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ANTIBACTERIAL ACTIVITY AND HEMATOLOGICAL PROFILE OF RAT (*Rattus norvegicus*) DUE TO ADMINISTRATION OF ETHANOL EXTRACT OF SOURSOP FLOWERS (*Annona muricata* L.) AND *Salmonella enteritidis* INFECTION

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ABSTRACT

This study aimed to determine the antibacterial activity of soursop flower extract (*Annona muricata* L.) and hematological profile of rats (*Rattus norvegicus*) due to administration of soursop flower ethanol extract and *Salmonella enteritidis* infection. The concentrations of soursop flower ethanol extract used for the antibacterial activity test were 25%, 50%, 75%, 100%, with the antibiotic ampicillin 10 µg/disk was used s a positive control (PC) and dimethyl sulfoxide (DMSO) 10% as a negative control (NC). For examination of hematological features, 15 male rat aged two months were used. All rats were divided into 5 treatment groups, each consisting of three rats. The NC group was not given soursop flower ethanol extract and *S. enteritidis* infection. The PC group was not given soursop flower extract but was given *S. enteritidis* infection. Groups P1, P2, and P3 were given ethanol extracts of soursop flower at a dose of 0.18, 0.36, and 0.72 g/rat/day peroral for a week using gastric sonde. On the following day (after administration of soursop flower extract) the first blood drawing was performed. All rats, except NC group, were then infected with *S. enteritidis* intraperitonially at dose of $3x10^8$ CFU/mL dose (0.5 mL McFarland). One week after being infected with *S. enteritidis*, a second blood drawing was performed. The results of the antibacterial activity test showed that there was no antibacterial activity was observed since no inhibition at various concentrations was formed. The administration of soursop flower extract at various dosage levels was able to maintain the number of leukocytes but reduced the number of erythrocytes, hemoglobin concentration, hematocrit value and the number of platelets in rats; whereas *S. enteritidis* infection decreased all the hematologic features of lab rats.

Key words: antibacterial, hematological features, *Salmonella enteritidis*, soursop flower extract

ABSTRAK

Penelitian ini bertujuan mengetahui aktivitas antibakteri ekstrak etanol bunga sirsak (Annona muricata L.) dan profil hematologi tikus putih (Rattus norvegicus) akibat pemberian ekstrak etanol bunga sirsak serta infeksi Salmonella enteritidis. Konsentrasi ekstrak etanol bunga sirsak yang digunakan untuk uji aktivitas antibakteri adalah 25%, 50%, 75%, 100%, dengan antibiotik ampicillin 10 µg/disk sebagai kontrol positif dan kontrol negatif dimetil sulfoksida (DMSO) 10%. Untuk pemeriksaan gambaran hematologi digunakan sebanyak 15 ekor tikus putih jantan berumur dua bulan. Semua tikus dibagi ke dalam 5 kelompok perlakuan yang masing-masing kelompok terdiri atas tiga ekor. Kelompok kontrol negatif (KN) tanpa ekstrak etanol bunga sirsak dan tanpa infeksi S. enteritidis, kelompok kontrol positif (KP) tanpa ekstrak etanol bunga sirsak hanya diinfeksi S. enteritidis, kelompok P1, P2, dan P3 masing-masing diberikan ekstrak etanol bunga sirsak dosis 0,18; 0,36; dan 0,72 gr/ekor/hari peroral selama seminggu menggunakan sonde lambung. Pada hari selanjutnya (pasca pemberian ekstrak etanol bunga sirsak) dilakukan pengambilan darah pertama, kemudian semua tikus kecuali kelompok KN diinfeksikan S. enteritidis secara intraperitonial sebanyak 0,5 ml dosis McFarland $3x10^8$ CFU/mL. Seminggu pasca diinfeksikan S. enteritidis dilakukan pengambilan darah yang ke dua. Hasil penelitian menunjukkan bahwa tidak ada aktivitas antibakteri. Pemberian ekstrak etanol bunga sirsak pada berbagai tingkat dosis mampu mempertahankan jumlah leukosit namun menurunkan jumlah eritrosit, konsentrasi hemoglobin, nilai hematokrit dan jumlah trombosit tikus putih, sedangkan infeksi S. enteritidis menurunkan semua gambaran hematologi tikus putih.

