NEPHROPROTECTIVE OF ANTHOCYANIN PIGMENTS EXTRACT FROM RED CABBAGE (BRASSICA OLERACEA L. VAR. CAPITATA F. RUBRA) AGAINST GENTAMICIN-CAPTOPRIL-INDUCED NEPHROTOXICITY IN RATS

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ABSTRACT

Objective: Red cabbage (Brassica oleracea L. var. Capitata f. rubra) has a fairly high anthocyanin content and is a source of powerful antioxidants. This study is basic for the development of a nutraceutical, which has nephroprotective activity.

Methods: Red cabbage was extracted using ethanol and water with a mixture of citric acid with a variation of 1, 2, and 3%. The total anthocyanin was determined with pH-differential method. The Wistar strain male rats were divided into five groups. Group 1 as a negative control, Group 2 as a positive control treated with Vitamin E dose of 400 mg/kg BW day. Groups 3–5 were treated with three different extract dose of 100, 200, and 400 mg/kg BW. Gentamicin was given intraperitoneally and captopril orally for 3 days. Extracts and Vitamin E were administrated orally for 15 days after induction of gentamicin-captopril. Nephroprotective activity was determined by measuring the levels of serum creatinine, blood ureum, and macroscopic kidney.

Results: The combination of 96% ethanol and citric acid 3% showed the percent of free radical 2,2-diphenyl-1-picrylhydrazyl arrest of 75.23% and contained 53.49 ± 5.01 mg/L of total anthocyanin. The anthocyanin pigment from red cabbage extract can decrease the levels of creatinine and ureum, which dose of 100 mg/kg BW showed the highest value of 48.72%. There were differences in the macroscopic morphology in the rat kidney.

Conclusion: Based on the results, we concluded that ethanol with 3% citric acid produced higher anthocyanin and showed nephroprotective activity.

Keywords: Anthocyanin, Brassica oleracea L., Captopril, Gentamicin, Nephroprotective, Creatinine.

INTRODUCTION

Red cabbage is one of the many vegetables that contain anthocyanins. It is a source of anthocyanin with potent antioxidant activity. The previous research showed that an extract of red cabbage anthocyanin pigments has IC50 values with hydrogen peroxide capture methods 19.90 mg/mL, nitric oxide 47.55 mg/mL, and 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical 6.02 mg/mL [1-3]. The potential of these antioxidants can be used in the treatment of oxidative stress in the body.

The antioxidant activity of the anthocyanin potentially inhibits the renal damage. An acute renal disorder can cause kidney malfunction. It is characterized by the higher serum creatinine levels on blood [4,5]. Anthocyanin improved the situation glomerulosclerosis characterized by kidney filtration dysfunction in diabetes treatment and reported it as antagonized glomerular angiogenesis due to chronic hyperglycemia and diabetes [6-9]. Pigment anthocyanin extracts can prevent oxidative stress and microvascular complications and rise kidney tissue damage in diabetics [10,11].

Various studies confirmed that red cabbage anthocyanins can be used as a nutraceutical ingredient, especially as a nephroprotective agent. Therefore, a study on the effects of the extract of anthocyanin pigments of red cabbage to improved kidney function was performed. Anthocyanin extract as the test sample is selected, based on the simple optimization of the extraction procedure. Nephrotoxicity in rats was performed by inducing gentamicin and captopril, followed the establish procedure [12-15]. Nephroprotective activity is determined by measuring levels of creatinine, ureum, and macroscopic kidney. Hopefully, this study can provide information about the nephroprotective activity of red cabbage anthocyanin extract in rats.

Moreover, it can be used as a basic to develop nutraceutical with a nephroprotective activity.

METHODS

Plant material
Flores red cabbage was collected from Cl iodé, Bandung, Indonesia, during February to April 2016. The red cabbage was then transferred in laboratory and stored at 2–8°C until extraction procedures.

Animals and ethical approval
Male rats (150–200 g) obtained from the pharmacology unit of our institution were housed under standard conditions with free access to commercial chow and water. All procedures described here were performed with approval from the Health Research Review Committee of Mohammad Hoesin Central Hospital and Faculty of Medicine, Universitas Sriwijaya, Palembang, Indonesia (Ethical approval certificate No. 114/keptsrhfkhunsri/2016).

Optimization of anthocyanin extraction
A simple optimization of extraction was performed by comparing the solvents. The solvents used were ethanol 90% and water with citric acid variation of 1, 2, and 3%. The extraction process was done with the same procedure. Each extract was obtained by testing the arrest of radical DPPH, yield calculation, and determination of total anthocyanin.

