Preventive Study Garlic Extract Water (Allium sativum) Toward SGPT, SGOT, and the Description of Liver Histopathology on Rat (Rattus norvegicus), which were exposed by Rhodamine B

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Abstract. Rhodamine B is a textile coloring materials which often mixed in food coloring. The use of Rhodamine B in food for a long time may result in liver dysfunction or cancer. However, when exposed Rhodamine B in large quantities in a short time it will be symptoms of acute poisoning of Rhodamine B. The allicin and alliin content in garlic works as an antioxidant that can neutralize free radicals there by lowering oxidative stress and help prevent an increase in SGOT and SGPT and improve depiction hepatic histopathology. The purpose of this study are to determine the effect of preventive therapy extract water of garlic (Allium sativum) on levels of SGOT and SGPT and liver histopathology description of rat (Rattus norvegicus) which were exposed by Rhodamine B. This study used male rats strain Wistar with 8 weeks of age and weight of 200 grams which were divided into 5 groups: group A (negative control), B (positive control), group C, D, and E were fed with rodhamin B and given preventive water with garlic juice with successive doses of 0.5 mL, 1 mL, and 1.5 mL. SGPT and SGOT level measurements performed by spectrophotometric method and observation of rat liver histopathology performed using light microscope. The data analysis activities of SGPT/SGOT using ANOVA, and the description of histopathology were analyzed descriptively. The results showed giving garlic extract with a dose of 1.5 mL/0.2 kg bw were able to decrease the activity of SGPT, i.e. respectively for treatments A to E, 18.89%, 17.3 %, 40.96% and SGOT 41.44%, 45.96%, 49.69%, and to improve the hepatocyte cells in rat exposed to Rhodamine B. The conclusion of this study is water garlic juice can be used as herbal therapy in mice which were exposed by Rhodamine B.

Keywords: rhodamine B, garlic (Allium sativum), SGPT, SGOT, liver histopathology

1. Introduction

Rhodamine B is a dye often used by food vendors. The synthetic dyes are often used because the color produced are brighter and more durable than natural food coloring. The use of Rhodamine B in food for a long time can lead to liver dysfunction and cancer. However, when exposed Rhodamine B in large quantities in a short time it will be symptoms of acute poisoning Rhodamine B. Rhodamine B that enters the body can cause several diseases, including cancer, poisoned, respiratory and digestive tract irritation [1].

In the structure of rhodamine B there is a bond with a compound of chlorine (Cl) in which chlorine atoms are classified as halogen compounds and properties of halogen in organic compounds are very dangerous and have high reactivity to achieve stability in the body by binding to substances in the body that cause toxic effects and lead to cancer in humans [2]. Some of the research results of toxicity tests indicate rhodamine B have LD50 more than 2000 mg/kg, and can cause strong irritation of mucous membranes [3]. Found in mice that the lethal dose (LD50) by mouth at 887 mg/kg, and the lowest dose of 500 mg/kg. Rhodamine B is carcinogenic and genotoxic [4].

The impact of consuming rhodamine B in large numbers and repeatedly dumps in the body that can cause irritation of the mucous gastrointestinal, and if inhaled can irritate the respiratory tract, skin...
irritation, eye looks redness and edema [1], as well as cause organ damage to the liver, kidneys and spleen [5,6]. Oxidative stress is damage to cells in the liver activates enzymes Serum Glutamate pyruvate transaminase (SGPT) and serum Glutamic Oxaloacetate transaminase (SGOT). The damaged cells hepatocytes in the liver will cause SGPT and SGOT excreted out of the cells of the liver and distributed into the bloodstream, in which serum glutamate pyruvate transaminase (SGPT) is an indicator of damage to the liver while the Serum Glutamate Oxaloacetate transaminase (SGOT) is an indicator of damage liver cells but in small amounts and is also contained in other cells such as in muscles and the heart.

Capacity and serves to reduce and prevent the emergence of free radicals in the body is garlic (Allium sativum). Garlic is a natural material which its utilization is already very widespread in many countries. Cultivation of garlic is easy and has done very spacious and become commodities that provide economic benefits. The use of empirically been followed up by scientific research to provide supporting evidence. This is particularly important when the garlic will be used in health care [7].

