The Effect of Salam Leaf Ethanol Extract on the Histopathology of Doxorubicin-Induced Rats and its Effectiveness in Protecting the Heart Compared with Positive Control (Vitamin E)

Dewi[1], Ermi Girsang[2], Ali Nafiah Nasution[3], Liena[4*]

[1,2,3] Universitas Prima Indonesia
[*]Corresponding Author:
Email: liena@unprimdn.ac.id

Abstract.
This study aims to determine the effect of bay leaf ethanol extract on the histopathological picture of the kidneys of rats induced by doxorubicin. To determine the administration of bay leaf ethanol extract is more effective in protecting the heart than the positive control (Vitamin E). The bay leaves used were obtained from the Harjosari II Medan Amplas area of Medan city. The independent variable in this study was Salam Leaf Ethanol Extract, while the dependent variable was kidney histopathology. This research was conducted at the Pharmacology and Toxicology Laboratory of the Faculty of Pharmacy, University of North Sumatra. The results showed that the administration of vitamin E together with doxorubicin showed a better renal histopathological picture. Vitamin E as a basic antioxidant is able to reduce the damage caused by doxorubicin which is seen in the absence of widening in the Bowman capsule.

Keyword : *Salam Leaf Ethanol Extract, Effectiveness, Vitamin E*

I. INTRODUCTION

Several studies have revealed the phytochemical components of S. polyanthum, but most of these studies have focused on the leaf parts of the plant. An initial phytochemical screening study conducted by Kusuma et al, (2017) revealed that the unripe leaves and fruits of S. polyanthum contain carbohydrates, tannins, alkaloids, steroids, triterpenoids, and flavonoids, while the ripe fruits contain saponins, carbohydrates, tannins, alkaloids, triterpenoids, and flavonoids. In fact, several studies have measured total phenolics and total flavonoids in various types of extracts from the bark and leaves of S. polyanthum. Lelono et al, (2009) found that the methanol-water extract from the bark of S. polyanthum had the largest total phenolic content (TPC) compared to the methanol and water extracts when measured as equivalents of catechins and gallic acid. In contrast, the methanol extract from the bark of S. polyanthum showed the highest total flavonoid (TFC) content, when measured as rutin and flavonol equivalents compared to the methanol-water and aqueous extracts. When the TPC of S. polyanthum leaves was compared with the bark of S. polyanthum from the previous study, the TPC of the former was found to be lower than the latter (Ismail

et al, 2019).One of the new plants that is widely used by the community to treat various diseases and their prevention is bay leaf (Syzygium polyanthum (Wight.) Walp.)

Which is a species of the Myrtaceae family. Apart from being an ingredient in cooking, bay leaf is also known as a traditional medicine. A number of studies have shown that the secondary metabolite content that has antioxidant properties such as phenolics, alkaloids, saponins, steroids, terpenoid tannins in bay leaves is able to detoxify the body, so it has the potential to treat gout. , lowers cholesterol levels, lowers high blood pressure (Verawati et al, 2017), acetylcholinesterase inhibitory, prevention of plaque on teeth (Ismail and Ahmad, 2019), treatment of haemorrhoids, diarrhea, gastric disorders (Silalahi, 2017). According to the Indonesian Herbal Pharmacopoeia (2009), bay leaves contain a total flavonoid not less than 0.40% which is calculated as quercetin (Rizki and Hariandja, 2015).Vitamin E is an antioxidant that can reduce the effects of tissue damage caused by free radicals so that vitamin E is useful in the treatment of neurological, cardiovascular, and malignant diseases such as tumors and cancer (Abdulkareem et al, 2015). The nephroprotective effect of Vitamin E is to protect cells from free radicals or oxidative stress (Ban et al, 2015). Tocotrienols are components of natural Vitamin E in addition to tocopherols, which are fat-soluble antioxidants that protect cell membranes from oxidative damage. Tocotrienols and tocopherols are combined ingredients to reduce the effects of free radicals, inhibit cancer growth, are cardioprotective, and premature aging (Reyes, 2006).

II. METHODS

The collection of bay leaves was carried out purposively, namely sampling was carried out without comparing the same plants from other areas. The bay leaves used were obtained from the Harjosari II Medan Amplas area of Medan city. The independent variable in this study was Salam Leaf Ethanol Extract, while the dependent variable was kidney histopathology.This research was conducted at the Pharmacology and Toxicology Laboratory of the Faculty of Pharmacy, University of North Sumatra.