Kata kunci: antibakteri, gambaran hematologi, Salmonella enteritidis, ekstrak bunga sirsak

INTRODUCTION

Infectious disease is one of the problems in the health sector that continues to grow. This infection can be caused by bacteria, fungi, viruses, or parasites. Salmonellosis is an infectious disease caused by bacteria. This disease is caused by *Salmonella enteritidis* (*S. enteritidis*) which is Gram negative bacterium (Omwandho and Kubota, 2010). *S. enteritidis* bacteria exist in the digestive tract and can also be found in feces and environment such as water, soil, plants, and dust (Portillo, 2000). Salmonellosis is a disease in humans that is transmitted through contaminated food and drink and raises more than one million cases each year with three thousands of them ending in death (Cao *et al*., 2008).

Various attempts have been made to prevent the emergence of diseases caused by pathogenic bacteria, including by the use of traditional medicines derived from nature. Soursop (*Annona muricata* L.) is one of the plants that have antibacterial potential. Soursop flowers which are extracted with ethanol solvents contain secondary metabolites such as alkaloid, phenolic and flavonoid (Zuraidawati *et al*., 2019) and are rich in phenolic antioxidants (Womeni *et al*., 2016). Flavonoid compounds directly act as antibiotics because they interfere with the function of organisms such as bacteria or viruses. Flavonoids also have antidiarrheal properties because they inhibit the intestinal chloride receptors thereby reducing fluid secretion to the intestinal lumen (Defrin *et al*., 2010).

The body's metabolic system, including hematological status, can be influenced by bacterial infections (Aboderin and Oyetayo, 2006). Blood functions to distribute nutrients, oxygen, and other substances to all organs so that the organs of the body

are able to carry out their functions. Blood function can be disrupted if blood parameters are not normal, resulting in animals easily affected by disease. Observation of the hematological profile as an indicator of a blood component in infectious disease is one way of examining to anticipate disease (Bastiawan *et al*., 2001).

Related to the content of secondary metabolites in soursop flowers, it is necessary to evaluate the antibacterial activity and hematological profile of lab rat (*Rattus norvegicus*) after administration of ethanol extract of soursop flower and *S. enteritidis* infection.

MATERIALS AND METHODS

Re-identification of Test Bacteria

Gram staining was done by taking one ose of bacteria and placing it on a glass object that had been dripped with physiological NaCl until it was even and fixed on a spiritus lamp. Bacterial preparation was stained with violet crystals for 1-2 minutes then rinsed with running water. The preparation was dripped with lugol and was left for 1 minute and rinsed with running water. Furthermore, the preparation was dripped with 96% alcohol for 10 seconds then rinsed with running water. Next, the preparation was stained with safranin for 1-2 minutes and rinsed with running water. The preparation was dried, immersed with oil, and observed under a microscope at 1000x magnification.

Antibacterial Activity Test

The antibacterial activity of soursop flower extract was tested at concentrations of 25%, 50%, 75%, and 100%. Suspension of *S. enteritidis* bacteria with a concentration of 1x108 CFU/mL (McFarland 0.5 mL standard) was evenly distributed on the surface of the Mueller Hinton Agar (MHA) media which was then affixed with paper disks that had been immersed in various concentrations on the media. Then the treated MHA media was incubated at 37° C for 24 hours. The diameter of the inhibition was observed after the incubation period.

Preparation of Soursop Ethanol Extract Dosage

The determination of the dosage of soursop flower extract was done according to the conversion of the dose from humans to rats, which was multiplied by 0.018, following the conversion table calculation of the dosage between animals according to the way of Laurence and Bacharach (1964) as cited in Santoso (2006). The dosages of soursop flower extract used in lab rats were 0.18, 0.36, and 0.72 g.

Treatment of Test Animals

After the preparation stage, the rats were divided into 5 treatment groups; negative control group (NC, rats were given standard food and *ad libitum*), positive control group (PC, rats were given standard food + *S. enteritidis* infection), P1 (rats were given standard food + 0.18 g of soursop flower ethanol extract), P2 (rats were given standard food + 0.36 g of soursop flower ethanol extract), and P3 (rats were given standard food + 0.72 g of soursop flower ethanol extract). The administration of soursop flower extract was done once a day for 1 week using gastric sonde. Blood was drawn after 1 week through the orbital sinuses using a microcapillary pipette and the hematological profile was examined using the Mindray BC 2800 Hematology Analyzer. After the first blood test, the rats were infected with *S. enteritidis* at dose of $3x10^8$ CFU/mL (McFarland 0.5 mL standard) intraperitonially. One week after infection with *S. enteritidis*, a blood drawing was carried out and the hematological profile was examined once again.

