

Hepatoprotective Activity Of Ethanolic Extract of Soursop (*Annona muricata* L.) through Review Testing of SGOT (Serum Glutamic Pyruvate Transaminase) and SGPT (Serum Glutamic Oxaloacetic Transaminase) *Rattus novergicus* Blood With Paracetamol Induced

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Abstract : Liver damage is caused by microorganisms such as viruses and bacteria, the use of drugs, alcohol, chemicals and environmental toxins. Paracetamol is widely used as an analgesic and antipyretic, can cause liver necrosis in humans. Soursop (*Annona muricata*) is a plant belonging to the familia annonaceae traditionally used to treat jaundice.

The purpose of this study was to prove Soursop leaf ethanol extract (*Annona muricata*) has a hepatoprotective effect and to know the effective dose of ethanol extract of soursop leaf that is able to protect the liver from paracetamol induction. Stages of research extract ethanol soursop leaves, giving treatment of extracts performed for 7 days in a row. Subsequent administration of paracetamol with a dose of 2.5 mg / kg BW on day 8 as inducer of liver damage. Further measurements of SGOT and SGPT levels of paracetamol-induced rat blood.

The results of this study indicate that there is a significant difference between treatment on negative control and treatment group given ethanol extract of soursop leaf where ethanol extract of soursop leaf with dose 100 mg / kgBW showed maximum result compared with dose 200 and 400 mg / kgBW but not different significant. So it can be concluded that ethanol extract of soursop leaf with dose of 100, 200 and 400 mg / kgBW has hepatoprotective effect against paracetamol-induced rats and ethanol extract of soursop leaf with dose of 100 mg/kg BW is the most effective dose in protecting rat liver from exposure paracetamol.

Keywords : Soursop, ethanolic extract, hepatoprotective, SGOT, SGPT, Paracetamol.

Introduction

The liver is one of the complex organs consisting of liver cells (hepatocytes) that play a role in the metabolism of nutrients, drugs and toxins.¹ Disease caused by damage or impaired liver function is a major problem in the world of health. According to WHO Data in 2013, liver disease affects hundreds of millions of people worldwide, causing acute and chronic illness and close to 1.4 million people die each year.²

Liver damage is caused by microorganisms such as viruses and bacteria, the use of drugs, alcohol, chemicals and environmental toxins.³ The chemical substances that can cause liver damage (hepatotoxicity) one of them is paracetamol (acetaminophen).⁴

Paracetamol is widely used as analgesic and antipyretic, can cause liver necrosis in humans.^{5,6} Paracetamol produces a toxic metabolite known as N-acetyl-p-benzoquinoneimine (NAPQI) that can deplete cellular glutathione (GSH) and form covalent bonds with macromolecules Tissues resulting in liver damage.^{7,8}

Soursop (*Annona muricata*) is a plant belonging to the familia annonaceae traditionally used to treat jaundice. A study conducted by Arthur, *et al* (2012)⁹ with *in vivo* method to determine the potential of *A. muricata* leaf water extract on decreasing bilirubin levels in rat blood. The results of the extract led to a significant decrease in hyperbilirubinemia of mice to nearly normal limits. Another study also conducted by Arthur *et al.* (2012)¹⁰, concluded that *A. muricata* leaf water extract had a hepatoprotective effect on histologically induced liver images of carbon tetrachloride and acetaminophen. The experiment was conducted using different concentration of extract extracts (50, 100, 200, and 400 mg / kg BW) for 7 days, where the concentration of extracts showing hepatoprotective effect was 100, 200, and 400 mg / kg BW.

Soursop is known to have high annonaceous acetogenins content.¹¹ Acetogenin is known to have high antioxidant activity. Lucius *et al* (2010)¹² study using the DPPH radical-scavenging activity method showed that acetogenin activity of the annonaceae family had high antioxidant activity proved by IC₅₀ which had the same level as ascorbic acid. The high antioxidant effects of acetogenin are thought to be correlated with their hepatoprotective effects. Because antioxidants can capture free radicals that can cause oxidative stress in the body. Oxidative stress can cause peroxidasilipide so that it can cause cell damage and cause degenerative diseases, such as liver disease.¹³

Liver damage is always associated with cellular necrosis, increased tissue peroxidation and thinning at the tissue GSH level, whereas at serum levels the damage can be determined by increasing levels of SGOT, SGPT, ALP and bilirubin.¹⁴ Research using high-dose chemical inducers will result in acute liver damage that can be seen clearly from elevated levels of SGPT (Serum Glutamic Pyruvate Transaminase) and SGOT (Serum Glutamic Oxaloacetic Transaminase).

