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Research Article

Effect of Andaliman (*Zanthoxylum acanthopodium* DC.) Methanol Extract on Rat's Kidney and Liver Histology Induced by Benzopyrene

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Abstract

Background and Objective: The andaliman fruits have anti-inflammatory, antioxidant activity and a strong inhibition in antitumor activity. The purpose of this study was to analyze the effect of extract andaliman on rat's kidney and liver histology induced by benzopyrene.

Materials and Methods: The rats model of cancer-induced benzopyrene. This research consists of 5 groups; K⁻: Control, K⁺: Cancer model rats, P₁: A dose of 100 mg per b.wt. per day of andaliman, P₂: A dose of 200 mg per b.wt. per day and P₃: A dose of 400 mg kg⁻¹ b.wt., per day for 30 days. On the 31st day, performed surgically on the subjects. **Results:** There were significant differences in the value of narrowing of the renal tubules (p<0.001), kidneys cells necrosis (p<0.01), hydrophilic degeneration (p<0.001), parenchymatous degeneration (p<0.01) and necrosis (p<0.001) in the liver after given the extract andaliman. **Conclusion:** Andaliman methanol extract repairs the damage of the liver and kidney of rats induced by benzopyrene. Andaliman can be recommended as a drug to repair the necrosis in the liver and kidneys caused by cancer.

Key words: Andaliman, cancer, liver, kidneys, benzopyrene, rats, zanthoxylum

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Andaliman is a unique spice from the province of North Sumatra, Indonesia. Andaliman extract contains alkaloids, steroids and terpenoids having antimicrobial activity against insects, bronchitis drugs, dyspepsia, antiviral, anticonvulsants, antifungals, analgesics, antibiotics, hepatoprotective agents, cancer and preeclampsia¹⁻⁶. The composition of andaliman is also used as an antioxidant can be presumed as a barrier to the growth of cancer cells^{7,8}. Andaliman can also have anti-inflammatory and antioxidant activity. The essential oil of *Zanthoxylum* showed there is a strong inhibition of the fungus growth and antitumor activity⁹⁻¹¹. The fruit Andaliman (*Zanthoxylum acanthopodium* DC.), is a spice in the Batak community, which can eliminate the fishy smell of raw meat and fish^{12,13}.

The utilization of traditional medicine in the treatment of humans has become the culture of society because it is proven to cure the disease. Therefore, traditional drugs of plant origin in Indonesia still need to be explored, researched, developed and inventoried¹¹⁻¹³. Research needs to be done about the plant that has potential as medicine and knowledge about the active ingredients contained in plants as well as the function and process in the body¹⁴⁻¹⁶. In addition to easily obtained, traditional medicine derived from plants generally has no side effects and the price is relatively more easily when compared with synthetic drugs¹³⁻¹⁷. The local potential that is beneficial to health there is still many who have not been assessed. Many herbs have been studied that have health benefits and one of them is plants Andaliman both fruit and leaves¹⁴⁻¹⁷.

The use of herbal medicine has been more developed and advanced rapidly, mainly due to the content of bioactive substances Andaliman recommended as a treatment for cancer and other diseases^{10,18}. For it is necessary to study whether the use of Andaliman is safe for the kidney and liver then how the standard recommended dose.

The purpose of this study was to analyze the effect of extract andaliman on rat's kidney and liver histology induced by benzopyrene, to see the safety effects of this herb to kidneys and liver in the development of cancer drugs.

MATERIALS AND METHODS

Study area: This study used 30 Wistar rats from the Animal House of Biology Laboratory, the University of Sumatera Utara (USU), Medan, Indonesia. This research project was conducted from February, 2019-January, 2020 in

Physiology Laboratory, Anatomical Pathology Laboratory and Biochemistry Laboratory, University of Sumatera Utara, Medan, Indonesia.