Data Analysis

The research data were analyzed using Analysis of Variance (ANOVA) and continued with Duncan test

RESULTS AND DISCUSSION

Antibacterial Activity Test of Soursop Flower Ethanol Extract against *S. enteritidis*

The antibacterial activity can be determined by measuring the diameter of the inhibition zone formed by the extract being tested. The results of testing the antibacterial activity of soursop flower extract against *S. enteritidis* bacteria up to 100% concentration did not have antibacterial power. These results indicate that the soursop flower ethanol extract has no antibacterial power and no potential as an antibacterial. The results of testing the antibacterial activity of soursop flower extract against *S. enteritidis* can be seen in Figure 1.

In Figure 1, it is clear that at all four concentrations from 25% to 100%, no inhibition zone was formed. This might be due to the soursop flower having only a small amount of secondary metabolites for antibacterial activity compared to the soursop leaves, roots, and bark. Another possibility was that compounds which are active as antibacterial were not extracted perfectly in ethanol solvents so that the antibacterial content in ethanol extracts does not exist. The absence of inhibition could be influenced by the quality of soursop flower extract. According to Sawitti *et al*. (2013), the quality of extracts can be influenced by biological and chemical factors.

Another factor that causes the soursop flower ethanol extract to have no antibacterial activitywas that *S. enteritidis* is a Gram-negative bacterium that has 3 layers (lipoprotein, phospholipid outer membrane and lipopolysaccharide) in its cell walls which causes it to have a selection system for foreign substances in its environment. The presence of phospholipid outer membranes makes it difficult for chemical components that are antibacterial to penetrate bacterial cell walls (Poeloengan and Praptiwi, 2010).

Leukocyte Count

The number of leukocytes in rats after treatment can be seen in Table 1. Based on the table, it can be seen that after administration of soursop flower ethanol extract, the number of leukocytes in rats increased compared to post *S. enteritidis* infection, but the

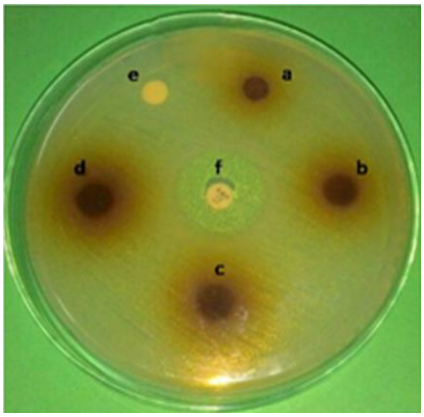

Figure 1. Observation antibacterial activity if soursop flower ethanol extract with concentrations. A= 25%, b= 50%, c= 75%, d= 100%, e= Positive control of ampicillin 10 µg/disk, f= Negative control of 10% DMSO

Parameter	Treatment group				
	NC	PC	P1	P2	P3
Number of leukocyte					
- Post extract administration	6.66±0.90[a]	11.16±1.34[a]	17.80±2.51[b]	9.33±0.46[a]	10.23±2.51[a]
- Post infection	5.66±2.04[a]	6.66±0.37[a]	5.23±0.30[a]	10.53±5.26[a]	9.23±7.58[a]
Erythrocyte count					
- Post extract administration	7.30±0.60[c]	7.44±0.39[c]	8.18±017[d]	6.05±0.30[b]	8.21±0.08[d]
- Post infection	6.85±0.66[c]	6.90±0.78[c]	4.93±026[a]	6.72±0.40[b]	5.02±0.19[a]
Hemoglobin concentration					
- Post extract administration	7.30±0.60[b]	7.44±0.39[b]	8.18±017[b]	6.05±0.30[b]	8.21±0.08[c]
- Post infection	6.85±0.66[b]	6.90±0.78[b]	4.93±026[a]	6.72±0.40[b]	5.02±0.19[a]
Hematocrit value					
- Post extract administration	42.30±0.91[b]	42.46±1.40[b]	38.63±0.66[b]	39.43±1.35[b]	46.56±0.50[c]
- Post infection	39.90±4.32[b]	39.70±4.60[b]	29.73±1.50[a]	39.50±2.85[b]	31.90±1.73[a]
Number of platelet					
- Post extract administration	793.33±21.36[a]	733.00±39.28[a]	476.67±20.77[a]	754.00±112.60[a]	596.33±55.07[a]
- Post infection	609.00±40.11[a]	465.00±21.00[a]	299.33±19.21[a]	485.33±74.60[a]	430.00±92.67[a]

[a, b, c]Different superscripts within the same row indicate significantly different (P<0.01)

increase that occurred was still in the normal range, which was 5-25x10^3/µL (Aboderin and Oyetayu, 2006). The highest result of leukocyte count after administration of soursop flower extract was at P1 treatment, which was 17.8x10^3/µL; however, after *S. enteritidis* infection, the number of leukocytes actually decreased.

