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Research article

EFFECTS OF ETHYL ACETATE FRACTIONS OF SURIAN LEAF (TOONA SURENI (BL.) MERR.) ON IMPROVEMENT OF KIDNEY DAMAGE CAUSED BY DEHYDRATION ON MALE WHITE MICE

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A B S T R A C T

The effect of ethyl acetate fraction from surian leaf (*Toona sureni* (Bl.) Merr.) to renal impairment due to dehydration has been tested in male white mice. The mice were dehydrated by not drinking, but still fed for 3 days. The fraction of ethyl acetate from leaves surian was given orally with variation of dose 2.5 mg / kg BW, 5 mg / kg BW, and 10 mg / kg BW once daily for 7 days. Parameters observed to see improvement in renal function due to dehydration are changes in serum creatinine and renal histopathology. The results showed that the application of fraction of ethyl acetate of leaf surian showed improvement of serum creatinine level and histopathology of male white mice compared to control ($p < 0.05$). Provision of fraction of ethyl acetate leaf surian can improve kidney damage in group dose 10 mg / kg BW.

INTRODUCTION

Surian plants (*Toona sureni* (Bl.) Merr.) contain many pharmacologically active compounds. Several previous studies have shown that leaflets contain triterpenes (Cuong *et al.* 2007), tetranortriterpenoid (Surenon and Surenin) (Kraus & Krypke, 1979), methyl gallate which have antioxidant activity (Ekaprasada *et al.*, 2010), and antibacterial (Ekaprasada, *et al.*, 2015). The plant of one genus with the Surian, *Toona ciliata*, also contains terpenoids (Chen *et al.*, 2009).

Traditionally the people of West Sumatra, especially Solok region, have been using leaf surian to treat bleeding after childbirth. In addition, the fraction of ethyl acetate from leaf surian can prevent the occurrence of atherosclerosis in the quail (Suhatri *et al.*, 2014). Surian leaf is also used as astringent, tonic, chronic diarrheal medication, accompanied by other intestinal diseases. While shoot leaf surian can also be used to overcome the kidney swelling (Yuhernita & Juniarti, 2011).

The occurrence of disorders of the kidneys can be caused by some serious diseases that slowly affect the damage to kidney organs. Some of these diseases include hypertension, diabetes mellitus, obstruction of the urinary tract, cancer, inflammatory abnormalities caused by infections causing renal glomerular damage, loss of dehydrated fluid (vomiting, bleeding, and burns) and other diseases. Factors causing dehydration are overheated environments, diarrhea, vomiting, diuretic drug use, lack of water or fluid intake and bleeding (Brunner & Suddarth, 2001).

This study aims to determine the effect of the fraction of ethyl acetate leaves surian against the prevention of kidney damage due to dehydration in white mice. Parameters observed to see improvement in renal function due to dehydration are changes in serum creatinine and renal histopathology.

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MATERIALS AND METHODS

Equipment

The tools used in this research are vacuum distillation, maceration bottle, flash column chromatography, TLC plate, rotary evaporator (Buchi), analytical scales (Precissa), animal weights (Ohaus), animal cages, glass tools (dropper glass, vial, funnel, intravenous bottle), oral needle, mortar, stamp, micro pipette (Eppendorf), tube rack, centrifuge (Hettich), uv-vis spectrophotometer (Shimadzu).

Material

The ingredients used are: leaf surian (*Toona sureni* (Bl.) Merr.), ethanol 70 % (AMS), n-hexane (Merck), ethyl acetate (Merck), methanol (Merck), Silica Gel 60 GF_{254} (Merck), sand, chloroform, anhydrous acetic acid, sulfuric acid (Merck), petroleum ether, concentrated hydrochloric acid (Merck), magnesium powder, iron (III) chloride, male white mouse, water, Diasys® creatinine reagent solution.

Sampling and Sample Identification

Leaves of surian are taken in Jorong Plasma 3, Luhak Nan Duo Sub district, West Pasaman District, West Sumatera. Samples were taken from the leaf and identified at the Herbarium of Andalas University.