Extract Characteristics

The ethanol extract of bay leaf obtained needs to be characterized to determine whether the extract obtained has met the quality requirements stated in the respective monographs. Organoleptic test of ethanolic extract of bay leaf showed that the extract obtained was dark green/blackish green, characteristic odor, thick texture and bitter and chelating taste. The manufacture of ethanolic extract of bay leaves using the maceration method by soaking 1000 g of bay leaf simplisa powder with 10 L of 96% ethanol so as to produce an extract of 123.5 grams with an extract yield of 12.35%. The amount of extract obtained depends on the amount of solvent used and the length of the

soaking time. The particle size of the simplicia powder also affects the ability of the filter liquid to attract secondary metabolites contained in the simplicia.

The smaller the particle size will produce a larger surface area so that the contact between the liquid filter and the powder will be more and more (Supomo et al, 2016).In this study, the extraction method used was maceration based on secondary metabolites contained in bay leaves which are unstable at high temperatures. Maceration is a method that does not require complicated equipment and is easy to process. The selection of the right solvent is one of the important factors in the process of extracting active compounds contained in plants (Azwanida, 2015).The solvent used in this study was ethanol 96% because ethanol is a solvent that can attract most of the active compounds contained in plants, both polar, semipolar and nonpolar including flavonoid and phenolic compounds. Ethanol also has a high level of safety and ease of evaporation, is not easily overgrown by microbes, is not toxic when compared to other organic solvents and is relatively inexpensive (Sulastri et al, 2015).

Doksorubisin (DOX)

Doxorubicin (DOX) is one of the most effective and important antineoplastic agents in clinical use, widely used for various types of cancer such as breast, endometrial, ovarian, testicular, thyroid, liver, lung, soft tissue sarcoma, solid tumors. and some cancers in children, including neuroblastoma, Ewing's sarcoma, rhabdomyosarcoma, osteosarcoma, including haematological malignancies. The United States Food and Drug Administration (FDA = Food and Drug Administration) has approved 132 anticancer drugs, which are the most widely used anthracyclines. Considered the mainstay of therapy for decades, conventional anthracycline-containing regimens have shown benefits in terms of response rates, time to disease progression, and overall survival (Winer et al, 2001; Carvalho et al, 2009).

Despite its wide clinical use, the mechanism of action of doxorubicin remains under intense debate. Increasing evidence supports the view that this drug can be a double-edged sword. Indeed, injury to untargeted tissues often complicates cancer treatment by limiting the therapeutic dose of Doxorubicin and reducing the patient's quality of life during and after Doxorubicin treatment. The literature indicates that the heart is the preferred target of doxorubicin toxicity. However, these anticancer drugs also affect other organs such as the brain, skeletal muscles, kidneys, and liver (Carvalho et al, 2009). In general, doxorubicin is used in combination with other anticancer agents (eg cyclophosphamide, cisplatin, and 5-FU) and clinical activity is increased in combination compared with single use (Chu, 2009).

Histopathological Analysis of Kidney Tissue

Analyzing the occurrence of kidney damage by proving the presence of histological damage due to loss of tubular structure and tubular cell death and analyzing the integrity of the glomerulus.

The stages in the preparation of preparations include:

1. Fixation

The purpose of fixation on tissue is to maintain the shape and chemical integrity of the cell during life; prevent damage to the shape, structure and relationships between cells as a result of decay and movement from one place to another; Harden the tissue to avoid trauma or the result of handling. Doing fixation should also consider; speed (network size), penetration (network type), volume (10-20 network size), fixation time (choose fixation as needed).

Using NBF material, which is a mixture of 10% formalin (1000 mL) + sodium acid phosphate (4 g) + Anhydrous disodium phosphate (6.5 g).

2. Trimming:

Trimming means slicing tissue into smaller pieces that aim to be included in a tissue cassette for the dehydration process. In trimming, it is very important to pay attention to which part of the network will be selected, so as to support the accuracy of the diagnosis.

3. Dehydration:

The dehydration process was carried out by immersing successively including: Immersion in 70% alcohol (2 hours), 80% alcohol (2 hours), 90% alcohol (2 hours), 96% alcohol (2 hours), absolute alcohol I (2 hours). hours), absolute alcohol II (2 hours), toluene I (2 hours), toluene II (2 hours), xylol 1 (2 hours), xylol II (2 hours), liquid paraffin I (2 hours), liquid paraffin II (2 hours).

4. Embeding + Blocking

5. Cutting / sectioning

Using a microtome, cutting thickness 4 – 6 m, close to a 56^{O}C water bath, for the development of tissue sections. If it has expanded, catch the tissue with a glass object, then dry it at room temperature, then put it in an incubator before coloring (Berata, 2018).