Experimental

Material

The ingredients used were Soursop leaves (*Annona muricata* L.) obtained in Lambaro, Ingin Jaya sub district Aceh Besar, Indonesia, ethanol 96%, Na CMC 0.5%, aquadest, chloroform and inspection kit SGOT / SGPT.

Animal Test

The animals used in this study were White Rats (*Rattus norvegicus*) male Wistar strain. Rats were taken as many as 25 tail with age 12-16 weeks with body weight 150-250 grams. Obtained from the Faculty of Veterinary Medicine of Syiah Kuala University of Banda Aceh. Indonesia

Preparing extracts

Simplicia of 200 g is inserted into a vessel. Added 1500 mL of ethanol liquid of the dancer, then closed. Then left for 5 days protected from light while stirring, diserkai, and squeezed. Then wash the dregs with liquid enough to get 2000 mL. After that it is transferred into a closed vessel, left in a cool place, protected from light for 2 days. Then it is precipitated, poured, then filtered. The whole maserate is collected, then the vapor with the vapor evaporator vapor rotary to obtain the viscous extract.

Preparation of 500 mg paracetamol suspension

The inducer of hepatotoxin to be used in this study was paracetamol with a dose of 2.5 g/kg BW of Paracetamol rat crushed until smooth. Then suspended in 0.5% CMC which has been made as much as 25 mL in a measuring flask.

Treatment dan Measurement of SGOT and SGPT level

25 male white rats in divided into 5 groups, each group consists of 5 tails. All the rats were coded and weighed. Rats are fasted for 18 hours and are only given a drink. Then the whole group was given treatment:

1. Group 1: Group I rats were given 1% (oral) CMC for 7 consecutive days and followed by 8 hours (oral) aquades after CMC 1% day 7 (normal control)
2. Group 2: Group II Rats were given 1% (oral) CMC for 7 consecutive days and followed by paracetamol

- dose 2.5 g / kg BW 8 hours (oral) after 7 days CMC (negative control)
3. Group 3: Rats of group III were given Soursop ethanol extract at doses of 100 mg / kg BW (oral) for 7 consecutive days and on day 7 given paracetamol dose 2.5 g / kg BW (oral).
 4. Group 4: Rats group IV were given Soursop ethanol extract at doses of 200 mg / kg BW (oral) for 7 consecutive days and on day 7 given paracetamol dose 2.5 g / kg BW.
 5. Group 5: Rats group V was given Soursop ethanol extract at doses of 400 mg / kg BW for 7 consecutive days and on day 7 given paracetamol dose 2.5 g / kg BW.

Blood collection is done 24 hours after administration of paracetamol. Blood is taken by direct withdrawal to the orbital sinus of an already drugged rat with chloroform using a syringe. Blood samples were collected by accommodating in an EDTA tube. The blood is then taken to the Hematology Laboratory of Harapan Bunda Banda Aceh for examination of its SGPT and SGOT levels.

Result and Discussion

SGOT and SGPT Level Serum Analysis

The *A.muricata* hepatoprotective effect test was performed by administering the Oral suspension for 7 consecutive days with a 24 hour interval. The doses used vary by 100, 200, and 400 mg / kgBW. The reason for giving extracts for 7 consecutive days before induction of hepatotoxin induction is to provide a protective effect (prevention) from liver damage that will be induced by inducing hepatotoxin. The inducer used was paracetamol at a high dose of 2.5 g / kgBW administered 8 hours after administration of the extract on the seventh day, after which 24 hours of rat blood was taken via mouse orbital sinus using hematocrit pipette. Blood that has been collected is then tested using a spectrophotometer to determine the levels of SGOT and SGPT mice. From the above treatment obtained results SGOT and SGPT levels of rat blood as follows table No 1.

Table 1. SGOT and SGPT levels of Rat blood

Group	Treatment	Average Treatment Group SGOT (U / I)	Average Treatment Group SGPT (U / I)
I	Normal Control	152,42±19,13	74,22±6.28
II	Negative Control	352,52±109,61	200,84±93,69
III	Dose of extract 100 mg / kgBW	194,14±7,91	87,86±3,75
IV	Dose of extract 200 mg / kgBW	219,94±7,28	99,120±3,01
V	Dose of extract 400 mg / kgBW	241,40±9,68	119,12±12,11

Based on the test results using ANOVA shown in the table and above graph, SGOT and SGPT value in group II (negative control) increased compared to group I (normal control). This indicates that paracetamol already gives damage effect on rat liver, but in treatment group III, IV and V (extract dose 100 mg / kgBW, 200 mg / kgBW and 400 mg / kgBW) SGOT and SGPT levels showed decrease compared to group II Although not yet equal to SGOT and SGPT group I which can be interpreted that giving *A.muricata* able to reduce liver damage caused by paracetamol.