Extraction procedures
Red cabbage as much as 10 g was added to 30 mL of solvent (ethanol/water) with a variation of citric acid (1, 2, and 3%) and then ground. Mass, in the form of a slurry, was put in a dark tube and 30 mL of solvent was added to it. Measurement of pH in each tube was performed.
Extraction was done by maceration at room temperature for 24 h. The supernatant was centrifuged for 10 min at a speed of 4000 rpm. It was taken and concentrated to obtain a concentrated extract [16-18].

**Anthocyanin identification**

The test method of anthocyanin identification was done using HCl and NaOH 2 M. A stable deep red color formation was resulted when tested with HCl, while with 2 M NaOH the red color turned green blue and faded slowly, indicating positive anthocyanin.

**DPPH free radical scavenging assay**

To quantitatively estimate free radical scavenging activity, 1 mL of 0.3 mM DPPH was added to 2.5 mL anthocyanin pigment extract, and the mixture was homogenized and incubated for 30 min. A blank was also prepared without using the extract. By spectroscopic method, the percentage of reduction of DPPH absorbance at maximum λ was calculated to determine the antioxidant activity [19-21].

**Total anthocyanin content**

The percentage of anthocyanin content was determined using the pH differential method [16,22-24]. Two samples were prepared, the first diluted with 0.025 M HCl, pH 1, and incubated for 15 min, and the second diluted with 0.4 M Na acetate, pH 4.5, and incubated for 5 min. Each solution’s absorbance at 700 nm and maximum wavelength was measured.

**Gentamicin-captopril-induced nephrotoxicity in rats**

Rats were acclimatized for 1 week. To induce kidney failure in rats, 100 mg/kg BW gentamicin (intrapерitoneally) and 10 mg/kg BW captopril (orally) were administered for 3 days [25,26]. Kidney failure was confirmed based on the levels of creatinine and ureum in urine [14,15,23,26].

**Nephroprotective activity**

The rats were grouped into five treatment groups, consisting of five rats in each group. In the negative control group, the rats were given distilled water. In the positive control group, the rats were given vitamin E 400 mg/kg BW once a day orally. In the Group 1, 2, and 3 as the treatment group of the extract, the rats were given extract anthocyanin pigments with doses of 100, 200, and 400 mg/kg BW orally daily, respectively. Provision of test compounds was initiated when the rat already had kidney failure or has elevated levels of creatinine and ureum. The increase of creatinine and ureum levels before induction day 0 and after induction day 4 is shown in Fig. 1. It was performed to monitor the process of induction (nephrotoxicity). All groups of test animals had increased levels of creatinine and ureum. The increase of creatinine and ureum processed statistically to see the significance.

**Macroscopic parameter of kidneys**

Phenobarbital injected rats were terminated after 10 min using the neck dislocation method and then dissected to extract the kidneys. Both of the kidneys were then weighed and their shape and color were observed.

**Statistical analysis**

The data obtained were presented in tables, charts, and images. Biochemical parameters (creatinine and ureum) were statistically analyzed, including the normality test and the one-way ANOVA test. There was a statistically significant difference if p<0.05. The significant differences between the groups were determined with LSD post hoc test and paired samples t-test.

**RESULTS**

**Optimization of extracts pigments anthocyanin**

Optimization was performed to obtain the extract with higher antioxidant activity and total anthocyanin content. Antioxidant activity assay of extracts from various acids was performed at λ max 515 nm. The result showed in Table 1, a mixture of ethanol 96% and citric acid 3% showed the highest percent inhibition at 75.23 ± 0.49%. The more the anthocyanin content, the higher the antioxidant activity.

Citric acid 3% was more effective for extracting anthocyanin pigments from red cabbage and showed a higher DPPH radical capture than the other citric acid concentration. The pH of the pigment decreased with the increasing of concentration of the citric acid that was added. Based on a previous research, we observed that citric acid 3% was the best acid in red cabbage anthocyanin extraction.

**Total anthocyanin**

The total anthocyanin content was measured using a pH differential method. The total sample dissolved in the two kinds of solutions which have different pH values, respectively. The difference of the two measurements will show the amount of anthocyanin [16,22]. This analysis was done using the ultraviolet-visible spectrophotometer at two wavelengths, 528 and 700 nm. A wavelength of 528 nm was the result of the measurement of the absorption spectrum λ max extract anthocyanin expected sianidin a type of anthocyanin. Absorption at 700 nm was used as a correction factor. The highest concentration of anthocyanin content presence in ethanol solvent with 3% acid citric was 53.49 ± 5.01 mg/L.