2. Materials and Methods

2.1. Materials

Materials used in this study were garlic, white rat (Rattus norvegicus) Wistar strain male aged 8-12 weeks and the mean body weight - approximately 200 grams, rhodamine B, sterile distilled water, and physiological NaCl (0.9%). The tools used in this research included freezer, pH meter digital, analytical balance, micropipette, aluminum foil, plastic filter, centrifuge (Sorvall Biofuge Thermo scientific Primo R Centrifuge), Eppendorf micropipette size of 10-100 mL, and spectrophotometers UV-Vis.

2.2. Work procedures

2.2.1. Preparation experimental animals. Animal model used is a white rat (Rattus norvegicus) male Wistar strain obtained from the State University of Brawijaya aged 8-12 weeks with an average body weight of 150-200 grams and has obtained a certificate of acceptance of ethics Research Ethics Committee of UB No. KEP-627-UB.

2.2.2. The making of water juice garlic (Allium sativum). Juice of garlic water was made by means of a blender. As much as 1 kg of garlic that have been peeled and blended was then placed on the filter paper and squeezed until only the dregs of garlic remains. The filtrate is ready to be given to the mice as a treatment or if not directly used it can be stored in refrigerator at 4 °C for extending the shelf life and to prevent further acidification [8].

2.2.3. Preventive therapy and Administration. The dose of water juice of garlic (Allium sativum) is 0.5 mL/0.2 kg bw, 1.0 mL/0.2 kg bw, and 1.5 mL/0.2 kg bw. Treatment was given for 21 days to mice before being given food that is mixed with rhodamine B. Therapy was administered orally.

2.2.4. Measurement of levels of SGPT and SGOT. Mice that had been dissected, then as much as 5 mL blood drawn through the heart. Blood was drawn accommodated in centrifuge tube and centrifuged at 3000 rpm for 10 minutes. Furthermore, serum which is located at the top are separated and taken to analyze the SGPT and SGOT levels. According to Deny (2013), SGPT and SGOT levels measurement was conducted using serum samples spectrophotometric by mixing with reagents. AST and ALT reagents consisting of reagent 1 and reagent 2. Blood serum and reagents SGOT/SGPT were mixed at room temperature (15-30 °C). Blood serum taken as many as 100 mL and incubated at 37 °C. After 60 seconds, the measured absorbance is recorded. The mixture was then brought back to room temperature and incubated at 37 °C for 60 seconds. The absorbance was then measured at minute 1, 2 and 3.
2.2.5. Observation of Histopathology. White rats (Rattus norvegicus) liver was made by preparation of hematoxylin eosin staining (HE). Histopathologic features hepatocytes and sinusoidal cells was observed using Olympus BX51 light microscope with 400x magnification.

2.2.6. Data analysis. The variables measured in this study including SGOT, SGPT, and liver histopathological level changes. Measurement of transaminase enzyme levels (ALT and AST) were analyzed quantitatively using pattern analysis of variance one-way ANOVA with the test pattern analysis data using two-way tables observations then followed by analysis of variance [9]. Meanwhile, hepatic histopathology observation was conducted using light microscope and HE staining.

3. Results and Discussion

3.1. Serum Levels of SGPT and SGOT

The measurement of Serum levels Gutamic Pyruvic transaminase (SGPT) in the blood of rats exposed to a concentration of 600 ppm rhodamine through feed and water given garlic juice is presented in Table 1.

| Treatment group | The average levels of ALT (U / L) (SGPT) | The increase of the negative control | The decrease of the positive control |
|-----------------|------------------------------------------|-------------------------------------|-------------------------------------|
| Group A (Control -) | 27.7±2.87 \(^{a}\) | - | - |
| Group B (Control +) | 84.7±12.73 \(^{c}\) | 205.77\% | - |
| Group C (feed rhodamine B to 600 ppm + 0.5 mL of water squeezed garlic/ 0.2 kg bw) | 68.7±5.12 \(^{bc}\) | - | 18.89\% |
| Group D (feed rhodamine B to 600 ppm + 1.0 mL of water squeezed garlic/0.2 kg bw) | 70.0±19.6 \(^{bc}\) | - | 17.3\% |
| Group E (feed rhodamine B to 600 ppm + 1.5 mL of water squeezed garlic/0.2 kg bw) | 50.0±10.7 \(^{ab}\) | - | 40.96\% |

Description: numbers with superscript (notation a, b, c) different shows their difference \(p<0.01\) between the treatment group.