Andaliman extract methanol: The Andaliman extract methanol. The fruit Andaliman used comes from the Bukit Gibeon Sibisa Parapat, District of North Sumatra. Andaliman is washed, then andaliman dries at room temperature for 3 days and blended until smooth. After that, the manufacture of the extract of andaliman with three steps as follows below:

- Drying of the crude drug: the fruit of andaliman cleaned and drained dry, then mashed with the blender
- Manufacture of andaliman extract: Powder, the fruit of andaliman macerated with methanol 96% for one night. The results of maceration and percolation botanicals to achieve a clear liquid. The results of the percolation concentrated with the evaporator until obtained the extracts are concentrated
- The manufacture of pharmaceutical suspension: given the extract of andaliman is used partly do not dissolve in water, to obtain a homogeneous mixture used a suspending agent CMC 1.5% as much as 1.0% or 1 mL in 150 mL of distilled water. The drugs are washed with solvent methanol 96% and then transferred in a closed container and left into a cool place protected from light for 2 days¹⁷

Animal: This research used 30 *Rattus norvegicus*, rats were taken and maintained in the Animal House Laboratory, University of Sumatera Utara Medan, Indonesia. The rat was made with the animal model of cervix cancer by inducing benzopyrene 50 mg b.wt. and let growing cancer until three months later¹⁹. The rats were divided into five groups. Group A was a control group, Group B was treated with a dose of 100 mg b.wt., group C was the treatment with a dose of 200 mg b.wt., group D was the treatment of a dose of 300 mg b.wt. and group E has treated a dose of 400 mg b.wt., of andaliman extract during 30 days administration^{10,20}. Rats dissected on day 30 after administration of andaliman extract, for blood, kidney and liver were taken and then the kidneys and livers were prepared for paraffin blocks and Haematoxylin Eosin (HE) staining.

Data analysis: Data calculated with the average change of the histopathological in the kidney and liver from five of the field view with the Manja Roenigk Histopathology Scoring model. Data analyzed with used ANOVA test and non-parametric data with the Kruskal Wallis test by using SPSS 22 program.

RESULTS

Histology of kidneys rats after given andaliman methanol extract:

There was a significant difference in the tubulous renal narrowing in a positive control group with negative control ($p < 0.001$). The administration of andaliman affected tubular constriction and the narrowing of tubules in the kidney in the benzopyrene-injection and treatment groups (P_1 , P_2 and P_3) had valued the same statistic (non-significant) compared to the K^+ group ($p > 0.05$) (Table 1). The narrowing tubules had most in the K^+ and P_1 groups, while the least in the K^- group (Fig. 1a-e).

There was a significant difference in the tubulous renal narrowing, between the positive control group with a negative control group ($p < 0.01$). However, an insignificant difference between the treatment of andaliman extract of dose P_1 compared K^+ groups ($p > 0.05$). While the Value of a statistical dose of P_2 and P_3 was significant compared with K^+ groups ($p < 0.05$) (Table 1).

The cells that had the most narrowed tubules were in the K^+ and P_1 groups, while the least in the K^- group (Fig. 1a-e). The kidney tissue of the benzopyrene injected group had an irregular cell shape, necrosis and many narrowed tubules (Fig. 1b), when compared to Fig. 1a, it is very different. The P_1 dose (Fig. 1c) had almost the same histologic damage as the benzopyrene-injected group. Kidney histologic changes at doses of P_2 and P_3 (Fig. 1d-e). However, insignificant differences in treatment of the extract of andaliman dose of P_1 , P_2 and P_3 ($p > 0.05$) compared with K^+ groups.

Table 1: Narrowing tubules and necrosis value in kidneys rats after given andaliman

Groups	Narrowing tubules	Necrosis
K^-	32 ± 7.59	71 ± 6.51
K^+	$80 \pm 9.36^{***}$	$96 \pm 8.94^{**}$
P_1	75 ± 3.55	88 ± 12.52
P_2	73 ± 7.5	$86 \pm 15.57^*$
P_3	68 ± 5.71	$85 \pm 20.61^*$

Value is expressed as the mean and standard error of the mean, the p-value was determined by ANOVA test, (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$). The values compared with K^+ groups