Extraction and Fractionation

4 kg of dried leaf samples were milled, then put into maceration, then soaked with ethanol 70% for 6 days with three repetitions. Next macerate filtered with cotton, ethanol filtrate evaporated with vacuum until thick to obtain a thick extract. The viscous extract was diluted with 500 ml distilled water then fractionated with different solvents of polarity in the separating funnel. Fractionation begins with the use of non-polar n-hexane 8 x 250 mL, so that the fraction of n-hexane and water fraction is obtained. In the same way fractionation was done using a semi-polar solvent of ethyl acetate 8 x 250 mL. Each fraction is vaporized vapor to obtain the n-hexane thick fraction and ethyl acetate fraction.

Chromatography Column of Ethyl Acetate Faction

The ethyl acetate fraction of 150 g was dissolved in ethyl acetate and added as much silica gel, and then the solvent was evaporated vacuum to obtain dry powder. This dry powder is sown evenly into the prefilled chromatographic column with dried silica gel of 300 grams and eluted with a stepwise phase whose motion is gradually enhanced. The elution process is assisted by the vacuum drawing (Flash Chromatography), the fraction out of the column accommodated with 100 mL infusion bottle.

Preparation of test animals

The experimental animals used were white male mice aged 2-3 months with a weight of 20-30 grams. The animal is acclimatized to the experimental environment for about a week by weighing daily, observing his behavior and feeding him the same food and beverage. The mice used were healthy mice and during acclimatization the weight did not change more than 10%.

Planning dose

In this study used dose fraction of ethyl acetate of surian leaf 2.5, 5, and 10 mg / kg BW. This dose was selected based on the dose used in previous studies that tested the effect of endothelial cell damage protection by the isolated compound of the ethyl acetate fraction of the leaf of surian (*Toona sureni* (Bl.) Merr.) in hypercholesterolemia state.

Preparation of Test Preparation

The fraction of ethyl acetate leaf surian weighed based on test dose and dissolved in water. Concentrations of test preparations prepared for doses of 2.5 mg / kg BW, 5 mg / kg BW, and 10 mg / kg BW were 0.025%, 0.05%, and 0.1%, respectively.

The Treatment of Animal Experiments

Each mouse was swallowed from drinking for 3 days, and then given the fraction of ethyl acetate leaf surian for 7 days. The grouping of mice was done based on the treatment given as shown in Table 1.

Table 1 Mice grouping based on given treatment

Group	Treatment	Dose (mg/kg BW)
1	Negative controls	-
2	Dehydration + water	-
3	Dehydration + ethyl acetate fraction	2.5 mg/kg BW
4	Dehydration + ethyl acetate fraction	5 mg/kg BW
5	Dehydration + ethyl acetate fraction	10 mg/kg BW

A total of 25 male mice divided into 5 groups (each group consists of 5 mice). After 7 days of treatment, all animal experiments were taken in their blood serum and checked for creatinine levels.

Blood Serum Taking

Taking blood serum is done by taking blood through the blood vessels of the neck. Blood is taken by cutting blood vessels in the neck of mice and accommodated with centrifugal tube and allowed to stand for 15 minutes andthen centrifugation with 3000 rpm for 20 minutes. The serum is taken with a disposable syringe and poured into a micro tube and stored in a freezer in an upright position.

Serum Creatinine Examination

Serum creatinine levels were measured by taking 50 µL serums with pipettes into a test tube, mixing with 1 mL of reagent solution (mixture of 4 parts of reagent 1 with 1 part of reagent 2). Homogenize with the vortex. The sample absorbance measurements were performed in the first minute (As1). The next measurement is done 2 minutes later (As2). Serum creatinine levels are determined by the formula:

$$Cr = \frac{As2-As1}{Ast2-Ast1} \times 2.05 \text{ mg/dL}$$

Sampling of Kidney Mice

After the mice are killed, the kidneys are removed (left kidney and right kidney), then the kidneys are inserted into 10% formalin solution to be fixed and labeled.

