Testing Of The Nephroprotective Effectiveness Of Sail Leaf (Syzygium Polyanthum (Wight.)Walp.) In Doxorubisin-Induced Male Wistar Rats

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Abstract.
Cancer is one of the leading noncommunicable diseases leading to death worldwide. Cancer treatment has progressed but has side effects including nephrotoxic effects. The toxicity of doksorubisin has been widely known which is likely to be chained by the metabolic conversion of doxorubicin to doxorubicinol. The main mechanism of doxorubicinol toxicity occurs due to its interaction with iron and the formation of ROS that damages cell macromolecules. One of the plants that are widely used by the community to deal with various diseases and prevention is bay leaves. Bay leaves contain secondary metabolites that are antioxidant in nature. Antioxidants are needed to protect the body's cells from oxidative damage, preventing various degenerative diseases such as cancer, cardiovascular disease. The study aimed to determine the content of nephroprotective EEDS chemical compounds in phytochemical screening, EEDS characterization and EEDS nephroprotective activity in doxorubicin-induced mice based on uric acid, ureum and creatine levels. The study was experimentally laboratory using mice as test animals. The treatment group consisted of CMC-Na 0.5%, Nature E, EEDS doses of 100 mg/kgBB, 300 mg/kgBB and 500 mg/kgBB followed by administration of doxorubicin. The results showed phytochemicalally EEDS contains chemical compounds that are nephroprotective namely flavonoids, steroids / triterpenoids, saponins . EEDS has a water soluble sari content value of 21.64%, total ash content of 1.79%, water content of 9.98%. Ethanol soluble juice levels were 45.38% and acid insoluble ash levels were 0.39%. EEDS doses of 100 mg/kgBB, 300 mg/kgBB and 500 mg/kgBB can lower uric acid, ureum and cretinine levels and can picture the histopathology of doxorubicin-induced mouse kidneys with an effective dose of 500 mg/kgBB. It can be concluded that ethanol extract of bay leaves has nephroprotective activity.

Keywords: *Nephroprotective, Gout, Ureum, Doxorubisin, Ethanol extract bay leaves*

I. INTRODUCTION

The incidence of cancer in Indonesia is 136.2/100,000 population and ranks 8th in Southeast Asia, while in Asia it is 23rd rank (Kemenkes, 2019). The biggest causes of cancer deaths each year are lung, liver, stomach, colorectal, and breast cancers (Primadi, 2015; Kemenkes, 2019).Chemo and radiation therapy are widely used treatments for cancer. Despite their antitumoral effects controlling both primary and metastatic tumors, both therapeutic modalities can produce toxicity in normal tissues and often, the associated side effects outweigh the clinical benefits and worsen the patient's quality of life (Lieet al, 2015).Kidneys are one of the important organs in the body, whose functions include regulating blood pressure, preventing the body from

lack of blood by producing red blood cells, producing vitamin D3 for bone needs, regulating osmotic pressure in the body by regulating fluid and electrolyte balance, homeostasis (regulating balance).

Acid-base), excretory function (i.e. removing waste from body metabolism in the form of urea, creatinine, uric acid; removing toxins from drugs and drugs), selective reabsorption function (glucose, amino acids, water and electrolytes and others are selectively reabsorbed in the body). renal tubules) (Karki, 2017). If kidney damage is severe and kidney function is very low, dialysis or a kidney transplant is required for survival. Kidney failure that is treated with dialysis or a kidney transplant is called end-stage kidney disease (ESKD). Treatment can slow the decline in kidney function and delay kidney failure.Based on the background of the high use of chemotherapy drugs such as anthracyclines in patients with malignancy, but it causes nephrotoxicity. And the antioxidant content in bay leaves which is very beneficial for human health, I will try to prove the nephroprotective effect of ethanolic bay leaf extract (EEDS) by measuring serum levels of urea, creatinine and uric acid as biomarkers of kidney function, as well as analyzing renal histological changes in induced rats. doxorubicin.

