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THE EFFECTIVENESS OF SOURSOP (*Annona muricata* L.) BOOKING WATER ON BLOOD BIOCHEMICAL LEVELS OF MOUSE (*Mus musculus*) INDUCED RHODAMIN B

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Abstract

This study aimed to determine the effect of giving soursop (*Annona muricata* L.) boiled water for 14 days on biochemical blood levels, including SGOT, SGPT, urea, and creatinine in the balb-C strain mice induced by rhodamine B orally. Twenty-five male mice were divided into five groups, namely groups I and II as negative control and positive control. Group III was treated with soursop leaf boiled water at 3.64 mg/mouse, and group IV was treated with soursop leaf boiled water at 7.28 mg/mice. Mice, while group V was treated with 10.92 mg/mouse of soursop leaf boiled water. The treatment was carried out for 14 days by giving soursop leaf boiled water seven days after administration of rhodamine B. On days 1, 7, and 14, the mice took intracardially, and then the levels of SGPT, SGOT, urea, and creatinine were measured. The data were then analyzed using the One way ANOVA test and continued with the LSD test. One-way ANOVA test results showed that giving soursop leaf boiled water had a significant effect on reducing SGOT, SGPT, urea, and creatinine ($p<0.05$). The results of the LSD test showed significant differences between levels of SGOT SGPT, urea, and creatinine in the group before treatment, after administration of rhodamine B, and after administration of soursop leaf boiled water. It concludes that the treatment of soursop (*Annona muricata* L.) cooked in the water had the most practical effect in neutralizing the damage to liver and kidney cells of mice exposed to rhodamine B. Because it contains antioxidants, the water that boiled with soursop leaf provided a cytoprotective effect by neutralizing the free radicals caused by rhodamine B.

Keywords: Effectiveness, soursop leaf (*Annona muricata* L.), Biochemistry

INTRODUCTION

Regulation of the Minister of Health of the Republic of Indonesia No. 239/Menkes/Per/V/85 states that one of the food coloring substances prohibited from being used as a food additive is Rhodamin B (Yamlean, 2011). Rhodamine B is a synthetic dye in the form of a crystalline powder that is odorless and green or reddish-purple. Rhodamine B belongs to the class of basic xanthenes dyes and is made from methadiethylaminophenol and phthalic anhydride as an inedible and highly fluorescing material. Rhodamine B compounds are commonly used in the textile industry as a dye and are dangerous when used as a food coloring (Dawile et al., 2013).

Consumption of rhodamine B can continuously irritate the respiratory tract, skin irritation, eye irritation, and digestive irritation, cause poisoning, kidney damage, impaired liver function, and even cause liver cancer (Eka, 2013). Rhodamine B is carcinogenic and is characterized by symptoms of enlargement of the liver, kidneys, and spleen, followed by anatomical changes in the form of organ enlargement. Liver damage can be indicated by increased secretion of transaminase enzymes in Serum Gulatamate Oxaloacetic Transaminase (SGOT) and Serum Gulatamate Pyruvate Transaminase (SGPT).

Radical compounds produced by rhodamine (ROS, Reactive Oxygen Species), which are the result of the metabolism of toxic substances, can also cause glomerular damage (Singh et al., 2006) and reduce the availability of the body's antioxidant reserves (Ercal et al., 2001). Renal tubular impairment due to consumption of Rhodamine B causes fluid retention, resulting in uremia, hyperkalemia, an increase in urea (BUN, Blood Urea Nitrogen) of about 25-30 mg/dl/day, and an increase in creatinine of about 2.5 mg/dl/day (Price and Wilson, 2005). Rhodamine B has also been shown to promote endoplasmic reticulum stress in the renal glomerulus, which leads to oxidative stress and inflammation in podocyte cells and the mesangial glomerulus (Inagi, 2009).

Various previous studies on traditional medicinal plants' benefits to health continue to develop. This natural or standard treatment is carried out in the hope that it can become an essential source of information to obtain new compounds capable of becoming specific receptor targets. The exploration of natural materials that have potential as medicinal plants is marked by the widespread movement of returning to nature (back to nature) as the right choice in overcoming difficulties with disease (Adjie, 2011; Rachmani et al., 2012; Dayeef et al., 2013)

Soursop (*Annona muricata* Linn.) is a plant from the class Dicotyledonae, family Annonaceae, and genus Annona (Adeyemi et al. 2006). The potential of soursop leaves as an alternative to traditional medicine is increasingly being studied because it contains various active compounds. The content of compounds contained in soursop leaves include tannins, calcium oxalate, muricin alkaloids, monotetrahydrofuran acetogenins such as anomurisin A and B, gigantetrosin A, annonasin-10-one, murikatosin A and B, annonasin and goniothalamicin (Suranto, 2011; Dayeef., et al. 2013), steroids, terpenoids, flavonoids, and coumarins (Ezejindu et al., 2014). Other soursop leaves contain calcium, phosphorus, carbohydrates, vitamin A,

vitamin B, vitamin C, saponins, flavonoids, phytosterols, anonol, and murisine alkaloids (Utami and Desi, 2013; Wulaningrum, 2014).