Microscopic Observation

Observations were made on the kidneys of mice by way of shooting and weighing. Then a longitudinal cut, the incision is opened and observed.

Data Analysis

Data analysis for this study used one way ANOVA for each treatment, and then continued with Duncan test to know the existence of significant difference in each treatment group. Scoring data was analyzed with non-parametric statistic using Kruskal-Wallis test.

RESULTS AND DISCUSSION

The identification of the results of surian leaf fractionation with ethyl acetate showed that the fraction of ethyl acetate of surian leaf was in the form of white brownish crystals with melting distance 181-184 °C. Analysis with thin layer chromatography gave spots at R_f 0.44 observed on thin layer chromatography plate with silica gel GF 254 and eluent ethyl acetate: n-hexane: methanol (5: 4.5: 0.5). The compound resulted in a positive reaction with iron (III) chloride and has a maximum absorption wavelength at λ 271 nm with an absorbance of 0.44.

Based on statistical test result, it can be seen that giving of fraction of ethyl acetate leaf surian with some dose influence serum creatinine level of mice ($p < 0.05$). Based on the Duncan test, it was found that the four treatment groups showed that there were differences in dehydration group + dose 10 mg / kg BW different from dehydration group + dose 5 mg / kg BW, dehydration group + 2.5 mg / kg BW and group dehydration + water.

The improvement in serum creatinine levels of mice was evident in the dose group of 10 mg / kg BW of 0.756 mg / dL. Serum creatinine levels at these doses were similar to normal serum creatinine levels (Table 2). Based on the score of the level of renal damage of the mice, each treatment group analyzed using non-parametric test Kruskall-Wallis obtained p value = 0.00 ($p < 0.05$). This means that there is a statistically significant difference to the histopathological structure of the renal changes in each group. The results of histopathology examination on the kidneys showed that in the dose group of 10 mg / kg BW for 7 days gave normal improvement in the renal kidney organ compared to the dose group 2.5 mg / kg BW, 5 mg / kg BW and negative control.

Figure 2 shows that the Bowman capsule is not clear because the glomerulus is enlarged. The distal and proximal tubules are not clearly defined; the lumen of the tubules is closed. Many cell nuclei are already blackening.

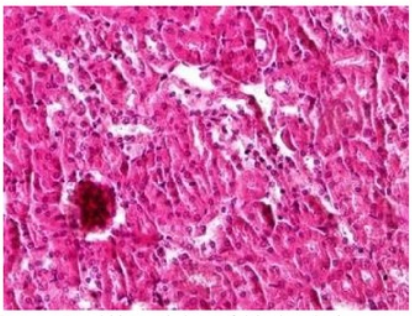

Figure 3 Renal histopathology of dehydrated mice + water

Figure 3 shows that the Bowman capsule is still narrowed because of the enlarged glomerulus. The distal and proximal tubules are still irregular, the lumen of the tubules still closing. The core of the tubular cells is still black.

Table 2 Serum creatinine levels of experimental animals (mg / dL)

Mice	Normal	Dehydration 3 days	Dehydration + water	Dose 2.5 mg/kg BW	Dose 5 mg/kg BW	Dose 10 mg/kg BW
1	0,67 mg/dL	4,02 mg/dL	2,56 mg/dL	2,61 mg/dL	2,32 mg/dL	0,72 mg/dL
2	0.83 mg/dL	3,05 mg/dL	2,51 mg/dL	2,57 mg/dL	2.98 mg/dL	0,60 mg/dL
3	0,67 mg/dL	4,00 mg/dL	2,34 mg/dL	2,60 mg/dL	3,70 mg/dL	0,75 mg/dL
4	0,70 mg/dL	4,00 mg/dL	2,24 mg/dL	2,41 mg/dL	2,85 mg/dL	0,80 mg/dL
5	0,89 mg/dL	3,88 mg/dL	2,38 mg/dL	2,66 mg/dL	3,10 mg/dL	0,91 mg/dL
Ave-rage	0,752 mg/dL	3,79 mg/dL	2,406 mg/dL	2,570 mg/dL	2,990 mg/dL	0,756 mg/dL