II. MATERIAL AND 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. This research was conducted at the Pharmacology and Toxicology Laboratory of the Faculty of Pharmacy, University of North Sumatra.

Simplicity Making

First, separate the bay leaf from the stem. The leaves that have been collected are washed clean of impurities with running water until clean and then drained. The leaves were spread on parchment paper until the water was absorbed and then the weight of the leaves was weighed as wet weight. Put in the dryer to dry for about 3 days. The dry weight of the bay leaf was weighed and the weight was recorded as dry weight. Puree the dry simplicia using a blender until a powder is obtained. The simplicia powder obtained was put in a tightly closed plastic container and stored in a dry place before use.

Making Bay Leaf Ethanol Extract

The method used in the manufacture of ethanolic extract of bay leaves is the maceration method by using 96% ethanol solvent as much as 10 times the amount of simplicia used. Weighed as much as 1,000 grams of bay leaf powder and then put into a glass container (maceration container). 75 parts of 96% ethanol (7.5 L) was added then stirred and closed tightly. Left for 5 days and stored in a dry place to protect from sunlight, stirring occasionally. Filtered and squeezed the dregs to obtain the filtered filtrate (filtrate I). Wash the dregs with 25 parts (2.5 L), filtered (filtrate II). The filtrate I and filtrate II were combined to obtain 100 parts (10 L). Transferred to a closed

vessel the obtained filtrate, left for 2 days in a cool place and protected from sunlight, then poured or filtered. The obtained maserate was concentrated with a rotary evaporator at 45ºC until an almost thick extract was obtained. Continue the process by evaporating the extract over a water bath until a thick extract is obtained (Depkes RI, 1979).

Extract Characteristic Check

Examination of the characteristics of the ethanolic extract of bay leaves that was carried out included determining the total ash content, determining the acid insoluble ash content, determining the water content, determining the water soluble extract content and determining the ethanol soluble extract content (WHO, 1995).

Determination of Total Ash Content

A total of 0.5 g of extract was ground and then weighed carefully. Put in a platinum crucible or silicate crucible that has been ignited and tared beforehand, leveled. The crucible is slowly ignited until the charcoal runs out at 600°C for 3 hours. Cool and weigh until a constant weight is obtained. The ash content is calculated on the material that has been dried in the air (Depkes RI, 1995).

Determination of Acid Insoluble Ash Content

The ash obtained in the determination of the ash content was cooled by adding 25 mL of dilute HCl for 5 minutes. Collect the part that is not soluble in acid and then filtered with ash-free filter paper. Washed with hot water, incandescent until the weight remains, cooled, weighed. The acid-insoluble ash content was calculated on the weight dried in the air (Depkes RI, 1995).

Determination of Water Content

The tools used in determining the water content consisted of a 500 mL round bottom flask, a 5 mL receiving tube with a 0.05 mL scale, a cooler, a receptacle, a connecting tube and an electric heater. The method used in determining the water content is the Azeotropic method (toluene distillation) in the following way:A total of 200 mL of toluene and 2 mL of distilled water were put into a round bottom flask. The container and cooler are installed and then distilled for 2 hours. Distillation was stopped and allowed to cool for 30 minutes. The volume of water in the receiving tube was read to an accuracy of 0.05 mL. A total of 5 g of extract that had been weighed carefully was put into the flask and carefully heated for 15 minutes. When the toluene boils, the drop rate is set to 2 drops per second until most of the water is distilled, then the distillation rate is increased to 4 drops per second. When all the water has been distilled, rinse the inside of the cooler with toluene. Distillation was continued for 5 minutes, then the receiving tube was allowed to cool at room temperature. Read the volume of water with an accuracy of 0.05 mL after the water and toluene have separated completely. The difference between the two volumes of water that is read is in accordance with the water content contained in the material being examined. Moisture content is calculated in percent (WHO, 1998).

