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Research article

Nephroprotective effect of virgin coconut oil on rats induced by doxorubicin

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Key words: VCO, Curcuma, Nephroprotective, Urea Creatinine.

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Abstract

Anthracycline doxorubicin (Dox) is a very effective anti-neoplastic agent, which intercalates in DNA and inhibits topoisomerase II. DOX is one of the most common systemic treatments to improve some cancers in adults and children alike, including hematologists and solid tumors. Unfortunately, Dox's clinical efficacy is hampered by dose-related organotoxic (heart, liver, and kidney) potential, which can be life-threatening. Induction of kidney damage using doxorubicin with an accumulative dose of 15 mg/kg for 21 days, with 5 mg/kg once a week. Before the treatment male wistar rats (*Rattus norvegicus*) was adapted for 14 days then continued with doxorubicin induction and treatment of experimental animals for 21 days. Then on the last day, the treatment of male wistar rats (*Rattus norvegicus*) was fasted for 18 hours, performed surgery on the test animals. Wistar rats (*Rattus norvegicus*) male fasted for about 18 hours (not given food, but still given a drink). Male wistar rats (*Rattus norvegicus*) were anesthetized with ketamine at a dose of 70 mg/kg bb i.v. Male wistar rats (*Rattus norvegicus*) are then tethered to the board on all four limbs. Blood serum is used for the examination of total urea and creatinine. Based on the results and discussion that has been presented, this study concludes that the provision of 6 ml of virgin coconut oil (VCO) solution in male rats can reduce serum creatinine and urea levels respectively by 1.35 ± 0.02 mg / dL; 83.66 ± 0.57 mg / dL.

Introduction

In Indonesia, the prevalence of cancer in the population of all ages in 2013 was 1.4% or estimated to be around 347,792 people. Yogyakarta D.I Province has the highest prevalence of cancer, which is 4.1. Based on the estimated number of cancer sufferers, Central Java and Central Java provinces are the provinces with the most estimated cancer sufferers, namely around 68,638 and 61,230 people (Ministry of Health Republic of Indonesia, 2015). [1].

Radiation and chemotherapy are treatments that are widely used for cancer. Despite their antitumoral effects in controlling primary and metastatic tumors, both therapeutic modalities can produce toxicity to normal tissue and often, associated side effects outweigh clinical benefits and worsen the patient's quality of life [2].

Anthracycline doxorubicin (Dox) is a very effective anti-neoplastic agent, which intercalates in DNA and inhibits topoisomerase II. DOX is one of the most common systemic treatments to improve some cancers in adults and children alike, including hematologists and solid tumors. Unfortunately, Dox's clinical efficacy is hampered by dose-related organotoxic (heart, liver, and kidney) potential, which can be life-threatening. Several cytotoxic mechanisms are involved in the pathogenesis of Dox-induced nephrotoxicity with a decrease in glomerular filtration rate that occurs in 15-30% of patients. Various mechanisms contribute to renal dysfunction after Dox exposure including tubular epithelial cell toxicity, vasoconstriction in renal microvasculature, and increase expression of proinflammatory cytokine [3]. However, a large amount of evidence indicates that oxidative stress induced by Dox remains the basis, as evidenced by reactive oxygen species (ROS) induces oxidative damage such as lipid peroxidation and protein, and over renin activity that produces angiotensin II. Angiotensin II, the main effector of the renin-angiotensin system (RAS), has been reported to have an important role in the pathogenesis of several cardiovascular and kidney injuries [4].

Also, nephrotoxicity is a common and severe side effect due to oxidative stress and Dox produces drastic cellular negligence in the kidneys, including focal necrosis and fibrosis in the cells of the kidney organs. Renal fibrosis occurs due to inflammation of the tubular and glomerular epithelium. This is indicated by the number of widening junction cells [5]. Dox-induced nephrotic syndrome is characterized by proteinuria and hypoalbuminemia. At present, there are no specific and effective therapeutic agents to avoid organ toxicity associated with Doxorubicin. Thus, studies of compounds that can increase the index of chemotherapy and radiotherapy that do not have side effects on healthy tissue and without affecting its anti-neoplastic effects are urgently needed [6-7].

Finding ways to predict and prevent kidney disease due to cancer drugs became the most important in the medical field for the previous several decades. The risk assessment method has been widely applied. In clinical practice, the risk model of identifying patients who are at risk of developing kidney failure due to the use of natural foods as primary prevention in kidney disease needs to be prioritized [8].

