Soursop leaves (*Annona muricata Linn*) ethanol extract prevents cisplatin-induced kidney injury of mice (*Mus musculus*) through SOD and MDA analysis

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**ABSTRACT**

**Background:** Cancer treatment has been carried out in various ways, including surgery, chemotherapy and immunotherapy. Cisplatin is often used for chemotherapy, but the use of cisplatin still has extensive side effects, including kidney injury. The ethanol extract of soursop leaves contains antioxidants expected to reduce ROS or induce SOD, glutathione and catalase, thereby preventing kidney injury. This study aims to explain the effect of soursop leaves ethanol extract on preventing kidney injury caused by cisplatin administration in animal models.

**Method:** This study is a true experimental study with a randomized, posttest-only control group design. We used 40 mice and randomly divided them into 5 groups, each group containing 8 mice. The K0 group was given aquadest, the K1 group was given cisplatin 20 mg/kgBW, the KP1 group was given cisplatin and soursop leaf ethanol extract 100 mg/kgBW, the KP2 group was given cisplatin and soursop leaf ethanol extract 200 mg/kgBW, the KP3 group given cisplatin and soursop leaves ethanol extract 400mg/kgBW. The extract was given orally for 5 days in a row. MDA and F2-isoprostane were measured using Immunohistochemistry. The data were all analyzed using an ANOVA test.

**Results:** Soursop leaves ethanol extract with 200 mg/kgBW is the most effective dose to prevent kidney injury from cisplatin exposure. Soursop leaves ethanol extract can increase the expression of SOD and decrease the MDA metabolites.

**Conclusion:** Soursop leaves ethanol extract can prevent kidney injury by increasing SOD and decreasing MDA.

**Keywords:** kidney injury, cisplatin, soursop extract, MDA, SOD.


**INTRODUCTION**

Cancer is the second leading cause of death worldwide after cardiovascular disease. Approximately 19.3 million new cancer cases appear worldwide, and about 10 million cancer deaths occurred in 2020. Overall, the burden of cancer and mortality is growing rapidly worldwide, including Indonesia. The prevalence of cancer in Indonesia showed an increase from 1.4 per 1,000 population in 2013 to 1.7 per 1,000 population in 2018.1 According to Global Burden of Cancer Study (2020), the new total cases of cancer in Indonesia reached 396,914 cases with a total death case is 234,511 cases.² Cancer treatment has been carried out in various ways, including surgery, chemotherapy and immunotherapy. Cisplatin is often used for chemotherapy, but the use of cisplatin still has extensive side effects, including kidney injury.³ According to the data obtained from several hospitals in Indonesia, cisplatin is used as a combination chemotherapy.¹⁶ Given the large number of cancer patients receiving cisplatin combination therapy, the incidence of kidney failure will increase if this is not given attention.

Kidney cells exposed to cisplatin will increase the production of reactive oxygen species (ROS).⁷ Anti-ROS will neutralize reactive oxygen species in kidney cells through the mechanism of radical superoxide (O₂⁻) converted into hydrogen peroxide (H₂O₂) by superoxide dismutase (SOD), whereas H₂O₂ is converted into H₂O by glutathione.⁸ If there is a decrease of anti-ROS, including SOD, catalase and glutathione, the cell will experience oxidative stress resulting in cell death characterized by malondialdehyde (MDA) and F2-isoprostane expression. It is necessary to prevent cell death by giving anti-reactive oxygen species (ROS).⁹

One of the ingredients that contain anti-ROS is soursop leaves. Soursop (*Annona muricata Linn*) is a plant with high antioxidant content, especially in the leaves and fruit. Soursop leaves contain acetogenin, tannin, phytosterol, calcium oxalate, murisin alkaloid, flavonoid and steroid.¹⁰ According to the previous study, soursop leaves water extract contains many active compounds, including alkaloid, acetogenin terpenoid, flavonoid, phenolic compounds, tannin and saponin.¹⁰ In vivo studies showed that soursop leaf extract could prevent the damage of pancreatic beta cells caused by oxidative stress and lower blood glucose levels.¹¹ To prevent kidney injury-cisplatin-induced, we proposed using soursop leaves ethanol extract, which contains antioxidants and is expected to reduce ROS or induce...
the increase of SOD, glutathione and catalase. This study aims to explain the effect of soursop leaves ethanol extract on preventing kidney injury caused by cisplatin administration in animal models.

METHODS

This study is a true experimental study with a randomized, posttest-only control group design. We used mice (Mus Musculus) as animal models. According to the sampling technique, we conducted 40 mice and randomly divided them into 5 groups, each group containing 8 mice. The K0 group was given the aquadest. The K1 group was given cisplatin 20 mg/kgBW. The KP1 group was given cisplatin and soursop leaf ethanol extract 100 mg/kgBW. The KP2 group was given cisplatin and soursop leaf ethanol extract 200 mg/kgBW. The KP3 group was given cisplatin and soursop leaf ethanol extract 400 mg/kgBW. The extract was given orally for 5 days in a row. MDA and F2-isoprostane were measured using Immunohistochemistry. The data were all analyzed using an ANOVA test.