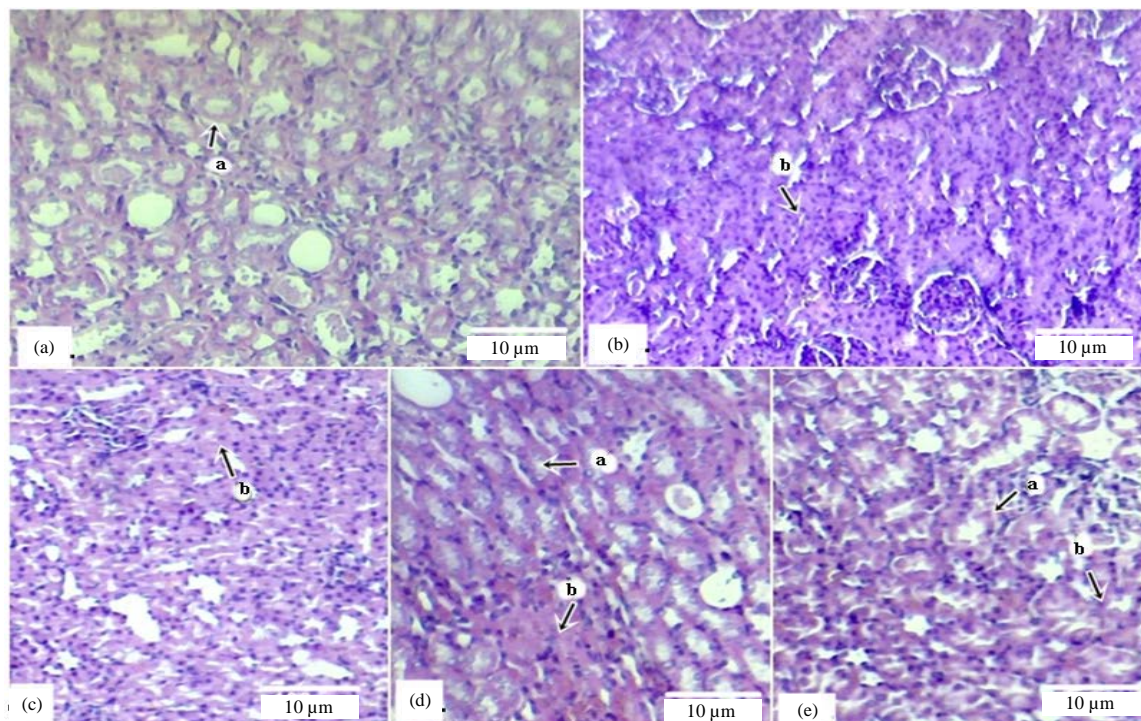


Fig. 1a-e: Histology of kidneys after given andaliman, (a) Control, (b) Cancer model rats, (c) Cancer model rats after given 100 mg kg^{-1} b.wt. andaliman extract, (d) Cancer model rats after given 200 mg kg^{-1} b.wt., andaliman extract and (e) Cancer model rats after given 400 mg kg^{-1} b.wt., andaliman extract
a: Normal tubules, b: Narrowing tubules ($40\times$)

