Yudishtira Journal: Indonesian Journal of Finance and Strategy Inside
p-ISSN: 2797-9733 | e-ISSN: 2777-0540
Vol. 2 No. 2 Mei - Agustus 2022

POTENCY OF SOURSOP LEAF (*ANNONA MURICATA* L.) DECOCTION WATER AS A TRIGGER FOR MICE (*MUS MUSCULLUS*) LIVER CELL REGENERATION MARKED BY CYCLIN D1 EXPRESSION DUE TO RHODAMINE B INFECTION

Pramita Wally[1]
[1]Biology Education Study Program, College of University Muhammadiyah Maluku, Ambon-Maluku, Indonesia
Email: pramitawally3@gmail.com

Abstract

Soursop leaf (Annona muricata L.) is one of the plants that contains chemical compounds such as acetogenins, flavonoids, saponins, tannins, triterpenoids and glycosides where this group of chemical compounds has the potential as antioxidants to ward off free radicals. This study aimed to determine the effect of soursop leaf decoction in regenerating liver cells of male mice of the Balb/C strain induced by rhodamine B through the expression of cyclin D1. The 25 mice were divided into 5 groups, namely group I was given standard feed, group II was given standard feed and rhodamine B 0.4 mg/ml, groups III, IV and V were given standard feed, rhodamine B 0.4 mg/ml, and water. soursop leaf decoction with doses of 3.64 mg/g BW, 7.28 mg/g BW, and 10.92 mg/g BW for each group. The treatment was carried out for 14 days by giving soursop leaf boiled water after rhodamine B induction for 7 days. The results showed that the boiled water of soursop leaves at a dose of 10.92 mg/g BW had a significant effect on reducing levels of SGOT and SGPT ($p<0.05$) and could regenerate mice liver cells through the expression of cyclin D1. Based on the results of the study, it can be concluded that the antioxidant content of soursop leaf boiled water can prevent liver cell damage in mice and increase cell regeneration power.

Keywords: *Soursop leaf (Annona muricata L.), cell regeneration, cyclin D1, rhodamine B*

INTRUDUCTION

Rhodamine B is a compound commonly used in the textile industry as a dye and is dangerous when used as a food coloring (Dawile, *et al*., 2013; Mayori *et al.,* 2013). Regulation of the Minister of Health of the Republic of Indonesia No. 239/Menkes/Per/V/85 states that rhodamine B is a food coloring substance which is prohibited to be used as a food additive (Yamlean, 2011). Consumption of rhodamine B continuously can cause irritation to the respiratory tract, skin irritation, eye irritation, irritation to the digestive tract, causing poisoning, kidney damage, impaired liver function and liver cancer (Eka, 2013; Mcheik, 2013). Rhodamine B is included in the organochlorine xenobiotic group, which during the metabolic process cannot be excreted properly so that when it accumulates in large quantities it causes cytotoxicity to cell apoptosis. Toxic metabolism due to rhodamine B causes membrane damage

by direct covalent binding to proteins and lipids resulting in the formation of reactive free radicals and an increase in Reactive Oxygen Species (ROS) (Sun *et a*l., 2010; Paliwal, 2011).

Umniyah (2007), stated that the mechanism of cell damage caused by free radicals is the same as the mechanism of cell damage in general. Free radicals first attack liver cell membranes which are composed of phospholipids, causing disruption of cell membrane permeability, due to impaired cell membrane permeability, there is an increase in calcium influx from extracellular sources as well as calcium release from mitochondria and endoplasmic reticulum. Increased calcium influx triggers an increase in a number of destructive enzymes such as proteases that can damage DNA so that polyribosomes increase and emptying of NAD occurs which results in inhibited ATP synthesis. Inhibition of ATP formation causes cell necrosis and apoptosis (Gupta et al, 2008; Dewi, 2012). In addition to an increase in protease enzymes, liver cell damage is also marked by an increase in the secretion of transaminase enzymes in the form of Serum Gulatamate Oxaloacetate Transaminase (SGOT) and Serum Gulatamate Pyruvate Transaminase (SGPT) (Eka, 2013). Liver cell damage due to free radicals can be overcome with endogenous antioxidant compounds but antioxidant compounds from the body are not used enough to overcome excessive oxidants in the body. Therefore, the body requires antioxidant compounds that come from outside the body (exogenous antioxidants) to reduce excessive oxidants in the body (Kurniasih *et al*., 2015).

The potential of soursop leaf (*Annona muricata* L.) as an alternative to traditional medicine is increasingly being studied because it contains various active compounds. The active compounds contained in soursop leaves are flavonoids, tannins, calcium oxalate, titerpenoids, murisin alkaloids, acetogenins such as anonamurisins A and B, steroids, terpenoids and coumarins (Suranto, 2011; Dayeef., et al. 2013; Ezejindu et al., 2014). Other content in the form of carbohydrates, protein, vitamin A, vitamin B, vitamin C, and mineral content (Utami and Desi, 2013; Wulaningrum, 2014). The content of these active compounds can inhibit the process of oxidative reactions caused by excessive ROS accumulation in cells. Zuhud (2011) revealed that compounds that act as antioxidants in soursop leaf boiled water are flavonoid compounds. The mechanism of action of flavonoids as antioxidants can occur directly or

indirectly. Flavonoids as antioxidants directly are by donating hydrogen ions so that they can neutralize the toxic effects of free radicals while flavonoids as antioxidants indirectly are by increasing the expression of endogenous antioxidant genes such as the SOD (superoxide dismutase) gene (Kusuma, 2015). In addition to flavonoids, alkaloid compounds, polyphenols, saponins and tannins in soursop leaves are also antioxidants that can counteract the presence of free radicals so as to prevent cell damage (Setiyadi *et al.,* 2014). The purpose of this study was to determine the liver cell regeneration of male mice (*Mus muscullus*) strain Balb/C induced by rhodamine B through Cyclin D1 expression after being treated with soursop leaf (*Annona muricata* L.) boiled water.

