB. Furthermore, the results of the study also showed that giving of pineapple extract had a significant effect on the erythrocyte sedimentation rate with Fcount 3.108> Ftable 2.67. Duncan's New Multiple Range Test results at the level of α = 5% indicate that the optimal dose of pineapple extract that can affect the erythrocyte sedimentation rate in male Wistar rats is 4 g / 200 g BB.

Keywords: influence, pineapple extract, inflammatory inhibition, erythrocyte sedimentation rate, male wistar rat.

INTRODUCTION

Pineapple (*Ananas comosus* (L.) Merr.) Is a plant of the Bromeliaceae family that is very important economically. This plant belongs to the subfamily Bromelioideae, Order Bromeliales, genus Ananas and species of comosus (Bartholomew *et al*., 2003).

Pineapple includes fruits that have a lot of nutritional content, namely vitamins (B1, B2, B3 and C), protein, fat, carbohydrates, calcium, phosphorus, potassium, magnesium, sodium, iron and fiber. Pineapple also contains several phytochemical compounds, namely bromelain, proteases, saponins, flavonoids and polyphenols (Ramayulis, 2013:47). The content of flavonoids that can provide anti-inflammatory effects because it can inhibit prostaglandin production (Sukmawati *dkk.*, 2015:131). In addition to flavonoids, bromelain can also provide anti-inflammatory effects by inhibiting prostaglandin production. Inhibition of prostaglandin, which is an inflammatory mediator can inhibit the proliferation of the inflammatory process (Sudjarwo, 2005:4).

Inflammation is a protective response from the body against tissue injury. This tissue injury can be caused by dangerous chemicals, physical trauma or microbiological agents. At the macroscopic level, inflammation is accompanied by clinical signs, namely erythema (redness), edema (swelling) and pain (Supriyatna *dkk.* 2015:223). Inflammation is one condition that causes an increase in the erythrocyte sedimentation rate. Increasing the value of erythrocyte sedimentation rate is probably due to changes in plasma components that occur during the inflammatory process (Tambayong, 2000:52; Mehta dan Hoffbrand, 2008:21).

Pineapple is widely produced in the Jambi city, precisely in the village of Tangkit Baru, which is located in Sungai Gelam, Muaro Jambi District. The Tangkit Baru Village is the largest producer of fresh pineapple in Jambi Province. Even in this village there is a monument called the pineapple monument. Covering an area of ± 1,000 ha from ± 1,811 ha, the Tangkit Baru Village is a pineapple plantation. This pineapple plantation can produce more than 50,000 pieces/day (Anonim, 2017). This abundance of pineapple production can cause problems. Pineapple fruit that is not sold and is not utilized will become waste.

This study aims to determine the effect of pineapple extract (*Ananas comosus* (L.) Merr.) to inflammatory inhibition and erythrocyte sedimentation rate in male Wistar rats (*Rattus norvegicus*), as well as the optimal dose of pineapple extract (*A. comosus* (L.) Merr.) which can give effect. This study is expected to provide information about the effect of pineapple extracts on decreasing inflammation and erythrocyte sedimentation rate in male Wistar rats.

MATERIALS AND METHODS

This research was conducted in the Laboratory of Instrument and Final Assignment at the Jambi University and the Biology Education Laboratory Jambi University. The study was conducted from January to November 2018. This type of research is an experiment that uses a completely randomized design. The study consisted of 5 treatments with 5 replications, so that 25 experimental

units were obtained. The test animals used were white rats (*Rattus norvegicus*) of 2-3 months old male Wistar strain weighing 150-200 g obtained from Palembang Rat Center.