Determination of Water Soluble Levels

A total of 5 g of bay leaf ethanol extract was weighed and then macerated for 24 hours using 100 mL of a water-chloroform mixture (2.5 mL of chloroform in 1000 mL of distilled water) in a corked flask while occasionally shaking for the first 6 hours, left for 18 hours, filtered. A total of 20 mL of the filtrate was taken and then evaporated to dryness in a flat-bottomed evaporating dish which had been heated and thawed before use. The remaining evaporation is heated at a temperature of 105°C until a constant weight is obtained. Calculated levels in percent soluble in water calculated on the material that has been dried in the air (Depkes RI, 1995).

Determination of Ethanol Soluble Extract Levels

A total of 5 g of extract was weighed and then macerated for 24 hours with 100 mL of 96% ethanol in a corked flask while occasionally shaking for the first 6 hours, left for 18 hours, filtered. 20 mL of the filtrate was taken and evaporated to dryness in a shallow dish with a flat base that had been tared and the remainder was heated at 105°C until a constant weight was obtained. The concentration of soluble extract in ethanol is calculated from the material that has been dried in the air (Depkes RI, 1995). Phytochemical Screening of Bay Leaf Ethanol Extract The phytochemical screening of the extracts was carried out at the Pharmacy Biology Laboratory, Faculty of Pharmacy, University of North Sumatra. The phytochemical screening of the ethanolic extract of bay leaves includes examination of compounds belonging to the glycosides, flavonoids, alkaloids, tannins, steroids/triterpenoids and saponins.

Glycoside Check

A total of 3 g of bay leaf ethanol extract was weighed and then extracted with a mixture of 95% ethanol with 30 mL of water (7:3) and 10 mL of 2N HCl, refluxed for 2 hours, cooled and filtered. A total of 20 mL of the filtrate was taken, 25 mL of distilled water and 25 mL of 0.4 M lead acetate (II) acetate were added, shaken and allowed to stand for 5 minutes and then filtered. The filtrate obtained was extracted 3 times with a mixture of chloroform-isopropanol (3:2) as much as 20 mL. The collection of juice on the isopropanol layer was evaporated in a water bath with a temperature of not more than 50ºC, the remaining evaporation obtained was dissolved with 2 mL of methanol to be used as an experimental solution. A total of 0.1 mL of the experimental solution was evaporated on a water bath, added 2 mL of water and 5 drops of Molish reagent to the remaining evaporation of the test solution, carefully added 2 mL of sulfuric acid to form a purple ring at the liquid boundary indicating the presence of sugar bonds (Departemen Kesehatan RI, 1995).

Flavonoid Examination

A total of 0.5 g of extract was weighed, extracted with 10 mL of methanol, refluxed for 10 minutes and filtered hot using small folded filter paper. The filtrate obtained was diluted with the addition of 10 mL of water. After cooling, 5 mL of ether was added, shaken carefully and then allowed to stand. The methanol layer was taken and evaporated at a temperature of 40ºC, the rest was dissolved in 5 mL of ethyl

acetate and then filtered. The filtrate was used for the flavonoid test in the following manner:

a. 1 mL of the experimental solution was taken and then evaporated to dryness. The remaining evaporation was dissolved in 1 to 2 mL of 95% ethanol, added 0.5 g of zinc powder and 2 mL of 2N HCl, allowed to stand for 1 minute. Added 10 mL of concentrated HCl, an intense red color will occur within 2 to 5 minutes which indicates the presence of flavonoids.

b. 1 mL of the experimental solution was taken and then evaporated to dryness. The remaining evaporation was dissolved in 95% ethanol as much as 1 and then 0.1 g of magnesium powder and 10 mL of concentrated HCl were added to a red orange color indicating the presence of a flavonoid group in the sample (Ditjen POM, 1989).

Alkaloids Check

A total of 0.5 g of bay leaf ethanol extract was weighed then added 1 mL of 2N HCl and 9 mL of distilled water, heated on a water bath for 2 minutes, cooled and filtered. The filtrate obtained was put into 3 test tubes for use in the alkaloid test. In each test tube:

a. 3 drops of filtrate is put into the tube, 2 drops of Mayer's reagent solution are added to form a white and yellow precipitate.
b. 3 drops of filtrate is put into the tube, 2 drops of Bouchardat reagent solution are added to form a brown-black precipitate.
c. 3 drops of filtrate is put into the tube, added 2 drops of Dragendorff's reagent solution will form a red or orange precipitate.