MATERIAL AND METHOD

Place and Time

The design used in this study was an experimental in vivo laboratory using a Completely Randomized Design (CRD) with the type of Post test only control group design, which was carried out on balb-C strain mice as subjects. This research was conducted for 1 month. The place for research is at the Zoology Laboratory of Pattimura University and the Maluku Province Health Laboratory and the Physiology Laboratory of the Brawijaya Faculty of Medicine, Malang. Soursop leaves used are obtained from the yard of the house. Rhodamin B was obtained from the Drug and Food Control Agency of Maluku Province.

Materials

Tools and materials used in this study were autoclave, aluminum foil, stove, analytical balance, spectrophotometer, water bath, erlenmeyer, oral sonde, measuring cup, 100 ml beaker glass, erlenmeyer, cuvette, spatula, spoon, pipette, syringe bottle, cage, 1 cc/ml syringe, 10 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 cameras. The materials used include soursop leaves, rhodamine B, balb/C male mice, paint hetmatoxylin (H) eosin (E), xylol solution,

aquadest, 70% alcohol, 95% alcohol, chloroform, formalin, liquid paraffin, NaCl, formalin, mice feed, husks, reagent kit for SGPT and SGOT examination.

Research Sample

The sample of this study was mice (Mus musculus L.) balb-C strain, totaling 25 males, aged ± 3 months with a body weight of 20-30 g. The mice were obtained from the Zoology Laboratory, Faculty of Mathematics and Natural Sciences, Pattimura University, Ambon, while soursop leaves (Annona muricata L.) were obtained from the yard. The leaves used are leaves that are not too old and not too young (leaves that are in the 4th, 5th or 6th order of the shoots). Supriyanto *et al*., (2014) stated that leaf age can affect the content of its phytochemical compounds.

Research procedure

Test Animal Preparation

Experimental animals were divided into 5 groups, each group consisted of 5 mice. Group I was the normal group, group II was treated with rhodamine B 0.4 mg/ml, group III was treated with rhodamine B 0.4 mg/ml and soursop leaf boiled water 3.64 mg/g BW, group IV was treated with rhodamine B 0.4 mg/ml and soursop leaf boiled water 7.28 mg/g BW, while group V was treated with rhodamine B 0.4 mg/ml and soursop leaf boiled water 10.92 mg/g BW. The treatment was carried out for 14 days by giving soursop leaf boiled water one week after administration of rhodamine B. During the study, mice were given standard feed and drank ad libitum. After 14 days, all mice were taken intracardially using a syringe and aspirated slowly, then the levels of SGOT and SGPT were measured and surgery was performed to take the liver and make histological preparations.

Making Soursop Leaf Boiled Water

Soursop leaves (*Annona muricata* L.) are washed and then sorted by raw materials (sorts of soursop leaves are 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 (± 5 minutes until the temperature reaches 900C) while stirring occasionally. Prabandari (2015) stated that

fresh soursop leaf extract with a boiling time of 5 minutes had high antioxidant activity. The next step, the boiled water is then put into a glass beaker and then given to each group of experimental animals for treatment (Restuati and Panggabean 2014). Making soursop leaf boiled water is done every 2 days because the boiled water can only last for 2 days in the refrigerator (Directorate General of POM, 2014).

Determination of the Dosage of Soursop Leaf Boiled Water

Determination of the dose is carried out based on the assumption of herbal therapy in humans weighing 50 kg, namely by boiling 10 soursop leaves in 3 cups of water (600 ml) to obtain 1 cup (200 ml) of soursop leaf boiled water with a concentration of 10% soursop extract. (Syariefa, 2011). This dose will be used to convert from the human dose to the mouse dose using the conversion formula of Laurence and Bacharach (1964). In this research, three dose levels were made, with the conversion results from humans to mice as follows; 70/50 x 0.0026 x 10% = 3.64 mg / 100 ml of water, 70/50 x 0.0026 x 20% = 7.28 mg / 100 ml of water and 70/50 x 0.0026 x 30% = 10.92 mg / 100 ml of water.

Determination of Rhodamine B Dosage

According to the International Agency for Research on Cancer (1978), oral administration of rhodamine B in a concentration of 20 mg resulted in all mice dying on day 42 due to multiorgan damage. The average weight of the mice used is 20 grams, so the dose calculation in this study is:According to the International Agency for Research on Cancer (1978), oral administration of rhodamine B in a concentration of 20 mg resulted in all mice dying on day 42 due to multiorgan damage. The average weight of the mice used is 20 grams, so the dose calculation in this study is:

$$\frac{20\text{mg}}{1000\text{g}} = \frac{\text{d}}{20\text{g}}$$

$$\text{d} = \frac{20}{1000}\,\text{x}\,20$$

$$\text{d} = 0.4\text{mg}$$

Each treatment group was induced by 0.4 mg/ml rhodamine B, the process was given orally with a volume of 2 ml for each test animal for 7 days.

Observation of SGOT and SGPT Levels

Blood was taken intracardially using a syringe and collected in an Eppendorf tube, then the blood sample was centrifuged to obtain the serum. After that, the levels of SGOT and SGPT were read with Kit's reagent using a 5010 photometer at the Maluku Provincial Health Laboratory, using the spectrophotometric method at a wavelength of 340 nm.