SGOT level of each groups were then tested using the Tuckey HSD test to see significant differences in each group. From the test using probability value <0,05, it was found that between group I and group II showed significant difference with probability 0.000, but group I did not show significant difference with test group *A.muricata* III, IV and V. In addition there were significant differences Between group II with groups III, IV, and V with a probability value of 0.001 and 0.001, respectively. Meanwhile, in the extract treatment groups (III, IV, and V) did not show significant differences with each other.

SGPT level of each groups were then tested using the Tuckey HSD test to see significant differences in each group. From the test, it was found that between group I and group II showed significant differences with the probability value of 0.001, but group I did not show significant differences with groups III, IV, and V. In

addition there were significant differences between group II and group III, IV, And V with value of probability respectively that is 0,003, 0,009 and 0,045. Meanwhile, in the extract treatment groups (III, IV, and V) did not show significant differences with each other

Discussion

Based on the measurements of SGOT and SGPT levels of rat blood above, it can be seen that the negative control group showed significantly higher levels of SGOT and SGPT than in the normal control group. This proves that paracetamol has been able to show hepatotoxic activity. Paracetamol activity that produces hepatotoxic effects is mediated by the resulting metabolite N-acetyl-p-benzoquinoneimine (NAPQI) that can deplete cellular glutathione (GSH) and form covalent bonds with liver tissue macromolecules causing damage, but in normal groups there is also elevated levels SGOT and SGPT, where the literature states that normal SGOT and SGPT levels of rats are 45.7-80,8 U / l and 17,5-30,2 U / l.²³ Increase in SGOT and SGPT levels in the normal group of possibilities Occurs due to a fight between rats during adaptation. Fighting may cause trauma to the skeletal muscle of rats because in addition to the liver, SGOT and SGPT are also found in skeletal muscle,²³ or due to abnormalities in liver organ suffered by previous rats. Therefore it is advisable to conduct further research, especially by looking at histopathology in rat liver organ.

All extract test groups showed hepatoprotective effect on rat liver organ, but not yet showed maximum protection effect because not yet reached SGOT and SGPT value of existing mice in normal group. This is probably due to the dose of *A.muricata* not at its optimum dose. Related to it can also be seen in the above results where the smallest dose of 100 mg / kgBW gives better hepatoprotective effect than the doses of 200 and 400 mg / kgBW which is characterized by low SGOT and SGPT values of rat blood, so the principle of increasing the dose is directly proportional With an increase in effect is not absolute, or can be said in this study increased dose inversely with the resulting effect. This is probably caused by the active compound content of *A.muricata* antagonist work together in the presence of antagonists where the higher levels of these compounds, the nature of mutually exclusive activity of each other higher, so it may be necessary to screen the active compounds contained in *A.muricata* so that its hepatoprotective effect will be even better.

The extraction of *Annona muricata simplicia* powder was performed in maceration using 96% ethanol solvent. The reason for the use of the solvent is due to the nature of the ethanol which can attract almost all the metabolite compounds in both polar and nonpolar simplicia. Based on the leaf literature *Annona muricata* contains carbohydrate, phenolic, flavonoid, alkaloid, coumarin, glycosies, phytosterols, quinones, steroids, proteins, saponins and terpenoids.¹⁶

The hepatoprotective effect *A.muricata* may be mediated by the chemical content of annonaceous acetogenins. Acetogenin is known to have high antioxidant activity. The high antioxidant effects of acetogenin are thought to be correlated with their hepatoprotective effects. Because antioxidants can capture free radicals that can cause oxidative stress in the body. Oxidative stress can cause peroxylipide so that it can cause cell damage and cause degenerative diseases, such as liver disease.¹³

Antioxidant compounds such as phenolics contained in *A.muricata* also have free radical neutralizing activity produced by paracetamol. The occurrence of liver damage due to the formation of bonds between the liver macromolecules with intermediary paracetamol metabolites undergoing biotransformation in the liver.¹³

Conclusion

Based on the research that has been done can be concluded that Soursop leaf ethanolic extract at doses of 100, 200, and 400 mg / kgBW had hepatoprotective activity in rat-induced liver paracetamol through SGOT and SGPT examination of the mice marked by lower levels of both compared with negative controls. The dose of ethanol extract of soursop leaves of 100 mg/kgBW showed the most effective hepatoprotective activity characterized by SGOT and SGPT levels of mice closest to SGOT and SGPT levels in the normal group.

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