The making of pineapple extract begins with collecting pineapple fruit obtained from the village of Tangkit Baru, Sungai Gelam, Muaro Jambi District, Jambi Province. Then the pineapple is peeled, washed and cut into small pieces. These pieces are then dried, blended, then macerated with methanol solvent for 72 hours while stirring occasionally. The pulp and macerated filtrate are separated. The pulp is macerated again until the filtrate obtained changes color. All obtained filtrate is combined and concentrated with a rotary evaporator until a crude extract is produced (Setyawati and Yulihastuti, 2011:192). Animal preparation trials were carried out by adapting rats in the enclosed environment for 1 week. Furthermore, the rats were fasted to eat for 18 hours. Previously rats were weighed and grouped randomly. The rats used in this study were grouped into 5 groups first. The P0 group is the control group. Group P1, the rats are made inflamed. P2-P4 group, rats given pineapple extract according to the prescribed dose (3 g/200 g BB, 3.5 g/200 g BB and 4 g/200 g BB) orally. Furthermore, rats are made to experience inflammation. Making inflammation in the rats refers to Ibad *et al.* (2013:159). Inflammation is made by compressing hot water to the body parts of rats that have been previously saved. Next will be seeing the emergence of erythema or redness in the body of the rats, which is a sign of inflammation.

Measurements for inflammation reduction were carried out on the first (early) and third (final) days. Erythema formed is photographed using a Nikon D3200 digital camera with a resolution of 24 megapixels. Photo processing obtained refers to Ibad (2013: 159) and Rinawati (2015: 3-4). The photo is processed using the Corel PHOTO-PAINT X7 program to find out the mean value of the erythema color intensity (hereinafter referred to as the inflammation value).

Then, the sedimentation rate measurements are carried out. This process begins with intracardiac blood sampling in the rats. After that, an erythrocyte sedimentation rate was examined by the Westergren method. According to Ariani *et al.* (2017), first EDTA blood pipette with a Westergren pipette to zero, then clean the outside of the pipette with a tissue. Next place the pipette on the Westergren rack in an upright state. After one hour, the sedimentation rate of the blood can be read by looking at the height of the plasma formed.Data analysis using variance test (ANOVA). If the results of the tests obtained indicate an influence, then proceed with the Duncan New Multiple Range Test at the level of $\alpha = 5\%$.

RESULTS AND DISCUSSIONS

Decrease in Inflammation

Inflammation is a form of the body's protective response to disorders that cause tissue injury (Supriyatna *et al.*, 2015: 223). According to Kee and Hayes (1996: 310), erythema is a sign that first appears when an inflammation occurs. At this stage, blood will gather in the area that has been injured as a result of the release of chemical mediators (quinine, prostaglandin and histamine). In this study, the value of inflammation was the mean value of erythema treated with the Corel PHOTO-PAINT X7 program.

Based on data on decreasing inflammation, the following is the average reduction in inflammation in each treatment (Figure-1).

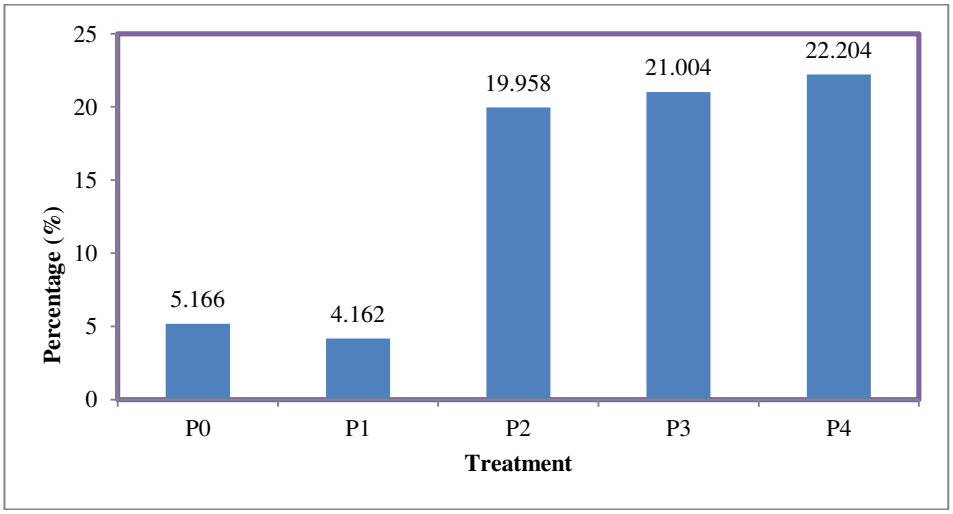

Figure-1. Graph of average reduction in inflammation in male Wistar rats.