Preparation of Liver Microanatomy Preparations

Liver tissue from the excision biopsy was fixed in 10% formalin solution, then dehydrated in graded concentration of alcohol, followed by a clearing process using xylol. The tissue pieces were then immersed in solid paraffin at a temperature of 56-58ºC, then paraffin blocks were made. The tissue in the paraffin block was then cut using a microtome with a thickness of 3-5 m and attached to an object glass. The tissue on the slide was stained using Hematoxylin-Eosin (H&E) staining. Furthermore, the preparations were observed under a microscope to obtain a descriptive analysis of the damage to liver cells caused by rhodamine B induction.

Preparation of Cyclin D1 . Expression Observation Preparations

Liver tissue that had been fixed, dehydrated and clearing was then paraffinized with xylene 3 times for 3 minutes. Rehydrate the preparations using 100% ethanol for 2 minutes, 95% ethanol for 2 minutes and 70% ethanol for 1 minute, finally with water for 1 minute. Next, soak in the peroxidase blocking solution at room temperature for 10 minutes and incubate the preparations in prediluted blocking serum at 25ºC for 10 minutes. Soak the preparations in Cyclin D1 monoclonal antibody for 10 minutes at 25ºC and wash the preparations again with PBS solution for 10 minutes. Incubate the preparations with secondary antibodies (conjugated to horse radish peroxidase) for 10 minutes at 25ºC, then wash the preparations with PBS solution for 5 minutes. Incubate the preparations with the chromogen Diaminobenzinidine at a temperature of 25ºC for 10 minutes and Hematoxylin-Eosin for 3 minutes. Clean the

preparations with running water and drip with mounting media using entelan, cover the preparations with coverslips and label them. Observe Cyclin D1 expression using a light microscope.

Data analysis

The research data were statistically analyzed using the Analysis of Variance (ANOVA) and continued with the Least Significance Different (LSD) Test at a significance level of 5% while the observational data on liver cell regeneration through cyclin D1 expression were analyzed using qualitative descriptive.

RESULTS AND DISCUSSION

Serum Glutamate Oxaloacetate Transaminase (SGOT) Levels

Measurement of serum SGOT levels in mice was carried out on the day of initial treatment, after induction of rhodamine B and after administration of soursop leaf boiled water for each treatment group. The data on the average SOPT levels of the mice obtained are presented in Table 1.

Table 1. Average levels of SGOT in mice (*Mus muscullus*) in each treatment

Group Treatment	SGOT Level (U/L) ± SD			Difference in Decreased Levels of SGOT (U/L)
	Beginning	After induction of rhodamine B	After Giving Soursop Leaf Boiled Water	
Negative Control	106 ± 3,60^{a}	107 ± 2,00^{a}	106 ± 2,64^{a}	-1 ± 0,57
Positive Control	106 ± 2,00^{a}	110 ± 2,64^{b}	112 ± 2,64^{ab}	2 ± 3,05
Dosage 3,64 mg/g BB	107 ± 2,64^{ab}	108 ± 1,00^{ab}	97 ± 6,08^{c}	-11 ± 6,08
Dosage 7,28 mg/g BB	108 ± 1,00^{b}	110 ± 3,00^{c}	93 ± 4,58^{cd}	-17 ± 9,29
Dosage 10,92 mg/g BB	106 ± 1,00^{a}	108 ± 2,64^{ab}	89 ± 2,64^{d}	-19 ± 10,44

Notes: Superscripts with the same letter showed no significant difference, ($p < 0.05$).

The results of the examination of SGOT levels (Table 1), showed differences. In the negative control group, the initial SGOT level was 106 U/L, increased after 1 week of the study

to 107 U/L and then decreased to 106 U/L after the 2nd week of the study, the positive control group of mice had an average initial SGOT level of 106 U /L, after induction of rhodamine B increased to 110 U/L and after the 2nd week SGOT levels increased to 112 U/L. The levels of SGOT in the group of mice that were given boiled water of soursop leaves at a dose of 3.64 mg/g BW decreased by 11 U/L so that the SGOT levels from 108 U/L to 97 U/L, the group with a dose of 7.28 mg/g BW on average SGOT levels from 110 U/L decreased by 17 U/L so that SGOT levels became 93 U/L, and the dose group 10.92 mg/g BW decreased by 19 U/L so that SGOT levels from 108 U/L to 89 U /L.

The high SGOT activity found in the positive control indicated the reactivity of rhodamine B in the process of liver cell damage which was marked by an increase in the level of the SGOT enzyme. Sulistina (2013) revealed that rhodamine B can increase the production of free radicals that cause liver tissue damage accompanied by an increase in lipid peroxidation in the liver so that high levels of SGOT will be detected in the blood. The increase in serum activity is proportional to the number of cells damaged. Lee et al., (2010) stated that if the SGOT enzyme value in serum reaches > 100 IU/L, the animal's liver tissue is damaged. Furthermore, Aleya and Berawi (2015) stated that if the SGOT level increased > 3-5 times the normal reference value, the test animals were experiencing acute hepatocellular damage.

The results of the Analysis of Variance (ANOVA) showed that giving soursop leaf boiled water had a significant effect on reducing SGOT levels in mice ($p<0.05$). Further test results showed that in each treatment group with a dose of 3.64 mg/g BW, 7.28 mg/g BW, and a dose of 10.92 mg/g BW, the negative and positive controls were significantly different. These data indicate that giving soursop leaf boiled water can prevent damage caused by exposure to rhodamine B with an indicator of decreasing levels of SGOT. Baskar et al., (2007) revealed that the antioxidant content of soursop leaf boiled water with a concentration of 1000 g/L showed maximum activity in inhibiting lipid peroxidation formed due to the presence of free radicals by rhodamine B. This antioxidant activity was caused by the presence of a specific compound known as rhodamine B. acetogenins. These compounds play an important role in inhibiting free radicals (Samin et al., 2016; Handayani et al., 2016).