Staining (Routine Staining– HE)

The preparations were immersed in xylol I, II and III for 5 minutes each, dehydrated with ethanol I and II for 5 minutes each, washed with distilled water for 1 minute, soaked in HE solution for 15 minutes, then rinsed with running water, then washed with Lithium carbonate for 15-30 seconds, rinsed with distilled water for 1 minute, immersed in 4 dips of acid alcohol, then rinsed with distilled water for 1 minute and 15 minutes, stained with Eosin for 4 minutes, the preparation was put in 70%, 80% alcohol and 96% for 3 minutes each, then immersed into ethanol III and IV for 3 minutes each, then into xylol IV and V for 3 minutes each. The preparations were dried and dripped with xylol IV and V for 3 minutes each. The preparation was dried and dripped with perount adhesive and covered with a cover slip, the preparations were ready to be read (Berata, 2018).

III. RESULTS AND DISCUSSION

Administration of doxorubicin followed by administration of Salam Leaf Ethanol Extract with various doses is known to improve the histopathological appearance of the kidneys. At the lowest dose of 100 mg/kgBW, Bowman's capsule widening, tubular dilatation, arteriolar vacuoles, inflammatory cell infiltration and tubular degeneration were seen. With the administration of Salam Leaf Ethanol Extract at a dose of 300 mg/kgBW the improvement was increasing with no dilation of Bowman's capsule. Only slight hemorrhage is seen with inflammatory cell infiltration. The highest dose of Salam Leaf Ethanol Extract given was 500 mg/KgBW showed even better improvement where the histopathological picture of the kidney showed a slight infiltration of inflammatory cells.Damage to rat organs including kidneys may be due to oxidative stress caused by the semiquinone reactive intermediate doxorubicin formed from doxorubicin. Anthracyclines are reported to form semiquinone radical intermediates, which react with molecular oxygen into reactive species that interact with cell macromolecules to cause serious cytological damage (Shivakumar, 2012).

Administration of vitamin E was successful in reversing the histological changes in the studied organs due to its free radical cooling activity.Histopathological changes may be related to the absorptive power of the renal tubules which initiates functional congestion of the nephrons due to damage that occurs in the kidneys (Khan et al, 2012). Histopathological examination should be performed to corroborate the biochemical findings found in the study. Administration of Acacia hydaspica ethyl acetate pharmacy was able to improve the histopathological picture of the kidney given doxorubicin due to its active antioxidant polyphenol metabolite content (Afsar et al, 2020). Another study showed that administration of thyme oil significantly improved the histological lesions of the kidney induced by doxorubicin. Histologically the kidneys were described by giving thyme oil there was mild congestion in the glomerular bundle and this improvement was due to the content of thymol compounds and other compounds that are antioxidants (Ahmed et al, 2020). The ability of bay leaf ethanol extract in repairing renal histology damage caused by doxorubicin administration can be attributed to the presence of chemical compounds in it such as flavonoids, steroids, triterpenoids, stigmasterol, phytol, squalane, hexadecanoic acid, -tocopherol, amyrin, quercetin, neophytadiene. has antioxidant and anti-inflammatory properties that support the ability of bay leaf ethanol extract as a nephroprotective.

Administration of vitamin E along with doxorubicin showed a better renal histopathological picture. Vitamin E as a basic antioxidant was able to reduce the damage caused by doxorubicin which was seen in the absence of widening in Bowman's capsule. Only slight inflammatory cell infiltration and tubular degeneration were seen. The nephroprotective effect of vitamin E is to protect cells from free radicals or oxidative stress that can cause kidney damage (Ban et al, 2015). Tocotrienols are components of natural Vitamin E in addition to tocopherols, which are fat-soluble antioxidants that protect cell membranes from oxidative damage.

Tocotrienols and tocopherols are combined ingredients to reduce the effects of free radicals, inhibit cancer growth, are cardioprotective, and premature aging (Reyes, 2006).

IV. CONCLUSION

Ethanol extract of salam leaf at doses of 100 mg/kgBW, 300 mg/kgBW and 500 mg/kgBW showed improvement in the histopathological features of doxorubicin-induced rat kidney. The most effective dose of bay leaf extract in reducing urea, creatinine, and uric acid levels as well as improving the renal histology of doxorubicin-induced rats was a dose of 500 mg/kgBW. Vitamin E (positive control) was more effective than ethanol extract of salam leaves at doses of 100 mg/kgBW, 300 mg/kgBW and 500 mg/kgBW. The administration of vitamin E together with doxorubicin showed a better renal histopathological picture. Vitamin E as a basic antioxidant is able to reduce the damage caused by doxorubicin which is seen in the absence of widening in the Bowman capsule

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