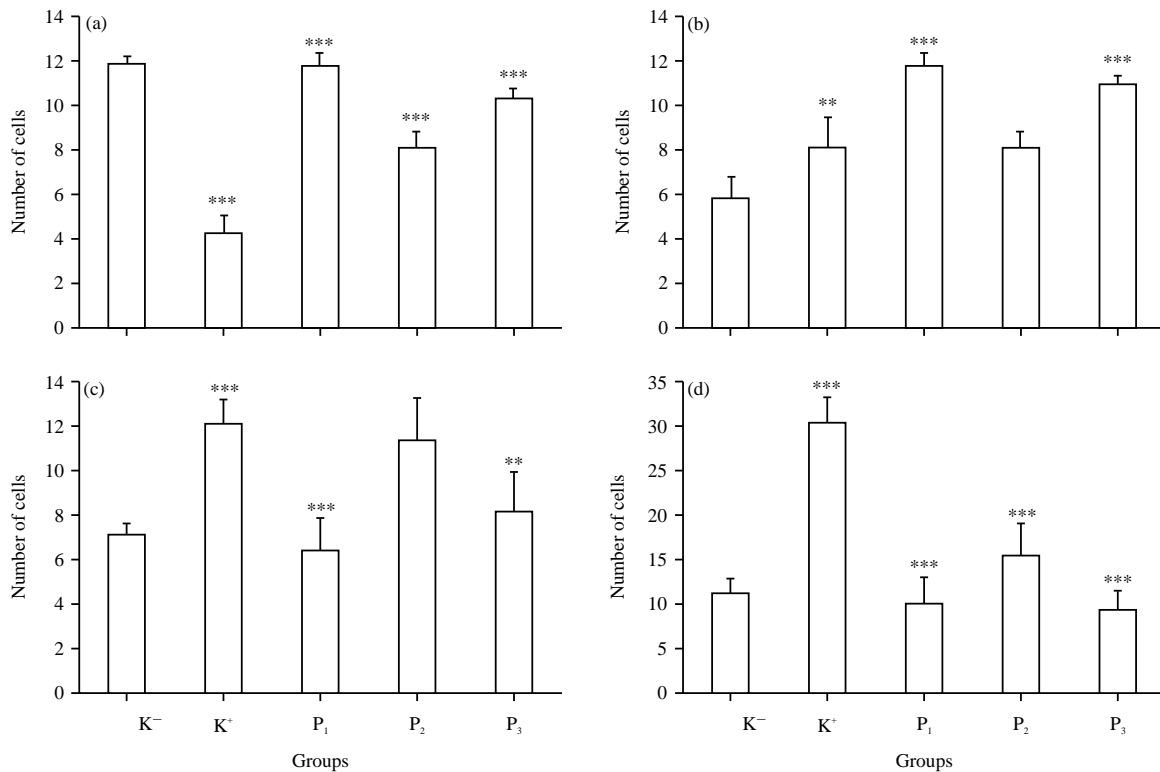


Fig. 2a-d: Graphical representation of hepatocytes cells after given andaliman, (a) Hepatocytes cells, (b) Parenchymal degeneration, (c) Hydrophilic degeneration and (d) Necrosis

K⁻: Control, K⁺: Cancer model rats, P₁: Cancer model rats after given 100 mg kg⁻¹ b.wt., andaliman extract, P₂: Cancer model rats after given 200 mg kg⁻¹ b.wt., andaliman extract, P₃: Cancer model rats after given 400 mg kg⁻¹ b.wt., andaliman extract, *p<0.05, **p<0.01, ***p<0.001

Histology of liver rats after given andaliman methanol extract:

There was a difference significant in the hepatocyte cells of the liver between the positive control group (p<0.001) compared K⁺ groups (Table 1), So it is known that the group does significantly, there were value equations hepatocytes normal with the group K⁻ and different from the group, there is an induction of benzopyrene (K⁺).

There was a significant difference in the degeneration parenchymatous in hepatocyte cells (p<0.01) compared to K⁺ groups (Fig. 2a). A significant difference also in liver histology a dose of P₁ and P₃ (p<0.001), But insignificantly differences in the treatment of the extract of andaliman dose P₂ (p<0.05) (Fig. 2b). There was a significant difference in hydrophilic degeneration in the cell's hepatocyte with p<0.001 (Fig. 2c). On a dose of P₁ (p<0.001) and P₃ (p<0.01), there was also a difference significantly in the treatment of the extract of andaliman against treatment induction benzopyrene (K⁺). But the insignificant difference in P₂ compared to K⁺ groups (Fig. 2a-c).

The histology of the liver also showed values necrosis with a significant difference (p<0.001) compared to the K⁺ group (Fig. 2b). It occurs at all dosage, there are also differences significantly with treatment extract of andaliman against treatment induction benzopyrene (K⁺) with p<0.001 value. The hepatocytes in the group injected with benzopyrene had irregular cell shapes and the cells had parenchymal, hydrophilic and necrotic degeneration, when compared to control tissue, hepatocyte cells in liver tissue were still normal (Fig. 3a-b). In the K⁺ group, there was also a stretch between the cells (Fig. 3b). Changes in parenchymal, hydrophilic degeneration and the number of necrosis in hepatocyte cells are getting better with increasing dose of andaliman (Fig. 3c-e). The Liver histology improved as same as the control group at the highest dose (Fig. 3e). It is known that the dose of the extract andaliman can significantly decrease the value of liver necrosis to the treatment group induction benzopyrene.