According to Redha (2010), besides acetogenin, soursop leaves are also rich in flavonoid compounds, tannins, and saponins. Flavonoids are one of the chemical constituents of soursop leaves that play an important role as medicine. This substance helps endogenous antioxidants in terms of treating injuries to liver tissue caused by free radicals by acting as a reductant that donates hydrogen atoms to free radical compounds so that these compounds become stable (Uyun et al, 2016). If the formation of free radicals is inhibited, the lipid peroxidation process in cell membranes cannot be formed (Wulandari 2016). Banu et al., (2009) reported that soursop leaf boiled water has protective activity against hepatocellular due to rhodamine B induction, namely by regenerating liver tissue by increasing protein synthesis or accelerating detoxification and excretion. Usunomena (2014) and Offor et al, (2015) stated that soursop leaf decoction also showed structural integrity of protection for hepatocyte cell membranes by regenerating damaged liver cells.

Serum Glutamate Pyruvate Transaminase (SGOT) Levels

Measurement of serum SGPT levels in mice was carried out on the day of initial treatment, after rhodamine B induction and after administration of soursop leaf boiled water for each treatment group.

Table 2. Average levels of SGPT in mice (Mus muscullus) in each treatment

Kelompok Perlakuan	Kadar SGPT (U/L) ± SD			Selisih Penurunan Kadar SGPT
	Awal	Sesudah Induksi rhodamin B	Sesudah Pemberian Air Rebusan Daun Sirsak	
Kontrol Negatif	45 ± 3,00^{a}	49 ± 2,64^{a}	47 ± 2,64^{c}	-2 ± 2,00
Kontrol Positif	52 ± 2,64^{ab}	80 ± 9,16^{d}	83 ± 3,60^{d}	3 ± 17,09
Dosis 3,64 mg/g BB	45 ± 2,00^{a}	65 ± 4,58^{c}	38 ± 4,00^{bc}	- 27 ± 14,01
Dosis 7,28 mg/g BB	49 ± 6,56^{a}	62 ± 6,24^{b}	34 ± 4,58^{b}	-28 ± 14,01
Dosis 10,92 mg/g BB	45 ± 4,36^{a}	55 ± 2,64^{b}	24 ± 5,29^{a}	-31 ± 15,82

Note: Superscripts with the same letter show no significant difference, ($p < 0.05$).

Based on the results of the Analysis of Variance (ANOVA) showed that the administration of boiled water soursop leaves had an effect on reducing the levels of SGPT in

mice ($p<0.05$). The results of the follow-up test (LSD) showed that in each treatment group the doses of 3.64 mg/g BW, 7.28 mg/g BW, 10.92 mg/g BW looked significantly different in both the negative control and positive control but at a dose of 3, 64 mg/g BW was not significantly different from the dose of 7.28 mg/g BW.

In this study, the data obtained indicated that the administration of boiled water from soursop leaves could reduce the effects of damage which was indicated by a decrease in SGPT levels. Manganese (2009) stated that the antioxidant substances contained in soursop leaf boiled water in the form of flavonoids, saponins, tannins, acetogenins, calcium, phosphorus, carbohydrate, vitamin A, vitamin B, vitamin C, phytosterols, ca–oxalates and murisine alkaloids can inhibit the process of oxidative reactions caused by excessive accumulation of ROS in liver cells. Puspitasari et al (2016) also revealed that flavonoid compounds are good reducing compounds by inhibiting various oxidation reactions both enzymatically and non-enzymatically as well as being a reservoir for hydroxy radicals and superoxide that can protect membrane lipids from damage. Thus, flavonoids as active components of plants can be used traditionally to treat liver function disorders. In addition to flavonoids, Mardiana and Ratnasari (2011) stated that the boiled water of soursop leaves also contains acetogenin compounds that are useful in treating various diseases. These compounds play a role in protecting the immune system and preventing deadly infections.

Results of Observation of Mice's Liver Microanatomy Structure

In this study, the study of liver anatomy focused on the description of hepatocytes due to rhodamine B induction. From the results of microscopic preparations of the liver of the test animals, the following data were obtained:

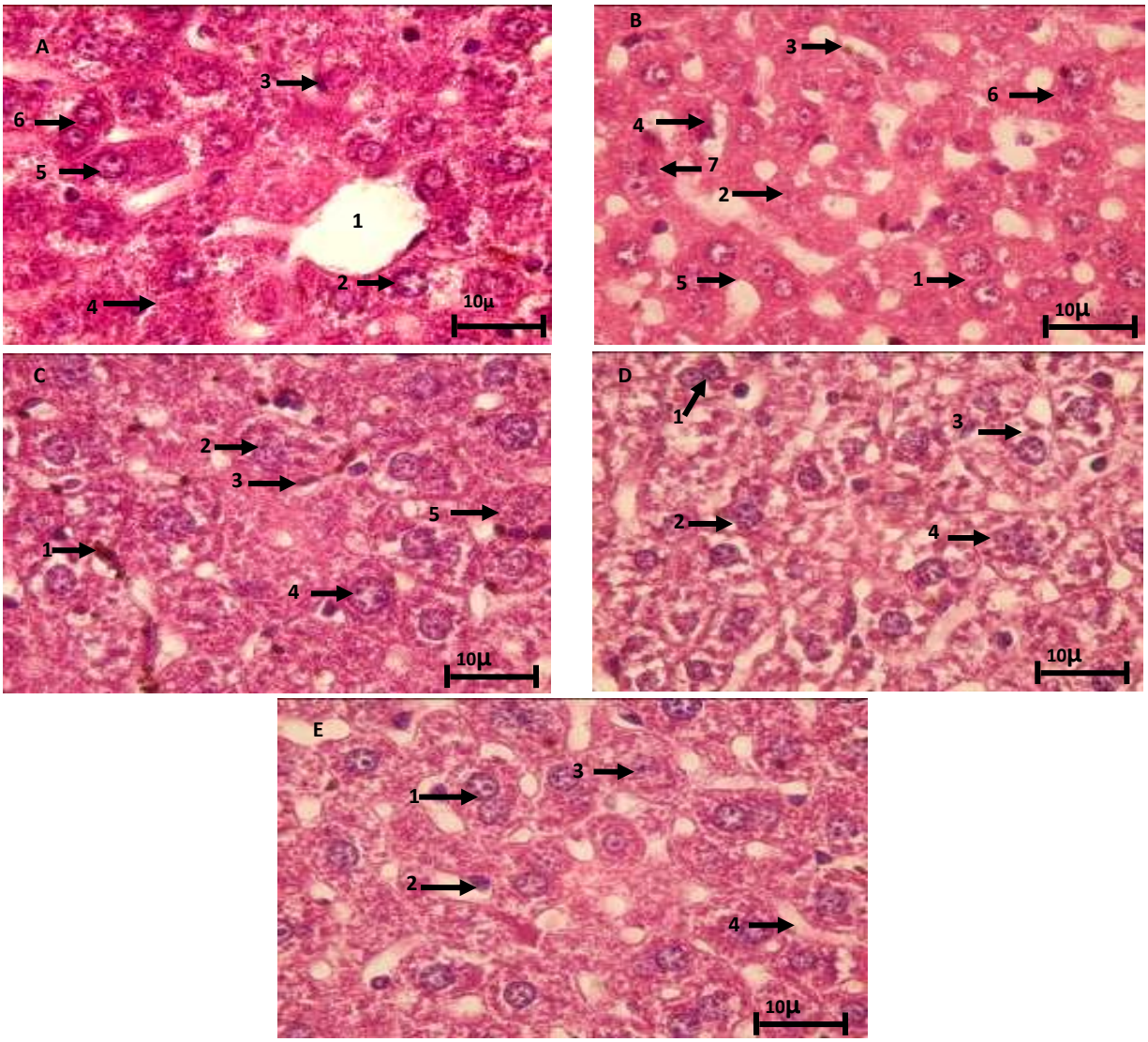

Figure 1. Photomicrograph of mouse liver cells (*Mus muscullus*) stained with Haematoxylin-Eosin (H&E) observed with an Olympus microscope at 1000x magnification. (A) Negative Control (Normal); 1. central vein, 2. hydropic degeneration, 3. Kupffer cells, 4. sinusoids, 5. hepatocyte nucleus, 6. hepatocyte binucleate (B) Positive Control (RB 0.4 mg/ml); 1. hydropic degeneration, 2. karyolysis, 3. sinusoidal congestion, 4. fat degeneration, 5. vacuolysis, 6. hepatocyte carioresis, 7. pyknosis (C) Dosage 3.64 mg/g BW; 1. Sinusoid congestion, 2. hepatocyte cariorexia, 3. Kupffer cells, 4. hepatocyte enlargement, 5. Karyolysis (D) Dosage 7.28 mg/g BW; 1. Binucleate hepatocytes, 2. Normal hepatocytes, 3. Sinusoid congestion and

erythrocyte infiltration, 4. cariorexic (E) Dosage 10.92 mg/g BW; 1. Liver cells with normal nuclei, 2. Kupffer cells, 3. hydropic degeneration, 4. Sinusoids.

Photomicrograph of liver cells, showed that the negative control group suffered damage in the form of hydropic degeneration. Cotran et al., (2003) revealed that hydropic degeneration occurs due to minor injury to the internal structures of the cell such as mitochondria and cytoplasm so that it will disrupt the cell's metabolic processes (Figure 3A). In the positive control group (Figure 3B), the damage that occurred varied, namely hydropic degeneration, karyolysis, cariorexis, pyknotic, erythrocyte infiltration, sinusoid congestion and fat degeneration. This condition also occurred in the 3.64 mg/g BW dose group, namely the damage caused by the induction of rhodamine B in the form of sinusoid congestion, cariorrhoea, karyolysis and hepatocyte enlargement (Figure 3C). The damage found in the liver incisions of mice was in the form of cell necrosis which was characterized by cariorexis, karyolysis and pyknosis. Necrosis is the loss of cell membranes and the rupture of the cytoplasm to form particles. Cell necrosis is characterized by a cytoplasmic condition that looks eosinophilic accompanied by clumping of nuclear chromatin and a shrinking nucleus (Cheville, 2006). Karyolysis (nuclear fragmentation) is the rupture of the nucleus into small parts that may be located in the original place or scattered, while karyolysis is characterized by the emptying of the cell due to the missing nucleus from within the cell, so that the cell is only an empty cavity or even if karyolysis occurs completely. then the cell will no longer be visible. Muhartono and Subeki, (2015) Pyknosis is the shrinkage of the cell nucleus so that the nucleus looks smaller than its normal size and usually cells that experience pyknosis will look dark in color. Erythrocyte infiltration can occur due to rupture of capillary blood vessels so that blood enters the sinusoids while congestion occurs due to an increase in blood volume due to widening of capillary blood vessels (Julio et al, 2013; Kumar et al, 2007). In addition to cell necrosis and erythrocyte infiltration, liver cell damage due to rhodamine B induction in experimental animals also resulted in increased fat degeneration in liver cells. Fat degeneration in liver cells can occur because the liver is the primary organ of lipid metabolism (Paul and Didia, 2012). Lesions in the form of fat degeneration can be caused by the presence of xenobiotics. Sulistina (2013), stated

that rhodamine B belongs to the organochlorine xenobiotic group where during the metabolic process it cannot be excreted properly and if it accumulates in large quantities it can cause cytotoxicity and even cell death.