RESULTS

Effect of soursop leaf ethanol extract on SOD

The results of SOD measurement are listed in Table 1. Data from the five experimental groups showed the highest SOD expression in the KP3 treatment group, giving 20 mg/kgBW of cisplatin and 400 mg/kgBW of soursop leaf ethanol extract. In contrast, the lowest SOD expression was found in the negative control group (K1), only cisplatin 20 mg/kgBW.

According to the normality and homogeneity test, it was found that the data were normally distributed so that the data presented as mean±SD. However, the data obtained was not homogenous, so the comparison test was carried out for the entire group using the Brown-Forsythe test. The result is there is a significant difference in SOD expression between the entire group (p<0.05), so it can be stated that at least one treatment of soursop leaf (Annona muricata Linn) ethanol extract affected increasing the number of kidney cells that expressed SOD. Then, a comparison test was conducted between the treatment and control groups using an independent t-test. It was found that there was a significant difference in SOD expression between the negative control group (K1), which was given 20 mg/kgBW of cisplatin and the KP2 group, given cisplatin 20 mg/kgBW and 200 mg/kgBW of soursop leaf ethanol extract and KP3 group that given cisplatin 20 mg/kgBW and 400 mg/kgBW of soursop leaf ethanol extract. Meanwhile, there is no significant difference between the normal control group and the KP1 group given cisplatin 20 mg/kgBW and 100 mg/kgBW of soursop leaf ethanol extract. There is a significant difference in SOD expression between the negative control group (K1) that was given cisplatin 20 mg/kgBW only with KP1 group that was given cisplatin 20 mg/kgBW and 100 mg/kgBB of soursop leaf ethanol extract.

Effect of soursop leaf ethanol extract on MDA

The results of MDA measurement are listed in Table 2. Data from the five experimental groups showed the highest MDA expression in the KP1 treatment group, giving 20mg/kgBW of cisplatin and 100 mg/kgBW of soursop leaf ethanol extract. In contrast, the lowest MDA expression was found in the normal group (K0), which is only given distilled water. According to data analysis, the results of this study showed that the administration of soursop leaf ethanol extract could increase the number of kidney cells that expressed MDA metabolites. Then, a comparison test was conducted between the treatment and control group using an independent t-test, and it was found that there was a significant difference in MDA expression between the negative control group (K1) given 20 mg/kgBW of cisplatin and the normal control group (K0) that given distilled water. Also, there is a significant difference between the KP2 group that was given cisplatin 20 mg/kgBW and 200 mg/kgBW of soursop leaf ethanol extract and the KP3 group that was given cisplatin 20 mg/kgBW and 400 mg/kgBW of soursop leaf ethanol extract. Meanwhile, there is no significant difference between KP1 given cisplatin 20 mg/kgBW and 100 mg/kgBW of soursop leaf ethanol extract.

DISCUSSION

The effect of soursop leaf ethanol extract on SOD expression

According to data analysis, the results of this study showed that the administration of soursop leaf ethanol extract could increase the number of kidney cells that expressed SOD.

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Note: *significance, α=0.05 (Brown-Forsythe test)

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expressing SOD. The 200 mg/kgBW dose is optimal for increasing the number of kidney cells expressing SOD.

The results of this study are similar to those of Palawe et al. (2021), who concluded that soursop leaf aqueous extract has an effect as a hepatoprotector because it can increase SOD levels and reduce liver MDA levels.12

Superoxide dismutase is a metalloenzyme that catalyzes the reduction reaction of the superoxide anion radical (O$_2^-$) into hydrogen peroxide (H$_2$O$_2$) and oxygen (O$_2$). This enzyme is unstable to heat, quite stable under alkaline conditions, and still has activity even though it is stored for up to 5 years at 5°C. The highest SOD activity was found in the liver, adrenal glands, kidneys, blood, spleen, pancreas, brain, lungs, stomach, intestines, ovaries, and thymus.13

**Superoxide Dismutase (SOD)** is one of the body's enzymatic antioxidants and metalloenzymes because its activity depends on metal cofactors such as Cu, Fe, Zn, and Mn. Based on this, SOD is grouped into Cu/Zn-SOD, Mn-SOD, Fe-SOD, and EC-SOD. Cu/Zn-SOD is found in the cytosol, chloroplasts of higher plants and possibly extracellularly. Mn-SOD is located in the mitochondria of eukaryotic cells and peroxisomes. Fe-SOD was found bound to chloroplasts. And also EC-SOD found in mammalian extracellular fluids.13

The degree of activity of each SOD is influenced by the degree of oxidative stress in the subcellular compartment. The action of the SOD enzyme can be seen in the number of lipid peroxidation products from each organelle. The low lipid oxidation products will illustrate the high activity of SOD.13

