

Antioxidant Activity Test of Ethanol Extract of Andaliman Fruit (*Zanthoxylum Acanthopodium* DC.) on Superoxide Dismutase (SOD) Levels in Rats

Arsiaty¹, Yulia Delfahedah², Andre Bastian Manik³, Nurasima Kurniati Damanik⁴

^{1,2}Lecture of Universitas Efarina, Simalungun, Indonesia

^{3,4}Student of Universitas Efarina, Simalungun, Indonesia

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Corresponding Author:

Arsiaty
Universitas Efarina,
Simalungun, Indonesia
Email:
arsiatyefarina@gmail.com

ABSTRACT

Free radicals are compounds or molecules that contain one or more unpaired electrons in their outer orbitals. If the production of free radicals is excessive, it can cause oxidative damage which ends in cell death resulting in the acceleration of various degenerative diseases. To neutralize the work of free radicals, external antioxidants are needed. Andaliman (*Zanthoxylum acanthopodium* DC) is a source of natural antioxidants that contain flavonoids that can neutralize free radicals. This research included sample preparation, examination of simplicia characteristics, screening of simplicia phytochemicals, extract preparation, determination of SOD activity spectrophotometrically using reagents. A total of 30 rats were divided into 6 groups consisting of the control group, the stress-induced group, the comparison group, and the three stress-induced and EEBA groups with doses of 75 mg/kg, 150 mg/kg, and 300 mg respectively. /kg bw. Stress induction was carried out by administering EEBA for 7 days and continued by administering EEBA together with doxorubicin for the next 2 days. The results showed that the average SOD level in the control group was (4.626 ± 0.2583), the doxorubicin group (1.956 ± 0.0879), the EEBA group 75 mg/kg body weight (2.444 ± 0.0844), the EEBA group 150 mg/kg body weight (3.052 ± 0.1139), the EEBA 300 mg/kg body weight (3.646 ± 0.1739) and the Routine 50 mg/kg body weight 5.594 ± 0.2056), EEBA had higher SOD activity when compared to the doxorubicin group. Based on the statistical results, SOD activity increased with the increase in the dose of EEBA given and showed a significant difference ($p < 0.05$) between the EPBA group and the doxorubicin group. Observations on liver tissue in the group given EEBA showed better conditions than the liver in the doxorubicin group.

Keywords:

Antioxidant, *Zanthoxylum Acanthopodium*, Superoxide Dismutase

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1. INTRODUCTION

Free radicals are compounds or molecules that contain one or more unpaired electrons in their outer orbitals. The presence of unpaired electrons causes these compounds to be very reactive looking for partners by attacking and binding the electrons of the molecules around them [5]. Free radicals are generated normally in the body by cell metabolism, inflammation, or when the body is exposed to environmental pollution [3]. If the production of free radicals exceeds the ability of intracellular antioxidants to neutralize them, the excess free radicals have the potential to cause cell damage. Often this damage is referred to as oxidative damage, namely damage to the biomolecules that make up cells caused by reactions with free radicals. An increase in oxidative stress has a negative impact on several components of the cell membrane, namely damage to the membrane lipids to form malonaldehyde (MDA). damage to proteins, carbohydrates, and DNA [9].

Oxidative damage caused by free radicals has implications for various pathological conditions, namely damage to cells, tissues, and organs such as liver, kidney, heart, both in humans and animals. This damage can end in cell death resulting in accelerated onset of various degenerative diseases [9].

External antioxidants can be in the form of natural and synthetic antioxidants. However, various kinds of synthetic antioxidants such as butylated hydroxytoluene (BHT) have been reported to have several side effects such as liver damage and mutagenesis. Therefore, alternative antioxidants are needed that have better and safer activities, namely from natural or plant ingredients [13]. One of the plants that can be used as an antioxidant is andaliman.

Research to determine the effect of andaliman fruit extract on superoxide dismutase (SOD) activity by spectrophotometry in rat blood has not been carried out, therefore researchers are interested in knowing the effect on SOD activity in rats through spectrophotometric blood measurements.

Formulation of the problem

The formulation of the problem in this study is:

- a. Can the ethanol extract of Andaliman fruit increase the activity of the SOD enzyme?
- b. Is the ethanol extract of Andaliman fruit able to prevent liver damage caused by doxorubicin?
- c. Does increasing the dose of Andaliman fruit ethanol extract increase SOD activity in rat blood?

2. METHOD

The research method used was experimental research. The research included collection and preparation of plant materials, sample identification, sample processing, simplista characterization, phytochemical screening, extract preparation for experimental animals, testing the antidiarrheal activity of ethanol extract of srikaya leaf (*Annona squamosa* L.) in male mice, and processing. data Research results data were analyzed by ANOVA (Analysis of Variance) using SPSS (Statistical Product and Service Solution) version 17.

Data analysis

The research data were analyzed using the SPSS version 17 program. The data were analyzed using the Kolmogorov Smirnov method to determine its homogeneity and normality. Then proceed to use the One Way ANOVA method to determine the average difference between groups. If there is a difference, it is continued by using the Post Hoc Tukey HSD test to see real differences between treatments.