Based on Figure-1, it is seen that there is a difference in the decrease in inflammation of each treatment. The decrease in inflammation of each treatment was P0 = 5, 166; P1 = 4,162; P2 = 19,958; P3 = 21,004 and P4 = 22,204. The results of variance analysis showed that giving of pineapple extract had a significant effect on the decrease in inflammation. Furthermore, based on the DNMRT test results at the level of $\alpha = 5\%$, it is known

that the inflammation treatment (P1) is significantly different from the pineapple extract treatment (P2 - P4).So is the control (P0) and pineapple extract treatment (P2 - P4) (Table-1). The decrease in inflammation increases with the increasing dose of pineapple extract until the treatment of pineapple extract 4 g / 200 g BB. However, there were no significant differences between the three doses. Based on the decrease in inflammation in pineapple extract treatment, it is said that the optimal dose of pineapple extract that can be used to reduce inflammation in male Wistar rats is 3 g/200 g.

Table-1. Data on the effect of pineapple extract on inflammation reduction in male wistar rats.

No.	Treatment	Decreasing Inflammation
1.	P0	5,166[a]
2.	P1	4,162[a]
3.	P2	19,958[b]
4.	P3	21,004[b]
5.	P4	22,204[b]

Description: The numbers followed by the same letters in the "Decrease Inflammation" column show no significant difference in the Duncan New Multiple Range Test at the level of $\alpha = 5\%$.

Based on Table-1, it is known that there are significant differences between controls (P0) and pineapple extract treatment (P2, P3 and P4). Likewise between the inflammation treatment (P1) and treatment with pineapple extract (P2, P3 and P4). Whereas between P0 and P1 there is no significant difference.

The increase in inflammation in the pineapple extract treatment (P2-P4) is caused by the presence of flavonoids and bromelain in pineapples which act as anti-inflammatory. According to Rinawati *et al*. (2015: 7), in the inflammatory phase, there is a process of tissue repair through hemostasis, which is temporary vasoconstriction in the blood vessels to supply blood and cells to damaged tissue (inflammed area) then form thrombocyte blockages and strengthened with fibrin fibers to make a clot. In addition, soft tissue responses, namely damaged tissue and mast cells release histamine and other mediators, causing vasodilation in undamaged blood vessels around the inflamed area and increasing blood flow to the inflamed area. This results in a feeling of warmth and redness (erythema) in the area of inflammation.

According to Rinawati *et al*. (2015: 8), the content of flavonoids that act as anti-inflammatory. The mechanism of flavonoids as anti-inflammatory is by promoting blood circulation and preventing blockages in blood vessels. Furthermore, according to Ibad *et al*. (2013: 160), as a reaction to damage (inflammation), the cells in the area experiencing inflammation will release phospholipids, among which are arachidonic acid. After free arachidonic acid, flavonoids will inhibit the lipooksegenase and cyclooxegenase pathways. Inhibition of the lipooksegenase pathway causes leukotriene inhibition. While the inhibition of the cyclooxegenase pathway causes inhibition of prostaglandin production, which is one of the chemical mediators. Prostaglandins synthesized in blood vessel walls are vasodilators and thromboginesis inhibitors, and thromboxane, which is a vasoconstrictor and strong platelet aggregation which induces the thrombogenesis process. Through this mechanism, cell viability can increase and give an effect in reducing erythema during the inflammation process.