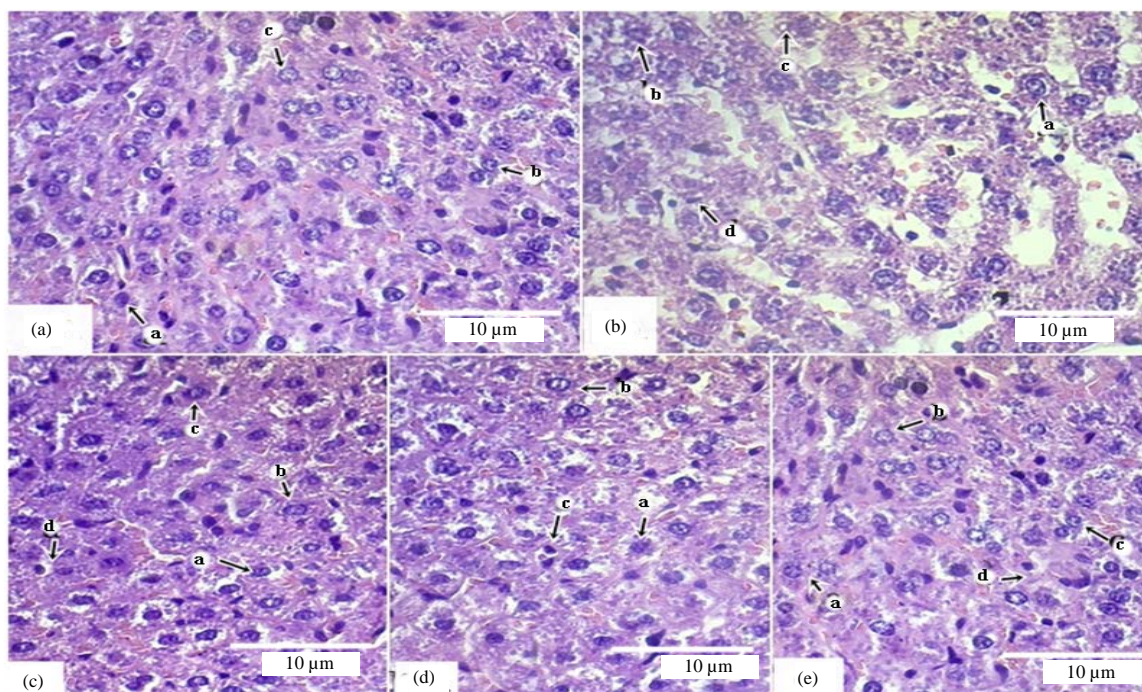


Fig. 3(a-e): Histology of liver cells after given andaliman, (a) Control, (b) Cancer model rats, (c) Cancer model rats after given $100 \text{ mg kg}^{-1} \text{ b.wt.}$, andaliman extract, (d) Cancer model rats after given $200 \text{ mg kg}^{-1} \text{ b.wt.}$, andaliman extract and (e) Cancer model rats after given $400 \text{ mg kg}^{-1} \text{ b.wt.}$, andaliman extract
 a: Hepatocytes cells, b: Parenchymal degeneration, c: Hydrophilic degeneration and d: Necrosis (40 \times)

DISCUSSION

Cancer patients are increasing from year to year, from the data obtained from the WHO, 2018 that new cases of cancer patients rise to 18.1 million and in 2018, 9.6 million deaths caused by cancer. Cancer patients increased and it is affected Cancer patients are increasing and it is influenced by many factors, including population growth and aging as well as changes in the prevalence of certain causes of cancer is associated with the development of social and economic. Rapid economic growth, where the observed shift of the cancers associated with poverty and infection in cancer-related to a lifestyle more typical of industrial countries²¹.

Herbal medicine began to be developed in the treatment of diseases such as cancer in the country-developed and developing countries as in Ogun State Nigeria, the results obtained showed the composition of elements in the examples of the plants studied vary greatly. Estimation of the content of concentrates on minerals and essential elements shows that some plants can be used as anti-cancer but need to do further research about how the impact on the system of other organs of the body²². The studies therapy anti-cancer

that the herbal remedies are applied as a combination therapy with conventional chemotherapy to enhance the therapeutic benefits and Quality of Life (QoL) as well as to reduce side effects or complications²³. Based on studies conducted by researchers where they research some plants that the results of the research indicate that, herbal extracts containing antioxidant compounds that can cause apoptosis and inhibit cell proliferation by a mechanism that is being studied²⁴.

Plants andaliman also contains many health benefits in addition to their function as a spice and food preservative. Many previews research about this andaliman leave and fruit. The result of the study has found that andaliman has cytotoxic activity on 4T1 cancer cells²⁵. Herbal nano belongs to the category of toxic lightweight so it can be used as herbal medicine then²⁶. Also based on the results extract andaliman has a high potential to be developed as co-chemotherapeutic for breast cancer with cell cycle termination²⁷.

From some research that has been done by previous researchers that andaliman can be used as a medicine. Organs that impact the process of use of this drug are the liver and kidney. It is necessary to research whether the andaliman is safe for kidney and liver. The kidney is the organ that is located

under the rib part of the back, near the middle of the back on either side of the spine and function in filtering substances in the body. While the Liver is the organ that is vital for the human body, which neutralizes toxins, regulates the circulation of the hormone, regulates the composition of blood that contains fats, sugars, proteins and other substances. The second organ is very important in the metabolism of the body.