A decrease in the number of leukocytes in rat after *S. enteritidis* infection was possibly due to the wound in the intestinal mucosa due to an infection so that the leukocytes were used for body defense. Based on statistical analysis, the treatment of soursop flower ethanol extract and *S. enteritidis* infection did not give a significant difference (P>0.05) in leukocyte count in rats, but there as significant difference (P<0.05) at the time of observation after the ethanol extract soursop flowers and post-enteritidis infection.

When there are foreign antigens, the number of leukocytes in the blood circulation will increase due to stimulation of myeloid tissue activity to produce leukocyte cells (Cahyaningsih *et al*., (2008) and Furman *et al*. (2014). Thus, giving ethanol extract of soursop flowers which contains flavonoid compounds, alkaloids and phenolics can maintain the number of leukocytes in rats. The number of leukocytes is also influenced by environmental conditions, age, and nutrient content of the food. The role of nutrients (protein) is very important in the process of leukocyte formation because protein is one of the blood components (Addas *et al*., 2012 and Etim *et al*., 2014).

Erythrocyte Count

The most numerous blood cells in normal blood circulation are erythrocytes. The main function of erythrocytes is to carry oxygen and food essence to be circulated throughout the body (Yakubu and Afolayan, 2009). The number of erythrocytes in the rats after treatment can be seen in Table 1. In the table, it can be seen that after administration of soursop flower ethanol extract, the number of erythrocytes in the rats in the NC, PC, P1, and P3 groups was still in the normal range, but in the P2 group the number of erythrocytes decreased. Likewise, after being infected by *S. enteritidis*, the average number of erythrocytes in the lrats decreased to below the normal range. The normal range of erythrocyte counts in rats is 7.2-9.6x10^6/µL (Aboderin and Oyetayu, 2006).

A decrease in the number of erythrocytes after *S. enteritidis* infection may be caused by *S. enteritidis* serovar having SEF21 fimbria which is manosa sensitive (can agglutinate erythrocytes) and can attach to glycoprotein receptor lectins from epithelial cells

(Sokja *et al.*, 1996). Damage to cell membranes due to the presence of pathogenic bacteria will disrupt the permeability of cell walls so that the cell leaks and loses several important metabolites which will end in a reduction in the number of erythrocyte cells (Aboderin and Oyetayo, 2006).

Statistical analysis showed that the treatment of soursop flower ethanol extract and *S. enteritidis* infection gave a significant difference (P<0.05) in the reduction in the number of erythrocytes. Reduction in the number of erythrocytes may be influenced by the content of flavonoids in soursop flower extract. Flavonoids function as antioxidants that counteract free radicals and protect membrane lipids so as to prevent erythrocyte damage (Sundaryono, 2011).

The role of erythrocytes is very important, ranging from identification, adhesion, and killing of pathogens so that they are able to regulate the immune system (Tian *et al*., 2013). The amount of erythrocytes in the circulation is influenced by the hormone erythropoietin which functions to stimulate erythropoiesis by triggering the production of proeritroblas from hemopoietic cells in the bone marrow. Vitamin B12 and folic acid affect erythropoiesis in the final maturation stage of erythrocytes, whereas hemolysis can affect the number of erythrocytes in the circulation (Meyer and Harvey, 2004).

Hemoglobin Concentration

Concentration of hemoglobin in rats after treatment can be seen in Table 1. In Table 1, the concentration of hemoglobin in rats was still in the normal range after administration of soursop flower ethanol extract, while in P2 group hemoglobin concentration decreased. Decreased hemoglobin also occurred after *S. enteritidis* infection. The administration of soursop flower extract and *S. enteritidis* infection showed a significant difference (P<0.05) in the decrease in rat hemoglobin concentration. Duncan test results showed no significant difference (P>0.05) in the NC and PC treatment groups, did not show any significant difference (P>0.05) in the P1, P2, and P3 treatment groups, but showed a significant difference (P<0.05) in the NC and PC treatment groups.