Statistical analysis showed that the juice garlic water provides highly significant effect \((p<0.01)\) on levels of Serum Glutamic Pyruvic transaminase (SGPT) in rats exposed rhodamine B through feed. The average levels of Serum Glutamic Pyruvic transaminase (SGPT) in the negative group is the standard used to determine the decrease or increase the levels of Serum Glutamic Pyruvic transaminase (SGPT) that occurs in the other treatment group. According to Schiff (2006), normal levels Serum Glutamic Pyruvic transaminase (SGPT) rat is 17.5 to 30.2 U/L [10].

In the positive control group (group B) an increase in the average levels of Serum Glutamic Pyruvic transaminase (SGPT) mice by 205.77\% as compared to the negative control group (group A). Increased levels of Serum Glutamic Pyruvic transaminase (SGPT) in the positive control group showed that exposure rhodamine B with a concentration of 600 ppm are given in the feed for 21 days can increase the levels Serum Glutamic Pyruvic transaminase (SGPT) in the rat. Serum Glutamic Pyruvic transaminase (SGPT) increased due to the exposure of rhodamine B to stimulate increased ROS (Reactive Oxygen Species). Based on statistical analysis known that the treatment of water provision garlic juice effect when compared to the positive control group (group B).

In the mice in group C were given the juice of garlic with volume 0.5 mL/ 0.2 kg bw shows the average levels of Serum Glutamic Pyruvic transaminase (SGPT) 68.7 ± 5.12 U/L with a decrease in
the positive control group (group B) amounting to 18.89%. Based on Tukey test results shown in Table 1 show that the administration of garlic juice volume 0.5 mL/0.2 kg bw differ significantly to the positive control group (group B). In group D mice that were given the juice of garlic with volume 1 mL/0.2 kg body weight shows the average levels of Serum Glutamic Pyruvic transaminase (SGPT) by 70 ± 19.6 U/L with a positive difference to the control group (group B) 17.3%. Based on Tukey test results in Table 1 show that administration of garlic juice 1 mL/0.2 kg bw significant difference against the group C in which the effect is different from the effect in rats of C group given garlic juice 0.5 mL/0.2 kg bw.

Serum levels of measurement results Gutamicoxaloacetic transaminase SGOT) in the blood of rats exposed to a concentration of 600 ppm rhodamine through feed and given the juice of garlic is presented in Table 2.

Statistical analysis in this study shows that there is a highly significant difference (p<0.01) between serum levels Gutamicoxaloacetic transaminase SGOT) negative control group (group A) and a positive control group (group B). Increased levels of serum Gutamicoxaloacetic transaminase SGOT) in the positive group (group B) showed that exposure rhodamine B through feed given for 21 days may increase serum levels of SGOT Gutamicoxaloacetic transaminase) in the blood.

The average value Gutamicoxaloacetic transaminase serum levels of AST) showed that the juice of garlic in mice exposed via feed rhodamine B in a very significant (P <0.01) were able to prevent an increase in serum levels of SGOT Gutamicoxaloacetic transaminase) in the blood when compared with the positive control group (group B), and advanced test Tukey test showed a difference between treatments. Juice of garlic on a volume 1.5 mL/0.2 kg bw showed elevated levels of serum Gutamic showed oxaloacetic transaminase (SGOT) were best compared with the volume of garlic juice others. The higher the volume of the juice of garlic is given, the lower the levels of serum Gutamicoxaloacetic transaminase SGOT) (Table 2).