Alkaloids are positive if there is a precipitate or turbidity in at least two of the three experiments above (Depkes RI, 1995).

Tannin Check

A total of 0.5 g of extract was weighed and then extracted with 10 mL of distilled water, filtered. The filtrate obtained was diluted with distilled water until it was colorless. Take 2 mL of the solution and add 1 to 2 drops of FeCl3 reagent. If a blue or blackish green color occurs, it indicates the presence of tannins in the extract (Farnsworth, 1996).Steroid/Triterpenoid Examination A total of 1 gram of extract was weighed and macerated with 20 mL of ether for 2 hours, filtered. The filtrate obtained was put into a vaporizer and evaporated. Added 2 drops of Lieberman-Bourchard reagent to the remaining evaporation. If a purple or red color is formed that changes to blue or green, it indicates the presence of steroids/triterpenoids contained in the extract (Farnsworth, 1966).

Saponin Check

A total of 0.5 g of bay leaf extract was weighed, put into a test tube. Add 10 mL of hot water, cooled, shaken for 10 seconds. If foam is formed as high as 1 -10 cm which is stable for not less than 10 minutes and does not disappear with the addition of 1 drop of 2N HCl, it indicates the presence of saponins contained in the extract (Depkes RI, 1995).

Preparation of Test Suspension Solution

Preparation of the solution includes the manufacture of 0.5% w/v CMC-Na suspension and the manufacture of EEDS (Balai Leaf Ethanol Extract) suspension at doses of 100 mg/kgBW, 300 mg/kgBW and 500 mg/kgBW.Preparation of 0.5% CMC-Na Suspension A total of 0.5 g of CMC-Na powder was weighed and sprinkled in a mortar containing 10 times the amount of hot water, allowed to stand for 15 minutes to swell, ground until a transparent and homogeneous mass was obtained. It is added with a little water and put into a 100 mL volumetric flask, then the volume is made up to the mark line (Anief, 1999).

Making of Salam Leaf Ethanol Extract Suspension

The ethanol extract of bay leaf was weighed as much as 100 mg, 300 mg and 500 mg. Put each extract into a different mortar, crushed. Added little by little 0.5% CMC-Na suspension while grinding until a homogeneous mass was obtained. It was put into a 10 mL volumetric flask and the volume was made up with CMC-Na suspension up to the marking line.

Nephroprotective Activity Testing in Test Animals

This test was carried out using male wistar rats as subjects. The experimental animals used in this in vivo test were 24 healthy male rats weighing about 170 g ± 10% which were divided into 6 treatment groups and each group consisted of 4 rats and had been adapted for 2 weeks in advance for followed by administration of doxorubicin induction, administration of test material for bay leaf ethanol extract (EEDS), administration of 0.5% CMC-Na, and administration of Nature E preparations for 21 days.

II. RESULTS AND DISCUSSION

Characteristics of bay leaf ethanol extract

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. The results of the characterization of the ethanol extract of bay leaves (EEDS) can be seen in Table 5 below.

Table 5. Characterization Results of Salam Leaf Ethanol Extract

No	Parameter	Results
1	Total Ash Content	1,79%
2	Acid Insoluble Ash Content	0,39%
3	Water content	9,98%
4	Kadar Sari Larut Air	21,64%
5	Kadar Sari Larut Etanol	45,38%

Phytochemical screening of bay leaf ethanol extract

Phytochemical screening is a test carried out on extracts with the aim of knowing the content of secondary metabolites using color reagents. The results of phytochemical screening of ethanol extract of bay leaves can be seen in Table 6 below.