According to Sudjarwo's research results in 2005, bromelain can act as an anti-inflammatory by inhibiting the production of one of the inflammatory mediators namely prostaglandin through inhibition of the cyclooxygenase enzyme activity. Prostaglandins are produced by arachidonic acid in the presence of cyclooxygenase enzyme activity. So that when the cyclooxygenase enzyme activity is inhibited, the production of prostaglandin by arachidonic acid is also inhibited. This is also the next cause of inhibition in the inflammation process.

Erythrocyte Sedimentation Rate

Erythrocyte Sedimentation Rate (ESR) is the rate of deposition of red blood cells in 1 hour. ESR can increase due to inflammatory disorders (Mehta and Hoffbrand, 2008: 21). In this study, rats were given inflammatory treatment to improve ESR. And it is said that if inflammation is the cause of an increase in the sedimentation rate of blood can be reduced, the sedimentation rate of blood will return to normal. The following is a graph of the average erythrocyte sedimentation rate of each treatment (Figure-2).

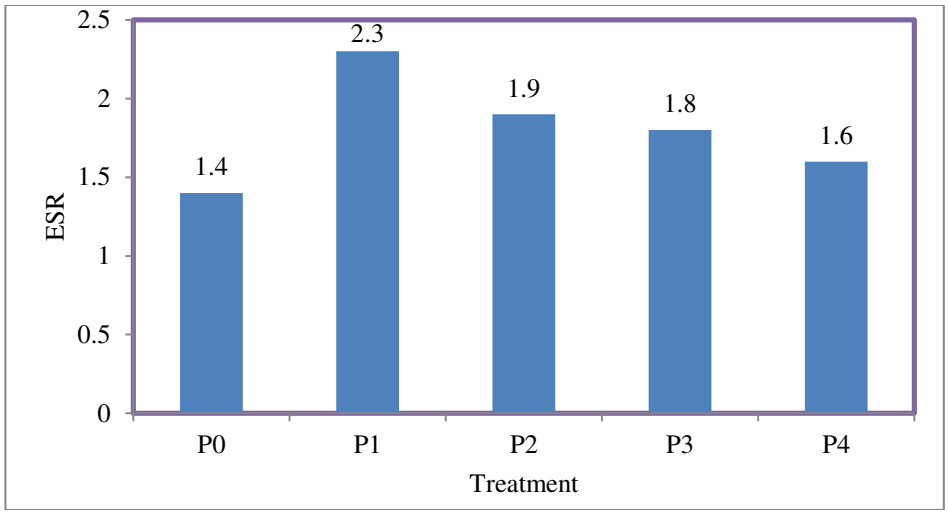

Figure-2. Graph of average of erythrocyte sedimentation rate in male Wistar rat.

Based on Figure-2, it is known that the ESR in the control (P0) is 1.4 mm / hour; Inflammatory treatment is 2.3 mm / hour and pineapple extract treatment (P2 - P4) is 1.6 - 1.9 mm / hour and the results of variance analysis show that giving of pineapple extract significantly affects the sedimentation rate of the blood. Then the Duncan's New Multiple Range Testwas carried out at the level of α = 5% and showed that there was a significant difference between the sedimentation rate in the inflammatory treatment (P1) and the control (P0) and the pineapple extract treatment with a dose of 4 g/200 g BB (P4). Based on the Duncan's New Multiple Range Test results at the level of α = 5%, it is known that the optimal dose of pineapple extract that can affect the sedimentation rate of blood in male Wistar rats is 4 g/200 g BB (Table-2).

Table-2. Data on the effect of pineapple extract on ESR on male wistar rats.

No.	Treatment	Erythocyte Sedimentation Rate (ESR)
1.	P0	$1,4^{a}$
2.	P1	$2,3^{b}$
3.	P2	$1,9^{ab}$
4.	P3	$1,8^{ab}$
5.	P4	$1,6^{a}$

Description: The numbers followed by the same letter in the Erythocyte Sedimentation Rate (ESR) column show no significant difference in the Duncan New Multiple Range Test at the level of α = 5%.

Table-2 shows that there are significant differences between the ESR on the inflammatory treatment (P1) and the ESR on the control (P0). Likewise between the inflammation treatment (P1) and the pineapple extract treatment dose 4 g / 200 gBB (P4).