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DISCUSSION

Total anthocyanin in the extract was influenced by the solvent. Polarity compound anthocyanin from red cabbage is lower compared with distilled water. A solvent with the relatively same polarity is suitable for the extraction of anthocyanin. Anthocyanin is classified as semipolar compound (dielectric constant 30–40), while the water is very polar (dielectric constant 80). So that, it complies with ethanol which has a dielectric constant of only 24.30.

**Table 1:** Optimization of extract pigments anthocyanin from various solvents and various acids

| Solvent and acid       | Yield extract (%) | % Inhibition DPPH | Total anthocyanin (mg/l) |
|------------------------|-------------------|-------------------|--------------------------|
| Ethanol+ citric acid 1%| 18.90±0.46        | 72.87±0.85        | 33.79±2.86               |
| Ethanol+ citric acid 2%| 24.19±1.22        | 73.30±0.82        | 46.03±4.62               |
| Ethanol+ citric acid 3%| 21.87±1.08        | 75.23±0.49        | 53.49±5.01               |
| Water+citric acid 1%   | 23.24±1.05        | 43.87±3.85        | 21.54±2.89               |
| Water+citric acid 2%   | 24.73±0.74        | 41.89±5.17        | 21.87±3.94               |
| Water+citric acid 3%   | 19.70±1.07        | 59.28±4.85        | 33.40±2.69               |

**Table 2:** Average value of creatinine and urea level decrease

| Sample                  | Blood urea (mg/dL)       | Blood creatine (mg/dL)       |
|-------------------------|--------------------------|------------------------------|
|                         | Average level            | Day 4                        | Day 20                        | Decrease          | Average level            | Day 4                       | Day 20                       | Decrease         |
| Group 1: Control negative| 89.58±7.84              | 75.80±3.95                  | 13.77                        | 2.97±0.40               | 2.81±0.33          | 0.16                        |
| Group 2: Control positive| 73.30±2.75              | 42.98±1.34                  | 30.32                        | 2.40±0.09               | 2.06±0.14          | 0.34                        |
| Group 3: Extract 100 mg/kg BW | 82.64±7.67            | 42.38±3.98                  | 40.26                        | 2.73±0.27               | 2.11±0.27          | 0.63                        |
| Group 4: Extract 200 mg/kg BW | 76.95±3.22            | 48.76±0.94                  | 36.18                        | 2.58±0.11               | 1.98±0.23          | 0.60                        |
| Group 5: Extract 400 mg/kg BW | 77.63±9.46            | 44.31±0.63                  | 33.32                        | 2.55±0.37               | 2.20±0.19          | 0.35                        |

**Fig. 1:** Profile blood creatinine and urea level various groups treatment, day 0: Before induced gentamicin-captopril; day 4: After induced gentamicin and day 20 after treatment
Consumption of anthocyanin allowed per day according to the acceptable daily intake is 0–0.2 mg/kg. Excessive consumption can cause toxicity. Toxicity is caused by the increase of antioxidant concentration. Increase of antioxidant concentration affects the rate of the oxidation rate. At high concentrations, the antioxidant activity of phenolic groups often disappears and even becomes prooxidant. Based on that information, the higher dose of anthocyanin can be excessive in the body so that it affects the ability to repair the kidney function.

The previous research used the Wistar strain in male rats weighing 150–200 g; it showed that the weight of a normal kidney is 1.1 g on the right side and 0.96 g on the left side with a red-brown color [33]. In early stage of kidney failure, the size of the kidney usually was normal, whereas in chronic renal failure it shows that the kidney size becomes smaller. This study showed that all the group of treatment has a lower kidney weight than a normal kidney. It was likely due to the differences in biological factors of rat, food, habitat, and exposure of inducers given.

CONCLUSIONS

Extract anthocyanin pigments of red cabbage were shown to repair the kidney function of rats induced with gentamicin-captopril. The extract of anthocyanin could accelerate the repairment of renal functions, in a dose of 100 mg/kg BW. Further studies should be done using the parameters of superoxide dismutase and malondialdehyde. Research on kidney tissue was performed microscopically to see any changes in the structure of important cells of the kidney due to oxidative stress.

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CONFLICT OF INTEREST STATEMENT

There is no conflict of interest.

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