Table 6. Screening Results of Salam Leaf Ethanol Extract

No	Secondary Metabolites	Results
1	Glycoside	+
2	Flavonoids	+
3	Alkaloids	+
4	Tannins	+
5	Steroids/Triterpenoids	+
6	Saponins	+

Examination of blood urea, creatinine and uric acid levels

The results of measuring blood urea levels in each group

Examination of urea levels in the blood is one of the clinical chemistry examinations carried out to determine the presence of abnormalities or diseases related to kidney function. The level of urea in the blood serum can reflect the balance between production and excretion. The results of the measurement of urea levels can be seen in Table 7 below.

Table 7. Results of measuring blood urea levels

No	Group	Average Ureum Level (mg/dL) ± SD
1	Normal	$28,33 \pm 4,04^{c}$
2	Induced Doxorubicin + Na-CMC 0.5%	$49,67 \pm 6,03^{a,b}$
3	Iinduced Doxorubicin + Vitamin E 1% BW	$31,67 \pm 8,08^{c}$
4	Induced Doxorubicin + EEDS 100 mg/kg BW	$39,33 \pm 6,66$
5	Induced Doxorubicin + EEDS 300 mg/kg BW	$34,33 \pm 1,53^{c}$
6	Induced Doxorubicin + EEDS 500 mg/kg BW	$33,33 \pm 1,53^{c}$

Results of measurement of blood creatinine levels for each group

Creatinine is an important parameter as a marker of damage to kidney function other than urea. The results of the measurement of creatinine levels can be seen in Table 8 below.

Table 8. Results of Measurement of Blood Creatinine Levels

No	Group	Average Creatinine Level (mg/dL) ± SD
1	Normal	$0,61 \pm 0,09^{c}$
2	induced Doxorubicin + Na-CMC 0.5%	$1,07 \pm 0,16^{a,b}$
3	induced Doxorubicin + Vitamin E 1% BW	$0,63 \pm 0,07^{c}$
4	induced Doxorubicin + EEDS 100 mg/kg BW	$0,74 \pm 0,08^{c}$
5	induced Doxorubicin + EEDS 300 mg/kg BW	$0,67 \pm 0,03^{c}$
6	induced Doxorubicin + EEDS 500 mg/kg BW	$0,65 \pm 0,03^{c}$

Results of Measurement of Uric Acid Levels in each group

Uric acid is a protein found in the body that flows with blood circulation which, when increased, will cause deposition in the joints and form small crystals that will cause pain. The results of measuring uric acid levels can be seen in Table 9 below.

Table 9. Results of Measurement of Uric Acid Levels

No	Group	Average Uric Acid Level (pg/mL) ± SD

1	Normal	2,73 ± 0,40[c]
2	induced Doxorubicin + Na-CMC 0.5%	7,40 ± 2,35[a,b]
3	induced Doxorubicin + Vitamin E 1% BW	3,83 ± 0,35[c]
4	induced Doxorubicin + EEDS 100 mg/kg BW	5,33 ± 0,78
5	induced Doxorubicin + EEDS 300 mg/kg BW	4,60 ± 0,36
6	induced Doxorubicin + EEDS 500 mg/kg BW	4,07 ± 1,05[c]

4.4 Kidney Histopathological Examination

Kidney histopathological results can be seen in Figure 20 below.