**The Effect of Soursop leaf ethanol extract on MDA expression**

This study's results show that administering soursop leaf ethanol extract can decrease the number of kidney cells expressing MDA metabolites. Soursop (*Annona muricata Linn*) is a plant that has high antioxidants, especially in its leaves and fruit. Soursop leaves contain acetogenin, tannin, phytosterol, calcium oxalate, murisin, alkaloid, flavonoid, and steroid.9

Tanin is a polyphenol compound proven to be an antioxidant that inhibits free radicals through the oxidation process of the electron transport chain.14

Flavonoid compounds can prevent damage caused by free radicals and stabilize ROS, which can bind with free radicals that can cause degenerative diseases. Flavonoid act as antioxidants because it has hydroxyl groups that can donate hydrogen atoms to free radical compounds and stabilize ROS.15

Hydrophilic degeneration is damage caused by exposure to toxic substances such as cisplatin. It can increase free radicals and disrupt the metabolism inside cells. The formation of ROS can damage lipid membranes into lipid peroxides, damage phospholipids from cell membranes, and lipoproteins by spreading the chain reaction.16 Lipid peroxidation impairs cellular function in two ways, i.e., impairing membrane function and forming reactive aldehydes/malondialdehyde (MDA) and F2 Isoprostane.8

Malondialdehyde (MDA) is an enzymatic product from the breakdown of prostaglandin endoperoxide and the end product of lipid peroxidation. MDA is a reactive molecule with the molecular formula C3H4O2 and is known as a marker of lipid peroxidation.17 Lipid peroxidase can damage the membrane structure, causing changes in permeability, inhibiting metabolic processes and changes in ion transport. Measuring the level of lipid peroxidation is done by measuring the final product, one of which is MDA. Malondialdehyde (MDA) is a highly reactive compound, and accumulation of MDA is an early indicator of cell and tissue damage mechanisms. Researchers have widely used the measurement of MDA as an indirect index of oxidative damage caused by lipid peroxidation.17

The principle of MDA measurement is the reaction of 1 molecule of MDA with 2 molecules of thiobarbituric acid (TBA) to form a complex MDA-TBA compound which is pink in color and the quantity can be read with a UV-Vis spectrophotometer at a wavelength of 532–533 nm.15

This study proved that the administration of ethanol extract of soursop leaves could reduce the number of kidney cells expressing MDA, resulting from lipid peroxidation. The antioxidant effect of soursop leaf ethanol extract is mainly due to its high flavonoid content. Flavonoids are polyphenolic compounds consisting of 15 carbon atoms configured C6-C3-C6, which means that the carbon skeleton consists of two C6 groups (substituted benzene rings), which are then donated by a three-carbon aliphatic chain.18

Flavonoids can prevent damage caused by free radicals and stabilize ROS that can bind to free radicals that cause degenerative diseases by deactivating free radicals. Flavonoids act as antioxidants because they have hydroxyl groups, which can donate hydrogen atoms to free radical compounds and stabilize reactive oxygen compounds (ROS) and have ketone hydroxyl groups, which have a role as metal chelators that function as catalysts in lipid peroxidation.19,20

The results of this study are similar to those of Palawe et al. (2021), who concluded that soursop leaves have an effect as a hepatoprotector, contain antioxidant flavonoids and can increase SOD levels and reduce liver MDA levels. The results of this study are also similar to those of Tazkia et al. (2019), who stated that soursop leaf water extract increased SOD levels and reduced hepatic MDA levels of Wistar rats induced by a high-fat and high-fructose diet.21-24

This study did not examine the histology of the animal model’s kidney, so how much the ethanol extract of soursop leaves can repair kidney injury is unknown. Further research needs to be carried out to see the histopathological appearance of the kidney given cisplatin and ethanol extract of soursop leaves. Further research is needed to study the bioavailability of soursop leaf ethanol extract.

**CONCLUSION**

The administration of soursop leaf ethanol extract can increase the number of kidney cells that express SOD in animal models given cisplatin. The administration of soursop leaf ethanol extract can also reduce the number of kidney cells that show MDA metabolites. Ethanol extract of soursop leaves at 200 mg/kg BW is the most effective dose for preventing damage to kidney cells exposed to cisplatin. Ethanol extract of soursop leaves prevents...
kidney cell injury exposed to cisplatin through increasing SOD and decreasing MDA metabolites.

CONFLICT OF INTEREST
The authors report no conflict of interest.

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ETHICAL APPROVAL
This research protocol received ethical approval from the animal care and use committee, Faculty of Veterinary Medicine, Airlangga University No: 2.KEH.004.01.2023

AUTHOR CONTRIBUTION
All authors contributed to this research. IMS and DS: Designing the research concepts and formulating research objectives. IMS: Coordinating all data collection and management, data validation, visualization, and analysis, and writing the initial draft of the article for publication. DA: coordinating technical and supervising the conduct of the research, IKS: supervising the research, editing and correcting the final article for publication.

REFERENCES