In this study, cell regeneration began to be seen in the treatment of soursop leaf decoction at a dose of 7.28 mg/g BW because the damage that occurred began to decrease including erythrocyte infiltration, carioresis and sinusoid congestion (Figure 3D), while the picture of liver tissue looked normal again in the dose group was 10.92 mg/gr BW because the cells began to regenerate (Figure 3E). Cell regeneration in this treatment group is thought to be related to the content of antioxidant compounds contained in soursop leaf boiled water. This opinion is in accordance with Bermejo's research (2015) which states that soursop leaf boiled water has antioxidant activity that has a protective effect on DNA due to the induction of toxic compounds such as rhodamine B.

Cyclin D1 Expression in Mice Liver Cells Through Immunohistochemical Test

The results of observations of CD-1 expression in liver tissue were carried out by observing cells stained with brown as shown in Figure 2.

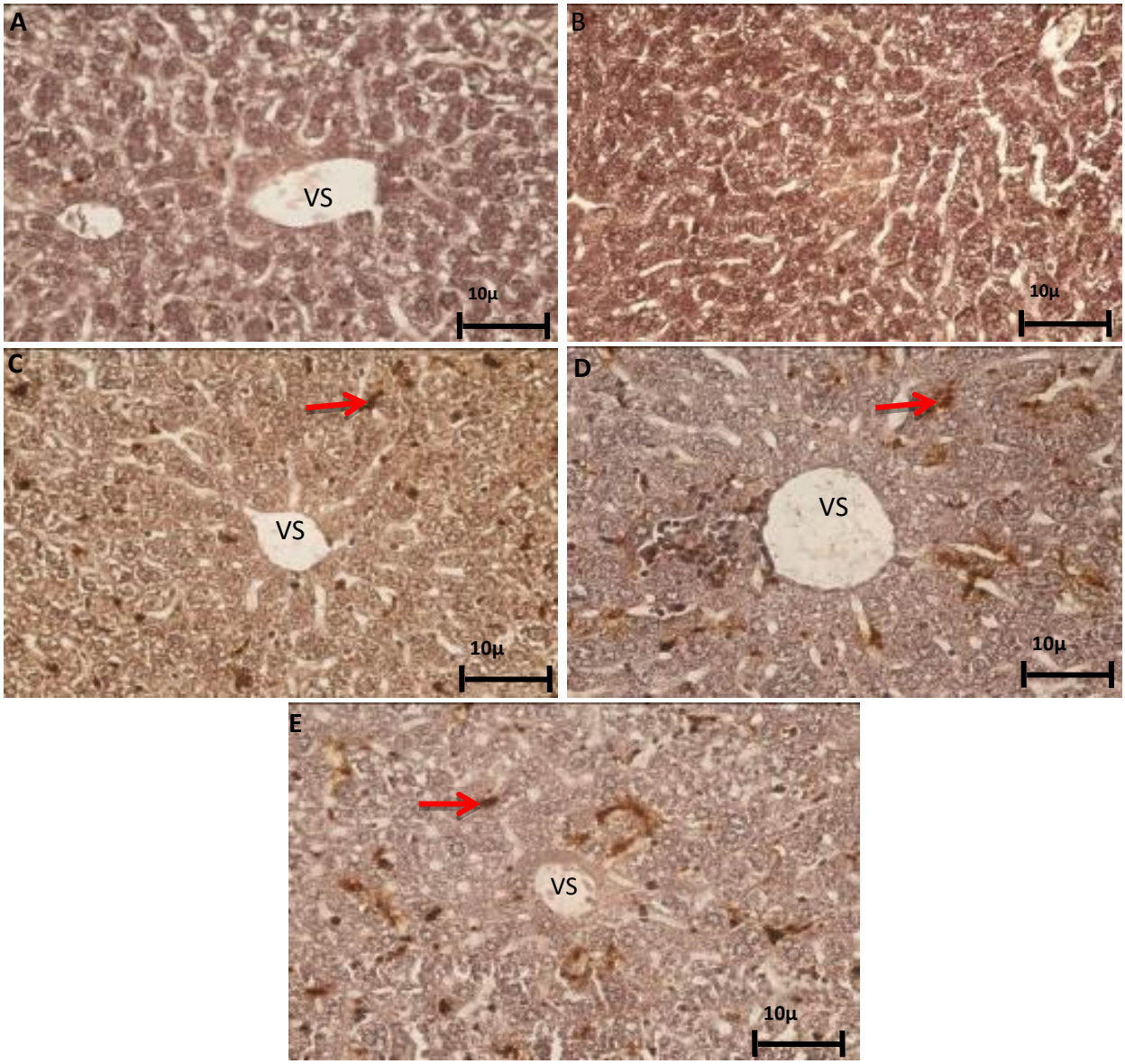

Figure 2. Histopathology of liver cells of mice (Mus muscullus) with immunohistochemistry (CPI) as observed with an Olympus microscope at 1000x magnification. (A) Negative Control, (B) Positive Control, (C) Dose 3.64 mg/g BW, (D) Dose 7.28 mg/g BW, (E) Dose 10.92 mg/g BW; () Cyclin D1 expression is marked in brown.