Virgin coconut oil (VCO) which appears as pure coconut oil is very beneficial to protect the kidney organ from drugs, especially anti-cancer, this has been linked to its strong natural antioxidants [9]. Likewise, the traditional plants of Curcuma (*Zingiberaceae*) which number more than 30 species and are found in Asia, where the rhizome of this plant is used as food and medicine. Among the Curcuma species; Curcuma longa, Curcuma aromatic, and Curcuma Xanthorrhiza are the most popular. The main constituents of the Curcuma species are curcuminoid and bisquane type sesquiterpenes. Curcumin is the most important element among the natural curcuminoids found in this plant [10]. Turmeric (Curcuma longa Linn) which is one of the plants rich in bioactive compounds whose properties have been proven long ago, the content of Curcumin in turmeric is a powerful polyphenolic antioxidant, so it can be beneficial for organ protective, especially liver and kidneys [11-12].

Virgin coconut oil (VCO) is a high-quality coconut product that is a mainstay product in coconut-producing countries. There are two kinds of coconut oil: VCO and ordinary coconut oil (refined, bleached, deodorized = RBD). VCO and ordinary coconut oil both contain beneficial medium-chain fatty acids (MCFA). But VCO has the added benefit of having fewer manufacturing processes experienced so that it still holds a lot of phytonutrients. Coconut oil has gained popularity in the world of health food because its health benefits are increasingly proven, including skin and hair care, stress relief, weight loss, lowering cholesterol levels, anti-diabetic effects, anti-cancer effects, immunomodulatory effects, maintaining blood pressure stability, circulation disorders and Alzheimer's disease, are anti-inflammatory, analgesic and antipyretic and topical treatment in minor burns such as sunburns to second degree burns [13-14].

The high polyphenol content in coconut oil can maintain normal levels of parameters in tissue and serum, by increasing antioxidant enzymes so that they function to bind and trap reactive oxygen in plasma and interstitial fluid from the arterial wall and prevent microsomal lipid peroxidation [15].

Based on the background of VCO and Curcuma Longayang which are very beneficial for human health, proving nephroprotective effects by measuring serum urea and creatinine levels in doxorubicin-induced mice.

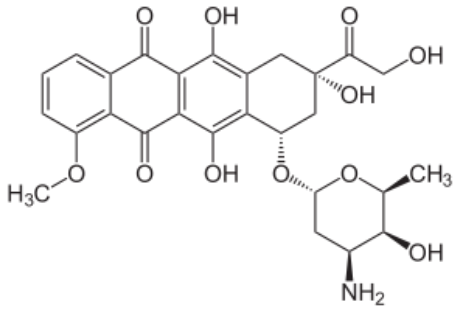


Figure 1. Structure of doxorubicin

Materials and method

Material

The tools used are surgical instruments, microtome, microscope, microplate reader, incubator, micropipette, automated plate washer, VCO, Doxorubicin, Ketamine, CMC-Na, capsules Super bio-curcumin 400 mg obtained from kalbefarma.

Animals

Animals used in research are male wistar rats (*Rattus norvegicus*) weighing 150 – 200 g. Before the study began, test animals were adjusted for one week with the condition of the room temperature (22-25 °C), under the cycle of 12 hours light/ dark, given the food and the drinking water. Ethics Commission from health and science commission, University of Sumatera Utara. Animal ethics committee approval number (0522/KEPH/-FMIPA/2019).

In vivo test nephroprotective effect of VCO and Curcuma

In vivo tested in an experiment by using 25 male wistar rats (*Rattus norvegicus*) weighing 150 – 200 g, as many as 20 and divided into 5 groups and each group consisted of 4 rats:

Negative control: Wistar rats (*Rattus norvegicus*) male + Na-Carboxy methyl cellulose (CMC).

Positive control: Wistar rats (*Rattus norvegicus*) male induced by doxorubicin 15 mg/kgbw + vitamin e 100 mg/kgbw.