The process of erythrocyte sedimentation rate is basically the interaction between the net charge of the erythrocyte surface and the net charge of plasma protein. Under normal circumstances, plasma proteins will be negatively charged. However, if an abnormality occurs, the plasma protein will be positively charged. The net charge of plasma proteins that have a positive value will cause the blood sedimentation rate to be faster or increase (Main, 2002 in Jacob and Rumlaklak, 2010: 159-160). A state of abnormality that can make a positively charged plasma protein is high levels of fibrinogen. Fibrinogen is a blood clotting factor and a sign of inflammation. When inflammation occurs, fibrinogen by thrombin will be converted into fibrin fibers which are useful for strengthening platelet blockages during the process of hemostasis (Rinawati *et al*., 2015: 7).

The results of this study indicate that the erythrocyte sedimentation rate in the inflammatory treatment (P1) has the highest value. According to Hasnawati in 2018, an increase in the sedimentation rate when there was inflammation was caused by an increase in fibrinogen. Fibrinogen is a plasma protein, which when levels increase will increase the formation of reuleaux. At reuleaux formation, erythrocytes will be close together like a pile of coins. And the amount of reuleaux formed causes the sedimentation rate to increase.

Furthermore, according to Litao and Kamat (2014), erythrocyte aggregation in the process of erythrocyte sedimentation rate is influenced by the surface load of erythrocytes and surrounding plasma proteins. In addition, erythrocyte aggregation is also determined by the concentration and symmetry of plasma proteins. When asymmetric proteins are large and positively charged the aggregation of erythrocytes and formation of rouleaux increases. The erythrocyte aggregate falls faster, thus increasing the erythrocyte sedimentation rate. Fibrinogen is one of the asymmetrical proteins and has the greatest effect on the sedimentation rate of the blood. This is also supported by the results of the study by Setyoningsih *et al*.

in 2016. Increased erythrocyte sedimentation rate is influenced by the composition of plasma proteins, especially fibrinogen. The protein affects the erythrocyte sedimentation rate by decreasing the erythrocyte negative charge (zeta potential). Zeta potential acts to keep the erythrocytes away from each other. When the zeta potential decreases, the erythrocytes will be arranged like coins piled up or form a rouleaux which can settle faster.

Based on the results of this study, it is known that pineapple extract has a significant effect on the erythrocyte sedimentation rate, which is characterized by the sedimentation rate of blood in the pineapple extract treatment (P2 - P4) which is lower compared to the ESR on the inflammatory treatment (P1). This is because the anti-inflammatory substances in pineapple extract can inhibit inflammation. This is supported by Ibad *et al*. (2003), Sudjarwo (2005) and Rinawati *et al*. (2015) which states that the content of flavonoids and bromelain in pineapple can act as an anti-inflammatory by inhibiting the production of one of the inflammatory mediators, prostaglandin. Inhibition of this inflammatory mediator can reduce blood supply to damaged tissue (inflamed area) and prevent blockages in blood vessels that should occur when inflammation. Smooth blood circulation shows no hemostasis process which causes an increase in fibrinogen levels, which can increase the erythrocyte sedimentation rate.

CONCLUSIONS

a) Pineapple extract (Ananas comosus (L.) Merr.) has a significant effect on decreasing inflammation in male Wistar rats (Rattus norvegicus).

b) Pineapple extract (Ananas comosus (L.) Merr.) Significantly affects the blood erythrocyte rate in male Wistar rats (Rattus norvegicus).

c) The optimal dose of pineapple extract (Ananas comosus (L.) Merr.) which can affect inflammation reduction in male Wistar rats (Rattus norvegicus) is 3 g/ 200 g BB, and the optimal dose of pineapple extract (Ananas comosus (L.) Merr.) Which can affect the erythrocyte sedimentation rate in male Wistar rats (Rattus norvegicus) is 4 g/200 g BB.

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