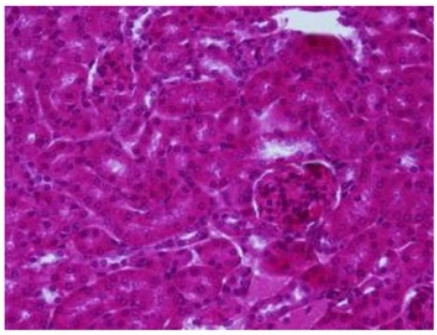

Figure 1 Normal renal histopathology of mice

Figure 1 shows that the normal glomerulus, good Bowman capsule, distal tubules and proximal tubules are also good, the cell nucleus is clearly visible and normal.

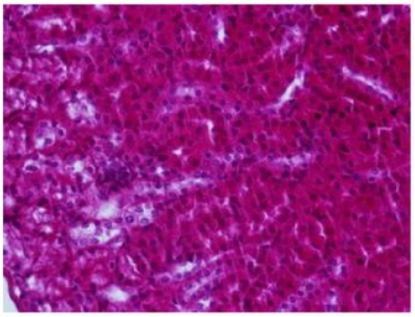

Figure 2 Kidney histopathology of dehydrated mice for 3 days

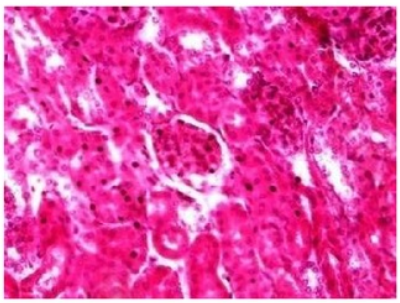

Figure 4 Renal histopathology of dehydrated mice + dose 2.5 mg / kg BW

Figure 4 shows that the Bowman capsule enlarges, because the glomerulus shrinks, the distal and proximal tubules are still irregular, the lumen of the tubules still closes. The cell nucleus is blackened and most of the cell nucleus in the tubule is gone.

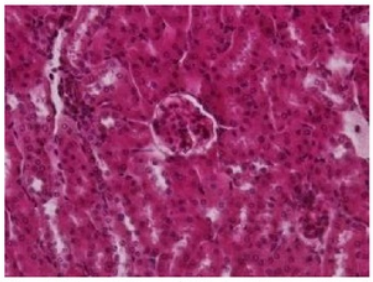

Figure 5 Renal histopathology of dehydrated mice + dose 5 mg / kg BW

Figure 5 shows that the Bowman capsule is still narrowed because of the enlarged glomerulus, the distal and proximal tubules are still irregular, the lumen of the tubules still closes. The nucleus of cells in the tubules is still black and most of them are gone.

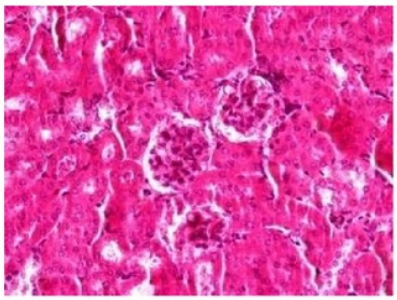

Figure 6 Renal histopathology of dehydrated mice + dose of 10 mg / kg BW

Figure 6 shows that the Bowman capsule begins to normal, the normal glomerulus, the distal and proximal tubules of its shape begin to clear and the cell nucleus is still apparent.

CONCLUSION

From the research results can be concluded that the fraction of ethyl acetate leaves surian (Toona sureni (Bl.) Merr) can repair kidney damage due to dehydration (not drinking) for 3 days. A 10 mg / kg BW dose with 7 days of administration may provide a serum creatinine value similar to that of normal animal serum creatinine.

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