Group I : Wistar rats (*Rattus norvegicus*) male induced by doxorubicin 15 mg/kgbw + 6 ml VCO

Group II : Wistar rats (*Rattus norvegicus*) male induced by doxorubicin 15 mg/kgbw + 7.2 mg superbiocurcumin

Group III : Wistar rats (*Rattus norvegicus*) male induced by doxorubicin 15 mg/kgbw+ 6 ml VCO + 7.2 mg superbiocurcumin

Induction of kidney damage was done by using doxorubicin with an accumulative dose of 15 mg/kg for 21 days, with 5 mg/kg once a week. Before the treatment male wistar rats (*Rattus norvegicus*) was adapted for 14 days then continued with doxorubicin induction and treatment of experimental animals for 21 days. Then on

the last day, the treatment of male wistar rats (*Rattus norvegicus*) was fasted for 18 hours, performed surgery on the test animals. Wistar rats (*Rattus norvegicus*) male fasted for about 18 hours (not given food, but still given a drink). Male wistar rats (*Rattus norvegicus*) were anesthetized with ketamine at a dose of 70 mg/kg bb i.v. Male wistar rats (*Rattus norvegicus*) are then tethered to the board on all four limbs. The chest cavity was dissected and 3 ml of blood from the heart was taken using a 5 ml syringe. The blood is then transferred in a blood tube. Then the blood is centrifuged for 10 minutes at a speed of 3000-4000 rpm to produce 2 layers, namely serum/supernatant and its sediment. The serum layer is then taken using a 1 ml syringe, stored in a microtube and stored in a refrigerator at -4°C. Blood serum is used for the examination of total urea and creatinine [16].

Determination of urea and creatinine

Measurement of the levels of urea and creatinine is performed by following the method of Lie. As many as 50 µl samples and 500 µl of urea reagent/creatinine mixed in a test tube. Then the initial absorbance read after 1 minute at a wavelength of 340nm. Next, the absorbance was measured again after 1, 2, and 3 minutes [17].

Statistical analysis

Test analysis was carried out by using one-way analysis of variance (ANOVA) followed by Post Hoc Test using the Tukey HSD test. P<0.05 was considered as statistical significance and also use IBM SPSS 20.

Result and discussion

Virgin coconut oil contents

Virgin Coconut Oil or known as VCO contains compounds of saturated fatty acids and unsaturated fatty acids that help the body's metabolism. These compounds can be seen in Table 1.

Table 1. Chemical compounds contained in VCO.

Chemical Component	Precentage (%)
Caproic acid	0.187
Caprylic acid	1.12
5-Cyclopropylpentanoic acid	0.54
Lauric acid	32.73
Myristic acid	28.55
Palmitate acid	17.16
Oleic acid	14.09
Stearic acid	5.68

Creatinine and urea level

In this study, serum creatinine and urea were examined from rat blood treated with Na-CMC suspension (group I), doxorubicin induction dose 15 mg / Kg BB (group II), doxorubicin induction dose 15 mg / Kg BB + 6 ml VCO (group III), doxorubicin induction dose 15 mg / kg BW + 7.2 mg super bio-curcumin (group IV), and doxorubicin induction dose 15 mg / kg BW + 6 ml VCO + 7.2 mg super bio-curcumin (group V). Serum creatinine examination was carried out in the Medan health area laboratory. The results of serum creatinine obtained can be seen in table 2.

Table 2. Creatinine level.

No.	Doses	Creatinine ± SD (mg/dL)
1.	Negative control	0.86 ± 0.01
2.	Positive control	1.49 ± 0.07
3.	Group I	1.35 ± 0.02
4.	Group II	0.65 ± 0.01
5.	Group III	0.54 ± 0.01

The data presented in the form of Mean ± SD. data obtained results based on the results of the statistical tests, the levels of creatinine on negative control normal have a significant difference ($p < 0.05$) with normal, positive control, treatment group I, II, III.

Based on table 2, it is known that the Na-CMC suspension group became the group with normal creatinine serum levels of 0.86 ± 0.01, the doxorubicin induction group at a dose of 15 mg / kg BW was above the normal value and became the group with the highest serum creatinine levels high of 1.49 ± 0.07, the group treated with 6 ml VCO test material experienced a decrease in serum creatinine value from the group without test material of 1.35 ± 0.02, super bio-curcumin as a comparison material used to give a good effect in reducing serum creatinine values by 0.65 ± 0.01, and so did the combination group between VCO and super bio-curcumin with serum creatinine values by 0.54 ± 0.01.

In addition to the decrease in creatinine value, the decrease in urea value from rat blood is also a parameter to determine the activity of the test material as a nephroprotective. The examination of serum urea values was carried out in the Medan health area laboratory. The results of serum urea obtained from various test groups can be seen in table 3.