3. RESULTS AND DISCUSSION

Simplicia Characteristics Examination Results

Macroscopic examination

The results of macroscopic examination of andaliman fruit showed that young fruit was green in color, and when ripe it was dark red to brownish red. The shape of the fruit is round and small, smaller than pepper, when bitten it gives off a distinctive aroma and sharp taste, and can stimulate saliva production. The seeds are in the fruit and hard. Macroscopic examination was carried out on the andaliman fruit simplicia, namely the simplicia was black in color, had a characteristic odor, and the seeds were coming out of the fruit. Examination of characteristics of andaliman fruit macroscopically was carried out to obtain simplicia identity. Results of macroscopic examination of andaliman fruit and andaliman fruit simplicia.

Microscopic examination

Microscopically, there are covering hairs, endosperm vascular bundles with oil drops, oil drops, and reddish orange seed coat fragments.

Simplicity characteristics

The results of the examination of water content, water soluble extract content, ethanol soluble extract content, total ash content and acid insoluble ash content.

Based on the results of the examination, the Andaliman fruit simplicia had a water content of 7.58%, this result met the water content requirements of the fruit simplicia in the book *How to Make Simplisia*, namely not more than 8% (Ministry of Health RI, 1985). The smaller the water content of the simplicia, the smaller the possibility of microorganism growth and hydrolysis of chemical compounds contained in the simplicia. The water-soluble essence obtained was 10.30% and the ethanol-soluble extract was 12.62%. Determination of the extract content is very useful to give an idea of the amount of dissolved material from simplicia. While the total ash content of the simplicia obtained was 7.06% and the acid insoluble ash content was 0.23%.

Simplicia powder extraction was carried out by maceration. Extracting 500 grams of Andaliman fruit simplicia using 96% ethanol produced 59.41 grams of extract with a yield percentage of 11.9%.

Phytochemical Screening

Phytochemical screening of simplicia and Andaliman fruit extract was carried out to obtain information on the class of secondary metabolites contained therein. Results of phytochemical screening of simplicia and andaliman fruit extract.

The Effect of Andaliman Fruit Ethanol Extract on SOD Levels in Rats

Examination of SOD levels was carried out quantitatively using the UV-Vis spectrophotometry method based on the Bioassay Systems procedure (*EnzyChrom Superoxide dismutase Assay Kit*) at a wavelength of 440 nm which can be seen in Appendix 10, page 70. This method is based on the colorimetric principle for determining SOD enzyme activity in quantitative biological samples. In the test, superoxide (O) is produced by a catalytic reaction of xanthine oxidase (XO). O₂ reacts with WST-1 dye to form a colored product. SOD collects O so that reduced O is useful for chromogenic reactions. Color intensity (OD440nm) is used to determine SOD activity in the sample. The higher the absorbance obtained (AAOD), the higher the SOD activity of the sample [1][2].

Histology Examination of Rat Liver Tissue

Hematoxylin Eosin (HE) staining . *Hematoxylin* is alkaline will color the tissue elements that are acidic (basophilic), namely the cell nucleus. Meanwhile, *eosin* is acidic so that it functions to color the cytoplasm which is alkaline (acidophilic)[18]. Results of histological examination of the liver.

Doxorubicin increases the apoptotic process in liver tissue, is induced by lipid peroxidation in microsomes and especially in mitochondria by the presence of Fe ions and includes damage to blood vessels and stenosis in bati cells [11]. Oxidative stress is the main pathogenetic event that occurs in several liver disorders, such as disturbances in cell metabolism to proliferate, and is the main cause of liver damage in ischemia [9].

High amounts of free radicals in the body attack biomacromolecules which are components of cell walls. As a result, the function of the cell wall decreases, causing cell damage in the form of degeneration as seen in the DOX-treated group [18]

Free radicals do not have an electron pair, so these free radicals will be free in the body and try to achieve stability by binding to nearby molecules. Bonds between free radicals and nearby molecules result in damage to the molecular structure. Damage to cell membranes by free radicals occurs through a series of covalent bonding processes between free radicals and membrane components, oxidation of thiol groups on membrane components by free radicals and lipid peroxidation reactions. The results of peroxidation of membrane lipids by free radicals, have a direct effect on damage to important macromolecules such as lipids, proteins and DNA [15].

If observed microscopically, hydropic degeneration is characterized by the presence of vacuoles in the cytoplasm of the cells so that the liver cells are swollen and have a paler color. Hydropic degeneration can occur due to disruption of the sodium potassium pump in regulating the entry and exit of ions. Hydropic degeneration includes mild damage because it can heal and liver cells become normal again (reversible) [16].

4. CONCLUSION

The conclusions obtained based on the results and observations are:

- a. Andaliman fruit ethanol extract was able to increase the activity of the SOD enzyme, where the SOD activity in the group given EEBA showed a significant difference with the group given doxorubicin (oxidative stress).
- b. Andaliman fruit ethanol extract can prevent liver damage caused by doxorubicin
- c. An increase in SOD activity occurred along with an increase in the dose of EEBA given. Where the most effective dose was EEBA dose of 300 mg/kg body weight, with an average SOD level of 3.646 U/ml.

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