Chemical compounds in soursop leaves have antibacterial, antifungal, and anticancer properties, fight various parasites, reduce high blood pressure, depression, and stress, and have been proven in vivo to normalize damaged nervous systems (Wijaya, 2012; Dayef et al., 2013). Soursop leaves also have antitumor, anti-inflammatory, antihyperlipidemic, and antihyperglycemic properties (Bermejo et al., 2005; Haijun et al., 2010 in Wahyunindiani, 2014). The various benefits of soursop leaves (*Annona muricata* L.), as described in the background above, become an essential consideration for researchers in developing the potential of this plant for studies of liver and kidney biochemistry of mice induced with rhodamine B compounds. This study aimed to determine the effect of soursop leaf decoction on SGOT, SGPT, urea, and creatinine levels in rhodamine B-induced balb-C mice.

METHOD

Soursop leaves used are boiled water obtained from the yard of the house—Rhodamine B as a carcinogen for induction received from the Food and Drug Monitoring Agency of Maluku Province. This type of research is an experimental in vivo laboratory using a Post-test only control group design, which was carried out on balb-C strain mice as the subject. The study was conducted at the Zoology Laboratory of Pattimura University and the Maluku Province Health Laboratory.

This research was carried out for one month, collecting raw materials and research samples. Materials such as soursop leaves, rhodamine B, balb/C male mice, paint contain

ing hematoxylin (H) and eosin (E), xylol solution, aqua dest, 70 percent alcohol, 95 percent alcohol, chloroform, formalin, liquid paraffin, NaCl, formalin, mice feed, husks, dip and read test strips (reagent strips) for bilirubin and proteinuria tests. The tools used in this study were autoclave, aluminum foil, stove, analytical balance, spectrophotometer, water bath, Erlenmeyer, oral sonde, measuring cup, 100 ml beaker glass, Erlenmeyer, spatula, spoon, pipette, syringe bottle, cage, syringe. One cc/ml, ten cc/ml syringe, tweezers, filter, cotton swab, razor blade, scissors, surgical instrument set, petri dish, microscope, object-glass, cover glass, rotary microtome, wire ram, porcelain bowl, mask, gloves, tissue, laboratory clothes, and digital camera.

Population and Sample

The population used in this study were all male mice of the balb-C strain (*Mus musculus*), and all soursop leaves (*Annona muricata* L.) obtained from the yard. The sample of this study was mice (Mus musculus L.) balb-C strain, totaling 25 males, aged ± three months with a bodyweight of 20-30 g. The mice were obtained from the Zoology Laboratory, Faculty of Mathematics and Natural Sciences, Pattimura University, Ambon. As for soursop leaves (*Annona muricata* L.) obtained from the yard, the leaves used are not too old and not too young (leaves in the 4th, 5th, or 6th order of the shoots).

Research Implementation

Test Animal Preparation

Experimental animals were divided into five groups, and each group consisted of 5 mice. Group I as a control, group II was given Rhodamine B 0.2 mg/mouse, group III was given rhodamine B 0.2 mg/mouse and soursop leaf boiled water 3.64 mg/mouse, group IV gave rhodamine B 0.2 mg /mice and soursop leaf boiled water 7.28. In comparison, mice in group V were given rhodamine B at a dose of 0.2 milligrams per mouse and soursop leaf boiling water at 10.92 milligrams per mouse. The treatment was carried out for 14 days by giving soursop leaves one week after administering rhodamine B. On days 1, 7, and day 14, the rats were drawn blood by taking blood directly to the heart using a syringe and aspirating slowly and then measuring the levels of SGOT, SGPT, Urea, and creatinine.

Making Soursop Leaf Boiled Water

Soursop leaves (Annona muricata L.) were washed and then sorted out the raw materials (selection of soursop leaves that were good and correct or not deformed and not rotten) and free of pests and dirt (Novianti, 2015). After washing thoroughly in running water, drain and put in a water bath or jug. Add 100 ml of clean water and place on the stove, then boil until boiling (approximately 5 minutes until the temperature reaches 900 C) while stirring occasionally. In the next step, the boiled water is then put into a glass beaker and given to each experimental animal group for treatment (Restuati and Panggabean 2014). Making soursop leaf boiled water is done

every two days because the boiled water can only last for two days in the refrigerator (Directorate General of POM, 2014).