Based on Figure 2. shows that the negative control group (normal) shows that there is no expression of cyclin D1, the same condition appears in the positive control group (Figure 4A, 4B). In the 3.64 mg/g BW dose group, there was an expression of cyclin D1 but in a small

amount (Figure 4C) while the 7.28 mg/g BW dose group showed an increase in cyclin D1 expression (Figure 4D). Furthermore, the 10.92 mg/g BW dose group showed higher cyclin D1 expression in liver cells (Figure 4E). This indicates that a dose of 10.92 mg/g BW is an effective dose to regenerate liver cells damaged by rhodamine B induction. This regeneration of liver cells is thought to be closely related to the antioxidant activity contained in soursop leaves. Prabandari (2015) revealed that soursop leaf boiled water contains compounds with strong antioxidants, namely flavonoids, tannins, saponins, titerpenoids, coumarins and acetogenin compounds. This acetogenin compound is known to only attack cells whose growth is not normal. Flavonoids are antioxidants that are very effective in repairing and protecting cell structures. The work effectiveness of flavonoids is also supported by -tocopherol, tannins, polyphenols, saponins, and minerals such as magnesium contained in soursop leaves (Wulandari, 2016, Robbinson, 1995). Flavonoids can also inhibit the oncogenesis process in three ways, the first is by inducing apoptosis and stopping the cell cycle through the topoisomerase enzyme inhibition mechanism, inhibiting cytochrome P-450 so that carcinogenic compounds become unreactive and increasing the expression of the glutathione S-transferase enzyme which can detoxify carcinogens so that quickly eliminated from the body (Lazarus and Schmitz, 2000). In addition, according to Gunawan et al, (2014) stated that small amounts of flavonoids can trigger an antiapoptotic effect through MAP kinase inhibition which causes suppression of the JNK-c-Jun/AP-1 pathway so that it can act as an intracellular antioxidant that protects cells from ROS damage (*Reactive Oxygen Species*).

As an antioxidant, vitamin C in soursop leaves is thought to be able to reduce the activity of Reactive Oxygen Species (ROS) and increase the production of proteins that play a role in cell growth. Vitamin C is able to induce various genes that play a role in the mitotic phase of the cell cycle, including genes encoding chromosomal segregation and proteins related to spindle formation. In addition, soursop leaves also contain carbohydrates and protein. Carbohydrates and proteins are known to act as nutrients that can be metabolized to produce ATP. ATP will be used for the preparation process for DNA synthesis in the G1 phase to the S phase in the cell cycle (Duarte et al., 2008). Minerals in the form of magnesium in soursop leaves also play a role

in extracellular media to accelerate cell regeneration, especially diploid cells. The presence of magnesium in the DNA duplication process acts as a polymerase and ligase that allows the formation of mitotic spindles and cytokinesis. Magnesium is also able to trigger cell cycle activation by increasing the regulation of cyclins D1 and Cdk4/Cdk6 and downregulating p21 and p27 so that DNA and protein synthesis increases. In addition, magnesium can function as an allosteric modulator of several enzymes, stabilize DNA, trigger DNA replication and transcription, influence RNA translation, and induce ribosome assembly (Gunawan et al., 2014). In addition to magnesium, soursop leaves are also known to contain iron. An increase in iron is accompanied by an increase in ribonucleotide reductase activity. Ribonucleutide reductase is an enzyme that is responsible for reducing ribonucleotides which are precursors of DNA synthesis. The increased activity of ribonucleutide reductase will be followed by increased cell regeneration.

In abnormal cell conditions, triterpenoids from soursop leaves work by blocking the cell cycle in the G2 phase to the M phase by stabilizing the spindle threads in the mitotic phase so that the mitotic process of abnormal cells can be inhibited and cells can regenerate by carrying out a normal cell cycle (Bishayee et al. In addition, there are also a number of important substances that act as drugs and are useful for the immune system in preventing deadly infections such as annocatacin, muricapentocin, annocatalin, annomuricin, annohexocin, anomurine, anonol, annomuricin, gigantetronin, caclourine, linoleic acid and gentisic acid. (Wulandari, 2016). Based on the results of the study, it is suspected that in the presence of these secondary metabolites, liver cell regeneration can occur.

CONCLUSION

Based on the results of the study, it can be concluded that the antioxidant content of soursop leaf boiled water (*Annona muricata* L.) is able to prevent damage to mouse liver cells and increase cell regeneration power through the expression of cyclin D1.

Suggestion

Suggestions that can be conveyed by the author in this writing is that further research is needed to determine the side effects of long administration of boiled water soursop leaves (Annona muricata L.) on the liver histopathology of male balb/C mice (Mus muscullus) and for those who are exposed to it. Oxidative stress can consume soursop leaf boiled water as an alternative treatment.

REFERENCES

Adjie, S. 2011. The Power of Soursop Kills Disease. Jakarta: Mother's Library

Aleya and Khairun N, B,. (2015). Correlation of SGOT/SGPT Laboratory Examination with Bilirubin Levels in Hepatitis C Patients in the Internal Medicine Room of Dr. H Abdul Moeloek Lampung Province. Journal of Majority, Vol. 4 No. 9 (https://doi.org/10.35816/jiskh.v11i1.240).