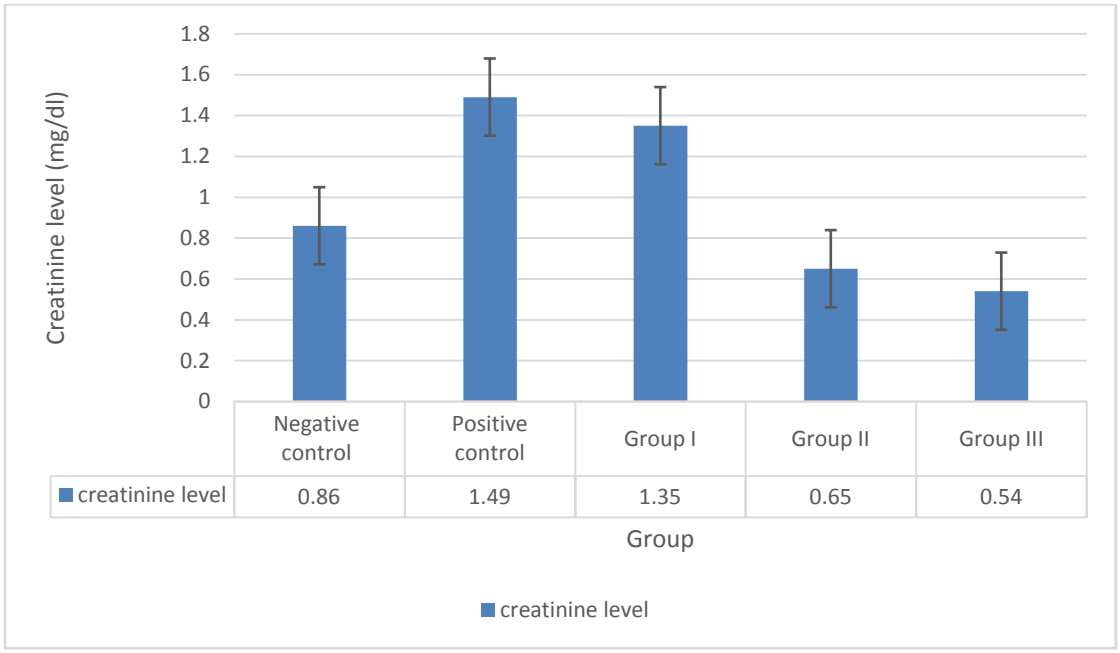
Table 3. Urea level.

No.	Doses	Urea Level ± SD (mg/ml)
1.	Negative control	44.00 ± 1.00
2.	Positive control	110.33 ± 1.53
3.	Group I	83.66 ± 0.57
4.	Group II	75.00 ± 2.00
5.	Group III	57.33 ± 1.52

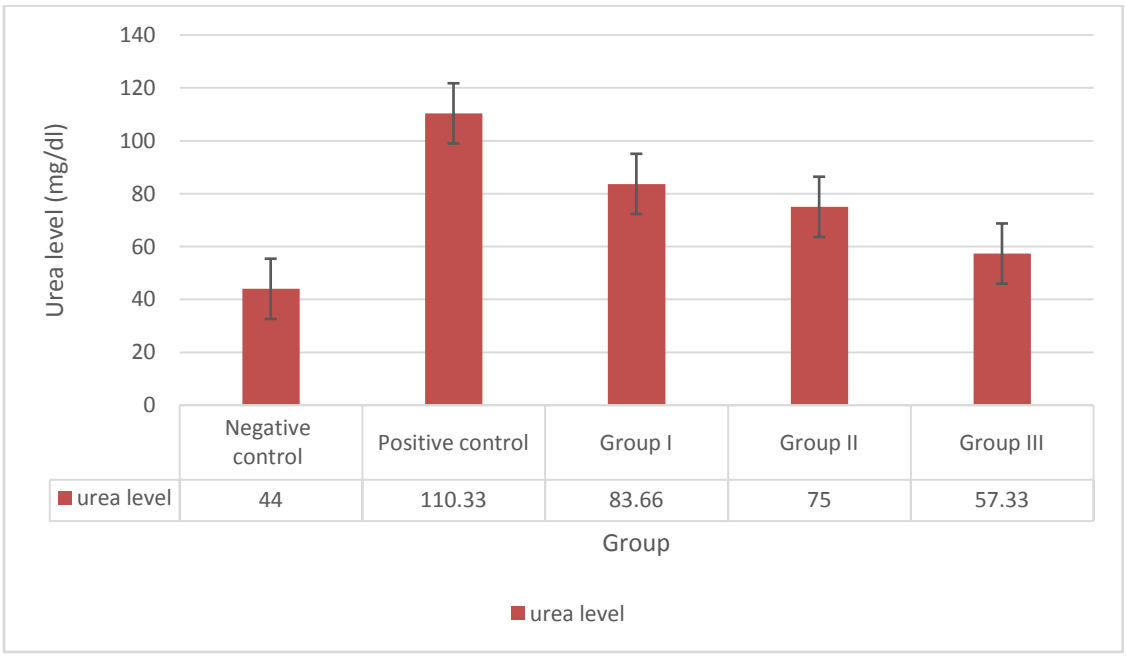
Based on table 3, it is known that the Na-CMC suspension group became the group with normal serum urea levels of 44.00 ± 1.00, the doxorubicin induction group at a dose of 15 mg / kg BW was above the normal value and became the group with the highest serum urea levels high of 110.33 ± 1.53, the group treated with 6 ml VCO test material experienced a decrease in serum creatinine values from the group without the test material by 83.66 ± 0.57, super bio-curcumin as a comparison