The average levels of serum Gutamicoxaloacetic transaminase SGOT) in the positive control group (group B) was 166.0 ± 62.1 U/L or an increase in the average levels of serum Gutamic shown in oxaloacetic transaminase (SGOT) were highly significant (P <0.01). Rhodamine B that goes through the feed may lead to an increase in productivity and a group of reactive oxygen (ROS). The reaction between ROS (Reactive

### Table 2. Serum levels Gutamicoxaloacetic transaminase SGOT) in the blood

| treatment group                                      | The average levels of AST (U/L) (SGOT) | Levels of SGOT | The increase of the control | The decrease of the positive control |
|------------------------------------------------------|---------------------------------------|----------------|-----------------------------|-------------------------------------|
| Group A (Control -)                                   | 61.0±13.2abc                         | -              | -                           | -                                   |
| Group B (Control +)                                   | 141.0±22.1abc                        | 131.14%        | -                           | 31.06%                              |
| Group C (feed rhodamine B to 600 ppm + 0.5 mL of water squeezed garlic/0.2 kg bw) | 97.2±8.0abc                          | -              | 36.38%                      |                                     |
| Group D (feed rhodamine B to 600 ppm + 1.0 mL of water squeezed garlic/0.2 kg bw) | 89.7±7.2abc                          | -              |                              |                                     |
| Group E (feed rhodamine B to 600 ppm + 1.5 mL of water squeezed garlic/0.2 kg bw) | 83.5±8.6abc                         | -              | 40.78%                      |                                     |

Description: numbers with superscript (notation a, b, c) different shows their difference p<0.05 between the treatment groups.

The average value Gutamicoxaloacetic transaminase serum levels of AST) showed that the juice of garlic in mice exposed via feed rhodamine B in a very significant (P <0.01) were able to prevent an increase in serum levels of SGOT Gutamicoxaloacetic transaminase) in the blood when compared with the positive control group (group B), and advanced test Tukey test showed a difference between treatments. Juice of garlic on a volume 1.5 mL/0.2 kg bw showed elevated levels of serum Gutamic shown oxaloacetic transaminase (SGOT) were best compared with the volume of garlic juice others. The higher the volume of the juice of garlic is given, the lower the levels of serum Gutamicoxaloacetic transaminase SGOT) (Table 2).
Oxygen Species) with unsaturated fats in cell membranes may result in peroxide compounds called lipid peroxidation that could potentially cause damage to the cell membrane so that the enzymes cytoplasmic out into the bloodstream. ROS are highly reactive also can bind to DNA in the mitochondria so caused cell necrosis. Necrosis of cells that occurs in hepatocytes cells will cause the enzyme GOT out into the bloodstream that can cause increased levels of serum Gutamicoxaloacetic transaminase (SGOT) \[11\].

In the mice in group C were given the juice of garlic with a volume of 0.5 mL/0.2 kg bw shows the average levels of serum Gutamicoxaloacetic transaminase SGOT) amounted to 97.2 ± 8.01 U / L with a positive difference to the control group (group B) amounted to 41.44%. Based on Tukey test results in Table 2 show that the administration of garlic juice volume of 0.5 mL/0.2 kg bw differ significantly to the positive control group (group B) in which the effects can prevent the rising levels of serum Gutamicoxaloacetic transaminase SGOT) in the blood.

In group D mice that were given the juice of garlic with a volume of 1 mL/0.2 kg bw shows the average levels of serum Gutamicoxaloacetic transaminase SGOT) of 89.7 ± 7.2 U / L with a positive difference to the control group (group B) amounting to 45.96%. Based on Tukey's test are shown in Table 2 show that the administration of garlic juice of 1 mL/0.2 kg bw significant difference against the group C in which the effect is different from the effect in rats of C group given garlic juice of 0.5 mL/0.2 kg bw.

In group E rats given garlic juice of 1.5 mL/0.2 kg bw shows the average levels of serum Gutamicoxaloacetic transaminase (SGOT) 83.5 ± 8.6 U/L with a positive difference to the control group (group B) amounted to 49.69%. Based on Tukey test results in Table 2 show that the group E by the juice of garlic 1.5 mL/0.2 kg bw differ significantly to the negative control group (group A). This proves that alisin contained the juice of garlic is able to bind free radicals in the body so as to suppress the occurrence of oxidative stress that would cause lipid peroxidation and inhibit the reaction of hydroxyl in the cell membrane, in which damage to the cell membrane is one of the causes of the issuance of Serum Gutamic Oxaloacetic Transaminase (SGOT).