Banu, G Sharmila, G Kumar and A G Murugesan. (2009). Effect Of Ethanolic Leaf Extract Of Trianthema portulacastrum L. On Aflatoxin Induced Hepatic Damage In Rats. Indian Journal of Clinical Biochemistry, Vol. 24 (4) 414-418 (https://dx.doi.org/10.1007%2Fs12291-009-0074-2)

Baskar R, Rajeswari, V., and Kumar S, T., (2007). In Vitro Antioxidant Studies In Leaves Of Annona Species. Indian J Exp Bio l45(5): 480-5. (https://pubmed.ncbi.nlm.nih.gov/17569293/)

Bermejo A, Figadere B, Zafra-Polo MC, et al. (2005). Acetogenins from Annonaceae: Recent Progress In Isolation, Synthesis And Mechanisms Of Action. Natural Product Reports 22(2):269-303. (https://dx.doi.org/10.1039/b500186m. Epub 2005 Mar 15)

Cheville, N. F. (2006). Introduction to Veterinary Pathology. 3rd Ed. Blackwell Publishing. USA.

Cotran, R.S., Michel, M.D., Ramzi, S. and Richard, N. (2003). Injury, Adaptation and Cell Death in Robins Pathologic Basic of Disease. Ed 7. Jakarta: EGC : 3-28

Dawile S, Fatimawali, Frenly W. (2013). Analysis of Rhodamine B Dyes in Crackers Circulating in Manado City. Pharmacon Scientific Journal of Pharmacy UNSRAT Vol.2 No.03. ISSN 2302–2493. (https://ejournal.unsrat.ac.id/index.php/pharmacon/article/view/2386)

Dayeef, M. Y, A., Setyawati K, Hidayat Sujuti. (2013). The Influence Of Annona Muricata Leaves Extract In Damaging Kidney Cell And Inducing Caspase-9 Activity. IOSR Journal of Pharmacy and Biological Sciences (IOSR-JPBS). Volume 8, Issue 5, PP 48-52. (www.iosrjournals.org)

Miranti, K, D., Laninka, A, U., Sadiah Ahmad. (2014). Effect of Soursop Leaf (Annona Muricata L) Water Extract on Body Fat Distribution in Obesity Model Wistar Male Rats. Proceedings of SNaPP Science, Technology, and Health. (http://proceeding.unisba.ac.id/index.php/sains_technology/article/view/544)

Directorate General of POM. (2014). Indonesian Pharmacopoeia. Edition V. Jakarta : Ministry of Health of the Republic of Indonesia

Eka, Reysa. (2013). The Secret to Knowing Dangerous Foods. Jakarta: Point Media Publisher

Ezejindu Dn, Udemezue Oo, Chukwujekwu Ie, Uchefuna Rc, Maduka So, Akingboye Aj, Ezejindu Cn. (2014). The Histological Effects Of Annona Muricata (Soursop). International Journal of Health Sciences and Research (https://doi.org/10.52403/ijhsr)

Gupta, S., Sekhon, L., Nabil Aziz, Ashok Agarwal., (2008). The Impact Of Oxidative Stress On Female Reproduction and ART: An Evidence-Based Review. Infertility and Assisted Reproduction. New York. 64:178-186 (https://www.levelandclinic.org/reproductiveresearchcenter/docs/publications/24_Gupta_et_al_The_impact_of_oxidative_stress_on_female_reproduction_and_ART_an_evidence_based_review.pdf)

Handayani H, Sriherfyna, H, F., Yunianta. (2016). Soursop Leaf Antioxidant Extraction Ultrasonic Bath Method (Study of Ingredients: Solvent Ratio And Extraction Time). Journal of Food and Agroindustry Vol. 4 No 1 p.262-272 (https://jpa.ub.ac.id/index.php/jpa/article/download/327/338)

Julio, E, H., Busman, and N. Nurcahyani. (2013). Histological Structure of Mice (Mus musculus L.) Liver in Response to Noise. National Seminar on Science & Technology V. Research Institute of the University of Lampung (http://repository.lppm.unila.ac.id/13471/1/27.%20Prosiding%20Saintek%20V%20Erangga%202013.pdf)

Kusuma, A, S, W., (2015). The Effect of Ethanol of Soursoup Leaves (Annona muricata L.) to Decreased Levels of Malondialdehyde. Journal Majority. 4(3)14-18. (http://juke.kedokteran.unila.ac.id/index.php/majority/article/view/543/544)

Kurniasih, Nunung., Mimin Kusmiyati, Nurhasanah, Riska Puspita Sari, Riza Wafdan. (2015). The Potential of Soursop Leaves (Annona muricata Linn), Binahong Leaves (Anredera cordifolia (Ten) Steenis), and Parasite Mango Leaves (Dendrophthoe pentandra) as Cancer-Preventing Antioxidants. Scientific Journal. Volume IX No. 1. ISSN 1979-8911

Laurence, J and Bacharach, M. (1964).. Analytical Toxicology. Philadelphia: CRC Press. pp. 125-127

Lee, S, J., Collard, R, H., Ganesh, Raghu., Matthew. P, Sweet., Steven. R, Hays., Guiherme. M, Campos., (2010). Does Chronic Microaspiration Cause Idiopathic Pulmonary Fibrosis. Am. J. Med. 123, 304–311 (https://doi.org/10.1016/j.amjmed.2009.07.033).

Manganese Y. (2009). Healthy Solutions to Prevent And Overcome Cancer. Agromedia Library : Jakarta

Mardiana, L., and Ratnasari, J. (2011). Soursop Herbs and Efficacy. Self-Help Spreader. Jakarta

Mayori, R., Marusin, N., and Tjong, D., (2013). Effect of Rhodamine B Administration on Kidney Histological Structure of White Mice (Mus musculus L.), Journal of Biology, Andalas University, 2(1), 43-49. (https://core.ac.uk/download/pdf/297218412.pdf)

Mcheik, A. H., M. M. Jamal. (2013). Kinetic Study Of The Discoloration Of Rhodamine B With Persulfate, Iron Activation.