material used to give a good effect in reducing the serum creatinine value by 75.00 ± 2.00, and so was the combination group between VCO and super bio-curcumin with a serum creatinine value of 57.33 ± 1.52.

The serum creatinine and urea data obtained were analyzed using SPSS 23 one way ANOVA program to see whether there were differences between the control group and the treatment group. Based on the analysis results obtained, that there are significant differences between the control group with the test material group. This is evidenced by the significance value obtained which is <0.05. Thus each treatment gives different results. The test treatment has a protective effect on kidney organ function if observed from a decrease in the value of serum creatinine and urea. A bar chart of the average measurements of serum creatinine and urea in male rats can be seen in figure 1a and 1b.



(a)



(b)

Figure 1a and 1b. Differences in serum creatinine and urea results from various test groups. (a): Serum creatinine level, (b): Serum urea level.

Evidenced by the results of research giving doxorubicin caused an increase in serum creatinine levels of 1.49 ± 0.07 mg / dL and serum urea of 110.33 ± 1.53 mg / dL. While the provision of VCO acts to reduce serum creatinine levels by 1.35 ± 0.02 mg / dL and serum urea levels by 83.66 ± 0.57 mg / dL. Better results are shown when VCO is combined with super bio-curcumin, where the serum creatinine level is 0.54 ± 0.01 mg / dL and the serum urea level is 57.33 ± 1.52 mg / dL.

The decrease in serum creatinine levels and serum urea levels is a sign of acute renal failure. In this study, doxorubicin is used as a material that causes damage to the kidneys, the use of VCO as a test material to obtain a protective effect and super bio-curcumin which is used as a positive comparison. Doxorubicin will cause destructive effects on kidney cells (nephrotoxic) due to increased Reactive Oxygen Species (ROS). Proximal tubular cells, endothelium, basement membrane, mesangial cells, glomerular visceral cells will be damaged and accompanied by an increase in serum creatinine and urea. The content of compounds in VCO that have antioxidant properties such as Medium Chain Fatty Acid (MCFA), unsaturated fatty acids, sterols, vitamin E, and polyphenol fractions such as phenolic acids are the reasons for reducing the number of ROS molecules formed by giving doxorubicin [18].

Virgin coconut oil based on the content of saturated fatty acids has the highest content of lauric acid. In the body, lauric acid is converted into monolaurin which contains natural antibiotics so that it can kill various types of germs, viruses, microorganisms by damaging the membrane that encloses cells that are composed of fatty acids. Virgin coconut oil is known to have a protective effect on cells [19].

Curcuma contains 3-5% v/v evaporated oil consisting of turmeron, zingiberen, ar-turmeron, slightly containing Fellandren, sesquiterpene alcohol, borneol, curcumin, Dezmethoxycurcumin, Bisdemethoxycurcumin, starch, tannin and resin [20].

Curcuma is characterized by phenolic compounds derived from diarilheptanoid or curcuminoids and sesquiterpenes. Achmad, 2009 reported that from the Curcuma longa (synonym C. domestica) rhizome found three phenol dyes derived from diarilheptanoid or curcuminoid. The three phenol compounds are the main phenol components, each of which is bisferuloilmetan or curcumin, 4-hydroxy-sinamoil feruloil methane or demethoxicurcumin and bis (4-hydroxisinamoil) -methane or bisdemectoxicurcumin. Besides that, an asymmetric curcuminoid derivative was also found, which is dihydrocurcumin [22-24].

Conclusion

Based on the results and discussion that has been presented, this study concludes that the provision of 6 ml of VCO solution in male rats can reduce serum creatinine and urea levels respectively by 1.35 ± 0.02 mg / dL; 83.66 ± 0.57 mg / dL.

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