3.2. Preventive studies Water Juice Garlic (Allium sativum) on Changes in Liver Histopathology in rats (Ratusnorvegicus) exposed Rhodamine B

In this study, in addition to using parameters SGPT and SGOT levels which is a biomarker of tissue damage, but also using histopathology imaging parameters. Observation of the histopathological picture is one of the critical success parameters to determine the level of damage that Occurs in mice exposed rhodamine B, as well as to see the results of the provision of the juice of garlic with a dose of 0.5 mL/0.2 kg, 1 mL/0.2 kg, and 1.5 mL/0.2 kg bw, is presented in Figure 1.

Based on the Figure below, it shows that the differences in hepatic histopathology between each treatment group. Histopathology features of the liver in the negative group (Figure A) can be observed that the nucleus of hepatocytes and sinusoidal cells in the negative control group did not reveal any damage, characterized by the hepatocyte cell membrane boundary is clearly visible, the nucleus intact and looks normal sinusoid. Seen in the cell nucleus numbered one hepatocytes. According to Utomo (2012) states that the organ normal liver histology indicated by the arrangement of the cells in radier the central vein, hepatocyte cells are round and oval, as well as hepatocyte cells have one nucleus or two nuclei. On histopathologic picture group A (Figure A) can be used as a reference in comparing the damage and repair that occurs in other treatment groups.

Histopathologic features positive liver group (group B) (Figure B) shows the sinusoidal dilation, Karyorrhexis and piknosis. Kaplowitz (2002) reported damage to liver cells caused by toxic materials such as rhodamine B, generally includes the participation of the toxic metabolites (rhodamine B), will now bring in the immune response, it can even directly affect the cell biochemistry. Damming vein usually starts from the central vein and then into the central part of the liver lobules.
Figure 1. Illustration of Liver Histopathology Rat (*Rattus norvegicus*) with HE staining of 400x. Description: (A) negative control mice; (B) Positive control mice; (C) Mice Feed rhodamine B to 600 ppm + 0.5 mL of water squeezed garlic/0.2 kg bw; (D) Mice with rhodamine B to 600 ppm feed + 1 mL of water squeezed garlic/0.2 kg bw; and (E) Mice with rhodamine B to 600 ppm feed + 1.5 mL of water squeezed garlic/0.2 kg bw. (→) Indicates Karyorrhexis (→ Shows the core piknotik, (↔) shows the sinusoidal dilation.

It appears that occurrence of necrosis of hepatocytes cells marked with the nucleus of cells undergoing Karyorrhexis. Karyorrhexis a malfunction in the cell nucleus that is characterized by cell nuclei began to disintegrate, the cell nucleus is not clear and hepatocyte cell boundary is not clear. Damage to the liver organ happens rhodamine B can prove toxic impact on the liver. Liver The damage can be caused by dyes, one of which is rhodamine B [4].

The observation of liver histopathological picture of rats which suffered exposure to rhodamine B to 600 ppm and got garlic juice 0.5 mL/0.2 kg bw (Figure C) has not shown any improvement that is still looks sinusoid dilatation and necrosis of hepatocytes cells. This suggests that therapeutic doses 0.5 mL/0.2 kg bw is still not able to provide maximum treatment.

Histopathologic features of the liver of white rats that experienced the exposure rhodamine B to 600 ppm and got the juice of garlic 1 mL/0.2 kg bw (Figure D) shows the histopathological picture looks like a group A (negative control), hepatocyte cells look normal, but still looks dilatation sinusoid. In the description of hepatic histopathology white mice who suffered exposure to rhodamine B to 600 ppm and got garlic juice 1.5 mL/0.2 kg bw (Figure E) shows the sinusoidal dilation wane, looks a little necrosis in hepatocytes cells characterized by the cell nucleus so that piktonik but the picture looks slightly darker histopathology (Figure E) show largely similar to the histology (Figure A). Improvements in liver organ is due to the influence of alisin compounds contained in garlic juice which act as antioxidants.

4. Conclusion
Based on the research that has been done can be concluded that provision of water juice of garlic can lower the levels of SGPT and SGOT and can improve liver histopathology in rats (*Rattus norvegicus*)
were exposed Rhodamine B. Further studies should be done by using garlic juice dose higher than the dose of 1.5 mL/0.2 kg body weight to obtain optimum results.

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