The occurrence of damage to the tubules the possible presence of toxic substances or foreign objects that get into the body that causes the cells of the epithelium of the tubules swell and eventually resulted in the tubule is damaged, it causes the lumen of the tubules narrowing. The narrowing of the tubules because induction benzopyrene disrupts the work of the kidneys so that the kidney is impaired (Table 1-2). Carcinogenic substances such as benzopyrene are agents that contribute to the acceleration of tumor development, regardless of the mechanism of action and the level of specificity of the effect. In other words, this is a substance that increases the possibility of cancer. In addition to causing cancer, these substances can affect the work of the organs in the body and the tubular is a marker of renal injury²⁸. On the other hand, damage of the kidneys can also be affected by the independent risk factors traditionally, the inhibition of insulin and the components of other metabolic²⁹, impaired transport of sodium tubular will enable feedback glomerular tubular (TGF) mechanism will cause the increase in vascular resistance and a decrease in the content in the GFR³⁰ and these conditions would later develop into the risk of impaired kidney failure chronic and end-stage kidney disease³¹. Tubular necrosis and parenchyma renal injury are a key role in acute kidney injury³² and cell death in renal disease caused by damage to the mechanism of tubular³³.

The liver is an important organ system and performs numerous vital functions, such as detoxifying the blood before it is supplied to the brain and break down proteins and lipids to maintaining blood chemistry tools neutralize and regulate the circulation of hormones, regulate blood composition containing fat, sugar, protein and other substances³⁴ and maintain homostasis³⁵. Hepatocytes are the cells parenchymal main on the liver that plays a role in many metabolic pathways. Hepatocytes are very actively synthesized proteins and lipids for secreted and have a lot of the endoplasmic reticulum and the Golgi bodies³⁴.

In the case of liver failure, a liver transplant may be the best choice in the future and can be seen from the progress of current research and the sophistication of the laboratory

equipment required and the progress of technology³⁶⁻³⁸. Although with the lack of progress, but there is no available targeted therapy, but the method of treatment of hepatitis keeps using the treatment methods in the era of 40 years ago to keep the nutrients balanced and the treatment with korticosteroid³⁹.

Liver damage is caused by metal and nano-titanium exposure in the environment, carcinogenic and the other substances that can cause oxidative stress⁴⁰⁻⁴². Disorders of the liver were always associated with necrosis of the cells and the administration of antioxidants exogen, it may be advantageous to protect can protect and nourish the liver. The natural antioxidant is known to have a particularly favorable effect on liver disorders. The provision of andaliman can reduce the damage to the liver and kidneys^{5,20}. For andaliman experience content important function in the health of the cells of the body. Andaliman has the properties of a unique taste and bioactive compounds. Chemical compounds in herbs are monoterpenes, hydrocarbon monoterpenes, volatile compounds main in the andaliman geranyl acetate and limonene^{43,44}. The andaliman harms the liver histology of rats²⁵.

A lot of the nucleus cells arise from cells of hepatocytes in the liver. The process when the toxic substances such as benzopyrene get into the hearts of the nucleus will die even more small parts of the chromatin fiber and reticular breed. Increased damage of hepatocytes from chemical compounds in the fruit andaliman. Protection herbs such as andaliman such compounds terpenoids that can be used as an insecticide and have effects very toxic in animals. The damage will cause the body's immune response and will be directly affected by the biochemistry of the cell. Then it takes an antioxidant such as andaliman. Based on the results and discussion it is known that benzopyrene that enters into the liver and kidneys will lead to impaired function of organs and the giving of andaliman as antioxidants are one way to fix it.

CONCLUSION

Andaliman extract can repair the damage of the liver and kidney of rats induced by benzopyrene and the provision of andaliman as antioxidants are one way to repair it. Andaliman can be recommended as a drug to repair the necrosis in the liver and kidneys caused by cancer. However, the dose andaliman needs to be analyzed in further research.

SIGNIFICANCE STATEMENT

This study discovers the possible effect of andaliman that can be beneficial for decreased necrosis in the liver and kidney of the cancer model rats. This study will help researchers to uncover that this herb may be beneficial in a decrease in liver and kidneys' problems and are still classified as safe on organs. Thus, a new theory on these herbs may be arrived at.

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