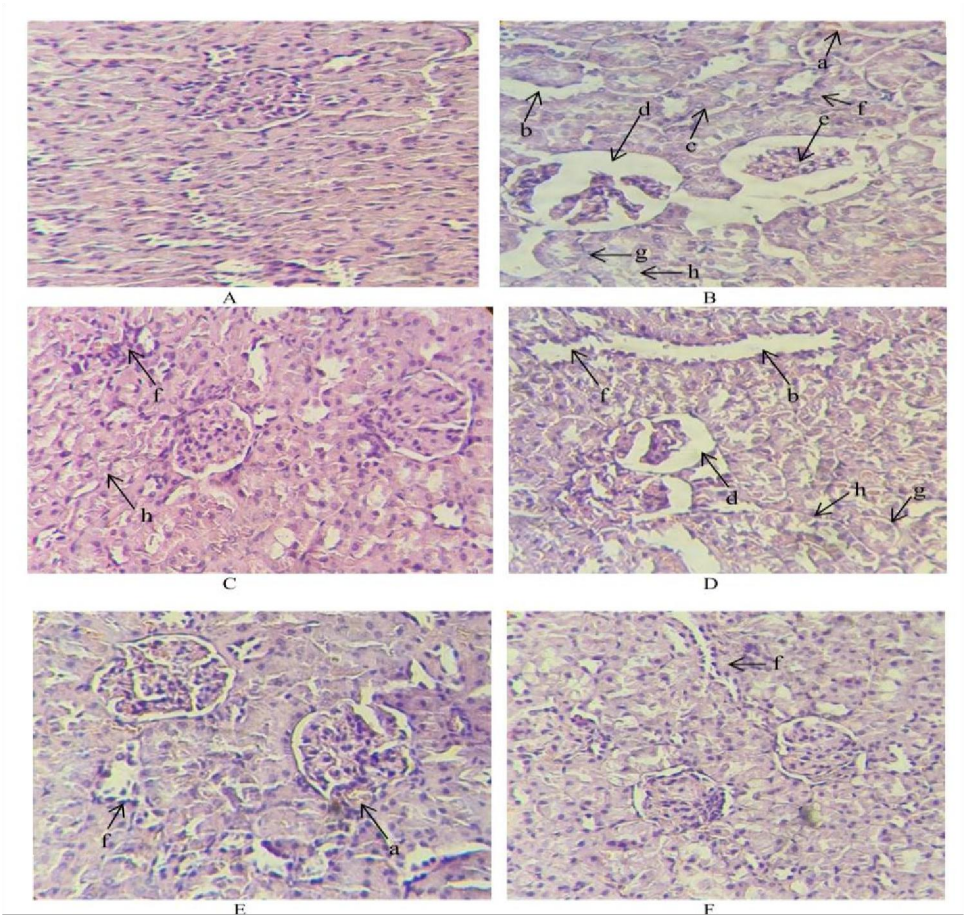

Fig 1. Histopathologic kidney

Description: A = Normal Group
B = Doxorubicin group + 0.5% Na-CMC
C = Doxorubicin group + Vitamin E
D = Doxorubicin group + EEDS 100 mg/kgBW
E = Doxorubicin group + EEDS 300 mg/kgBW
F = Doxorubicin group + EEDS 500 mg/kgBB
a = Hemorrhage
b = tubular dilatation
c = Picnotic

d = Dilation of Bowman's capsule and capillaries
e = Hypocellular glomerulus
f = Infiltration of inflammatory cells
g = arteriolar vacuole
h = tubular degeneration

From the picture above, it can be seen that in the normal group there was no histological damage to the kidneys. The results of this study show that the group

IV. CONCLUSION AND RECOMMENDATION

a. Ethanol extract of bay leaf (EEDS) has met the characterization requirements, namely water soluble extract content of 21.64% (> 7.4%), total ash content of 1.79% (<5.5%), water content of 9.98% (<10%), ethanol soluble extract content 45.38% (>7.8%) and acid insoluble ash content 0.39% (<1.8%)

b. Ethanol extract of bay leaf (EEDS) contains alkaloids, flavonoids, tannins, glycosides, saponins and steroids/triterpenoids.

c. Ethanol extract of bay leaf (EEDS) doses of 100 mg/kgBW, 300 mg/kgBW and 500 mg/kgBW can reduce urea, creatinine and uric acid levels in rats induced by doxorubicin.

d. Ethanol extract of bay leaf (EEDS) at doses of 100 mg/kgBW, 300 mg/kgBW and 500 mg/kgBW showed improvement in the histopathological features of doxorubicin-induced rat kidney.

In future research, it is better to use other parameters such as MDA, GSH, GPx and immunohistochemical methods with iNOS and Nf-κB parameters.

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