Blood serum collection and measurement of Biochemical Levels

Blood was taken directly from the heart intracardially or using a syringe and collected in Eppendorf, and then the blood sample was centrifuged to obtain the serum. The spectrophotometric method uses to do this. Then, at the Maluku Provincial Health Laboratory, the activity of SGOT and SGPT is measured with Kit's reagent and a 501 photometer using the spectrophotometric method at a wavelength of 340 nm. The urea activity and creatinine level are measured at a wavelength of 546 nm.

Data analysis

The research data were analyzed statistically using the Anova test and continued with the LSD (*Least Significance Different)* test with the SPSS version 22.

RESULTS AND DISCUSSION

Examination of SGOT, SGPT, urea, and creatinine activity was carried out on days 1, 7, and 14. Based on the study results in the table above, on day seven or after rhodamine B induction, the group before treatment had lower SGOT activity than the group before treatment. However, the activity of urea and creatinine SGPT enzymes was higher when compared to the pre-treatment group. After administration of Rhodamine B, the SGOT enzyme's concentration was lower than the SGPT enzyme. This study was because the proportion of the SGOT enzyme was more abundant in other organs such as skeletal muscle, pancreas, heart, and kidneys than in the liver (Kaplan and Pesce, 2003). 1998). It can conclude that giving rhodamine B 0.2 ml/BW in mice can cause liver and kidney damage.

Table 1 The results of the average levels of SGOT, SGPT, Urea and Creatinine

Group	**(Mean ± SD (U/L)**			
	SGOT	**SGPT**	**Ureum**	**Kreatinin**
Before Treatment	107.40 ± 1,67	47,20 ± 3,19	43,80 ± 4,38	0,51 ± 0,03
After administration of rhodamine B	100.40 ± 5,03	60,40 ± 16,81	52 ± 11,02	0,68 ± 0,05
After giving boiled water	71 ± 13,22	46,60 ± 3,91	22,40 ± 5,07	0,41 ± 0,02

Source: Primary Data

Paliwal (2011) states that the toxic substance of Rhodamine B can induce the production of Reactive Oxygen Species (ROS), which causes lipid peroxidation, DNA damage, and loss of cells that have antioxidant systems. This result triggers the emergence of free radicals in the form of lipid peroxide. In addition, according to Umniyah (2007), the mechanism of the liver cell damage caused by free radicals is the same as the mechanism of cell damage in general. First, free radicals attack liver cell membranes composed of phospholipids, disrupting cell membrane permeability. Due to impaired cell membrane permeability, there is an increase in calcium influx from extracellular sources and calcium release from mitochondria and endoplasmic reticulum. Increased calcium influx triggers the activation of several destructive enzymes such as proteases that can damage DNA. When DNA damage increases, polyribosomes, and NAD empties occur, resulting in inhibited ATP synthesis. Inhibition of ATP formation causes liver cell damage or necrosis.

Liver cell damage due to free radical compounds is overcome with antioxidant compounds. However, the antioxidant compounds in the body are not used enough to overcome excessive oxidants in the body (Arnelia, 2004). Therefore, the body needs an antioxidant blend that comes from outside the body (exogenous antioxidant) to reduce the presence of excessive oxidants in the body. In this study, the soursop leaf was used (*Annona muricata* L.)

With an average SGOT enzyme level of 71 13.22 mg/dl, the soursop leaf ethanol extract treatment decreased SGPT activity. When the SGPT test, the results showed a drop, with an average of 118.1372.69U/I for the enzyme SGPT. So, we can say that boiling the leaves of the soursop plant (Annona muricata L.) affects the levels of SGPT and SGOT in Mus musculus caused by rhodamine B in vivo.

In contrast to the levels of SGOT and SGPT, creatinine and urea are essential parameters to determine the state of acute kidney failure and see the form of kidney function (Jorres et al., 1999). Based on the measurement results, urea and creatinine levels as indicators of kidney examination also increased in treatment after rhodamine B administration. After rhodamine B administration, urea levels became 52 ± 11.02, and creatinine levels 0.68 ± 0.05. An increase in urea and creatinine levels indicates that kidney damage occurs due to the induction of rhodamine B. Meanwhile, the average results of urea and creatinine levels decreased again when soursop leaf boiled water gave, namely urea levels 22.40 ± 5.07 and creatinine levels 22, 40 ± 5.07.

The evidence indicates that soursop leaves have a diuretic effect. The decrease in blood creatinine indicates that the boiled water of soursop leaves can maintain blood urea and creatinine levels in normal conditions by increasing the glomerular filtration rate of the kidneys so that the rate of disposal of metabolites, including creatinine, becomes smooth. The increased renal glomerular filtration rate is due to the diuretic properties in soursop leaves. It is in line with the research results from Sukandar et al. (2010) that soursop leaf decoction given to balb-C strain mice showed an increase in the volume of urine excreted.