Hemoglobin concentration after administration of soursop flower ethanol extract ranged from 11.76-14.23 g/dL, whereas after *S. enteritidis* infection ranged from 8.43-11.56 g/dL. Rat hemoglobin concentrations after *S. enteritidis* infection showed results below the normal range of 12.48-14.63 g/dL (Charles River Laboratories, 1998). Damage to red blood cells due to infection can reduce the concentration of hemoglobin (Paim *et al*., 2011). Clinically, a decrease in the number of erythrocytes will result in a decrease in hemoglobin and the occurrence of anemia (Tian *et al*., 2013).

Hematocrit Value

The post-treatment hematocrit value of rats can be seen in Table 1. Table 1 showed that the average hematocrit value of rats in the NC group in this study was 41.10%, which was still within the normal range. Likewise, the average hematocrit value of rats in the CP group was 41.08%. However, in the treatment groups P1, P2, and P3, the average hematocrit value decreased. Decrease in hematocrit values in P2 and P3 groups was still in the normal range, while in the P1 group the hematocrit value decreased to below the normal range. The normal range of hematocrit values is 39-53% (Aboderin and Oyetayu, 2006).

Sodium and potassium ions contained in body fluids, both extracellular fluid (blood) and intracellular fluid (cytoplasmic fluid), are able to influence the work of epinephrine in suppressing excessive splenic contractions, so that contractions that occur in erythrocytes become stable. Sodium and potassium ions also keep the hematocrit value in the blood in the normal range (Von-Borell, 2001). The hematocrit value is related to the number of erythrocytes. The greater the number of erythrocytes, the greater the hematocrit value in the blood. Vice versa, a decrease in hematocrit values can be caused by damage to erythrocytes. Hematocrit value is very dependent on the number of erythrocytes, because erythrocytes are the largest cell mass in the blood (Virden *et al*., 2007).

Statistical analysis showed that administration of soursop flower extract and *S. enteritidis* infection showed a significant effect (P<0.05) on the decrease in hematocrit value. The treatment of soursop flower ethanol extract and *S. enteritidis* infection decreased the hematocrit value of rat. Other causes that can reduce the hematocrit value are due to technical errors when taking, handling, and delivering blood samples, and lysis of blood cells due to a long storage time (Hohenhaus, 2007).

Platelet Count

The average number of rat platelets after treatment can be seen in Table 1. In Table 1 it can be seen that after administration of soursop flower ethanol extract, the average number of rat platelets in all treatment groups was still within the normal range, but after infection of *S. enteritidis* in the P1 treatment group, the average number of platelets in rats below the normal range. The average number of blood platelets in rat is 430-1450x10^3/µL (Weiss and Wardrop, 2010).

Based on the results of statistical analysis, the treatment of soursop flower ethanol extract and *S. enteritidis* infection had a significant effect (P<0.05) on the number of platelets in rats. Furthermore, Duncan's test in these treatments showed that the average number of platelets in rats in P1 group experienced a significant difference (P<0.05) compared to the P3 group, whereas the treatment on PC and P2 groups did not showed a significant difference (P>0.05), but the treatment in the NC, P1, and P3 groups gave a significant difference (P<0.05).

The pathogenic activity of *S. enteritidis* is able to lyse the intestinal mucosal walls, resulting in bleeding. If a wound occurs, the damaged tissue platelets will release thromboplastin that reacts with prothrombin and calcium to form thrombin. Thrombin will react with fibrinogen to form fibrin which will cover the injured

tissue. The result will be a decrease in the number of platelets in the blood circulation in the group infected with *S. enteritidis*. Giorgi (2000) stated that flavonoids in the blood can inhibit excessive platelet function. Clotting of platelets in the blood can cause excessive blood clotting or hypercoagulation which includes increased platelet function and disruption of fibrinolysis. Flavonoids can reduce excessive platelet production by preventing inflammation in the body thereby reducing damage to blood vessels.

CONCLUSION

Based on the results of the study it can be concluded that the ethanol extract of soursop flower does not have antibacterial activity against *S. enteritidis* bacteria. The administration of soursop flower ethanol extract at various dosage levels is able to maintain the number of leukocytes but reduce the number of erythrocytes, hemoglobin concentration, hematocrit value and platelet count in lab rats. Meanwhile, *S. enteritidis* infection decreases all hematological features of lab rats.

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