Based on statistical analysis, the results of the normality test (Kolmogorov-Smirnov) showed that the levels of SGOT, SGPT, urea, and creatinine in the blood of mice on day 1, day seven, and day 14 were usually distributed ($p>0.05$).

And homogeneity test (Levene) showed data that levels of SGOT, SGPT, urea, and creatinine varied homogeneously ($p> 0.05$).

Because the conditions for normality and homogeneity had been met, a one-way ANOVA analysis continued.

ANOVA test results obtained $p = 0.000$ for SGOT and $p = 0.025$ for SGPT, $p = 0.000$ for urea and $p = 0.000$ for creatinine. Based on these data, it can be interpreted that the p-value <0.05 states that the hypothesis can be accepted based on a significant difference in the results of the ANNOVA test, which is shown in the following table.

Table 2. One Way ANOVA Test Results

ANOVA

SGOT levels in mice in mg/dl

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	3730.533	2	1865.267	27.552	.000
Within Groups	812.400	12	67.700		
Total	4542.933	14			

SGPT levels in mice in mg/dl

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1045.200	2	522.600	5.085	.025
Within Groups	1233.200	12	102.767		
Total	2278.400	14			

Ureum levels in mice in mg/dl

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	2335.600	2	1167.800	21.041	.000
Within Groups	666.000	12	55.500		
Total	3001.600	14			

Creatinine levels in mice in mg/dl

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.189	2	.095	69.759	.000
Within Groups	.016	12	.001		
Total	.206	14			

Source: Primary Data

The ANNOVA test results show the levels of Serum Sugaramate Oxaloacetate Transaminase (SGOT) and Serum Sugaramate Pyruvate Transaminase (SGPT). Based on the way ANOVA test, groups found that giving soursop leaf boiled water (Annona muricata L.) in experimental animals affected decreased levels of SGOT, SGPT, urea, and creatinine. This study is indicated by a significant difference between groups in each week of treatment.

The enzymes commonly used in diagnosing liver damage are aminotransferases (SGPT and SGOT). The enzymes GPT and GGT are present in cells of various body tissues, but the primary source is liver cells. The presence of SGPT activity in plasma reflects liver cell damage. While the GOT enzyme itself is spread in the cells of the body's organs, starting from the most abundant, which is found in the heart muscle, then the liver, body muscles, kidneys, and pancreas. So when viewed based on distribution specifications, SGPT and GGT are the most specific markers for liver damage.

Post Hoc LSD analysis shows significant differences between groups for decision-making based on probability values. If the p-value <0.05, then there is a significant difference, while if the p value> 0.05, then there is a non-significant difference which can be seen in the following table:

Table 3. Post Hoc LSD Test Results SGOT, SGPT, Urea, and Creatinine levels

Time	**Data distribution of SGOT**	**Homogeneity Test**	**Parametric**	
			ANOVA	**Post Hoc LSD**
Day 1	TN	DH	BB	0,203

7th day	TN	DH	BB	0,00
Day 14	TN	DH	BB	0,00
Waktu	**Distribusi data of SGPT**	**Homogeneity Test**	**Parametric**	
			ANOVA	**Post Hoc LSD**
Day 1	TN	DH	BB	0,19
7th day	TN	DH	BB	0,927
Day 14	TN	DH	BB	0,016
Waktu	**Distribusi data of urea**	**Homogeneity Test**	**Parametric**	
			ANOVA	**Post Hoc LSD**
Day 1	TN	DH	BB	0,107
7th day	TN	DH	BB	0,01
Day 14	TN	DH	BB	0,00
Waktu	**Distribusi data of creatinine**	**Homogeneity Test**	**Parametric**	
			ANOVA	**Post Hoc LSD**
Day 1	TN	DH	BB	0,00
7th day	TN	DH	BB	0,01
Day 14	TN	DH	BB	0,00

Information :

TN: normally distributed

DH: homogeneous data

BB: different means

Table 3 shows the activity of SGOT, SGPT, urea, and creatinine. The group before treatment was statistically different from that in the treatment group after rhodamine B gave, and boiled water soursop leaves were given to each group at doses of 3.64 mg./kg BW, 7.28 mg/kg BW, and 10.92 mg/kg BW on day 1, day 7, and day 14.

Based on these results, it can seem that giving soursop leaf boiled water in each dose group can improve liver and kidney damage which is characterized by a decrease in the levels of SGOT, SGPT, urea, and creatinine in each experimental group. This study indicates that the test material is not toxic and can repair damaged liver and kidneys caused by rhodamine B induced.

CONCLUSION

In the treatment of boiled water, soursop leaves (*Annona muricata* L.) have the most practical effect in neutralizing damage to liver and kidney cells of mice exposed to rhodamine B. The content of antioxidants in water-boiled soursop leaves can overcome free radicals triggered by rhodamine B, so exert a cytoprotective effect.

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