

THE EFFECT OF VITAMIN E (α -TOCOPHEROL) ADMINISTRATION ON GLOMERULUS AND PROXIMAL KIDNEY TUBULES DAMAGE WHICH RECEIVED CISPLATIN EXPOSURE ON SPRAGUE DAWLEY MICE

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ABSTRACT

Objective: To analyze the protective effect of Vitamin E on cisplatin toxicity in Sprague Dawley mice nephrons. **Material & Methods:** This is an experimental study using post-test only control group design, the subject was white male mice (*Rattus Norvegicus*) adult Sprague Dawley strain (10-12 weeks) of 24 rats divided into four groups. Negative control group (CN) got normal saline 0.9% intraperitoneal 1 cc, Positive control group (CP) got cisplatin 5 mg/kgBB, group P1 got vitamin E 50 mg/kgBB and Cisplatin 5 mg/kgBB, and P2 group got vitamin E 200 mg/kgBB plus cisplatin 5 mg/kgBB intraperitoneal. Cisplatin is conducted in the third week in each treatment group through intraperitoneal injection. Vitamin E is administrated per sonde for the first three weeks resumed on the fourth week to the seventh week. At the end of the seventh week, nephrectomy was performed on the treatment group to analyze the kidney damage. Histopathological observation is performed using a light microscope with a magnification of one hundred and four hundred times magnification. **Results:** Cisplatin administration resulted in significant tubular and glomerular damage compared to the control group. Increasing the dose of vitamin E in mice that received cisplatin resulted in significant nephron damage compared to the group who received cisplatin alone. **Conclusion:** Cisplatin administration results in nephrotoxicity in mice. The administration of high dose Vitamin E resulted in increased nephrotoxicity in mice that received cisplatin.

Keywords: Cisplatin, vitamin E, glomerulus, kidney tubules, nephrotoxicity.

ABSTRAK

Tujuan: Menganalisis kemampuan proteksi isomer vitamin E pada nefron tikus Sprague Dawley yang terpapar cisplatin. **Bahan & Cara:** Penelitian ini merupakan studi eksperimental laboratorium dengan post test only control group design, subjek penelitian adalah tikus putih jantan (*Rattus Norvegicus*) jenis Sprague-Dawley (10-12 minggu) sejumlah 24 ekor terbagi dalam empat kelompok. Kelompok kontrol negatif (CN) mendapatkan normal saline 0.9% intra peritoneal 1 cc, kelompok kontrol positif (CP) mendapatkan Cisplatin 5 mg/kgBB, kelompok P1 mendapatkan vitamin E 50 mg/kgBB dan Cisplatin 5 mg/kgBB, dan kelompok P2 mendapatkan vitamin E 200 mg/kgBB ditambah cisplatin 5 mg/kgBB intra peritoneal. Pemberian cisplatin dilakukan di minggu ketiga pada tiap kelompok perlakuan melalui injeksi intraperitoneal. Vitamin E diberikan per sonde selama tiga minggu pertama dilanjutkan pada minggu keempat hingga minggu ketujuh. Diakhir minggu ketujuh, nefrektomi dilakukan pada kelompok perlakuan untuk dilakukan analisa kerusakan ginjal. Pengamatan histopatologi dilakukan menggunakan mikroskop cahaya dengan pembesaran seratus dan empat ratus kali. **Hasil:** Cisplatin mengakibatkan kerusakan tubulus dan glomerulus ginjal secara signifikan dibandingkan dengan kelompok kontrol. Peningkatan dosis vitamin E pada tikus yang mendapatkan cisplatin mengakibatkan terjadinya kerusakan nefron secara signifikan dibandingkan dengan kelompok yang mendapatkan cisplatin saja. **Simpulan:** Cisplatin mengakibatkan terjadinya nefrotoksisitas pada tikus. Pemberian Vitamin E dengan dosis tinggi mengakibatkan terjadinya peningkatan nefrotoksisitas pada tikus yang mendapatkan cisplatin.

Kata Kunci: Cisplatin, vitamin E, glomerulus, tubulus ginjal, nefrotoksisitas.

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INTRODUCTION

One of the most effective antineoplastic drugs and is most often used as a chemotherapy

agent for cancer today is Cisplatin. Cisplatin works by inhibiting deoxyribonucleic acid (DNA) replication by forming the interstrand crosslinks with DNA. The Clinical use of cisplatin has been

shown to successfully provide a better prognosis and reduce the mortality rates of cancer patients. But the greater the dose of cisplatin, the more severe the side effects are inflicted.¹

Nephrotoxicity is a side effect of cisplatin which needs special attention and treatment. Induced nephrotoxicity due to the use of cisplatin can lead to decreased kidney function and is permanent.² Recently, the pathophysiology of cisplatin nephrotoxicity has been focusing on the understanding of cellular and molecular mechanisms. Tubular cell exposure to cisplatin will activate complex signaling pathways that cause damage and death from tubular cells. Meanwhile, many inflammatory responses are stimulated which will cause impairment in kidney tissue. Cisplatin was also known to induce impairment in renal vascularization so that it will ultimately reduce blood flow and ischemic damage from the kidneys, which contributes to a decrease in glomerular filtration (GFR). This results in loss of kidney function in the cephalic cisplatin process and triggers kidney failure.

Oxidative stress such as reactive oxygen species (ROS) has been shown to be a significant factor in cisplatin nephrotoxicity.³ Some mechanisms regarding the role of ROS in this pathological condition are this substance causing accumulation of ROS and oxidative stress cells which can continue to mitochondrial dysfunction and increased the production of ROS through a disrupted respiratory chain. A rise of reactive oxygen species (ROS) was successfully observed when cisplatin was exposed to renal tubular cell cultures, kidney fragments, and in all parts of animals in vivo.^{4,5} ROS directly attack molecular components of cell including lipids, proteins and DNA and damage their structure.⁶ In the presence of cisplatin, ROS is produced and involved in the pathogenesis of cisplatin-induced kidney damage.⁷

Vitamin E is a fat-soluble vitamin and is included in non-enzymatic antioxidants. Vitamin E prevent or reduce the cell damage caused by oxidative stress by preventing the occurrence of fat peroxidation reactions by ROS in membrane cells.⁸ Oral supplementation of vitamin E is known to be effective in lowering the incidence and severity of cisplatin-induced nephrotoxicity.⁹ It is important to know that natural products from antioxidants may detoxify ROS in the kidneys, without affecting the anticancer efficacy of cisplatin, even though the active ingredients still remain unknown, but the protective effects on kidneys have been proven to be true in humans and vitamin E may have potential therapeutic applications.

OBJECTIVE

To analyze the role of vitamin E α -tocopherol on cisplatin toxicity in Sprague Dawley mice kidney.

MATERIAL & METHODS

This research is a laboratory experimental study with post-test only control group design. The subjects of the study were Sprague Dawley white male rats aged 10-12 weeks old with a weight of 200-250 grams. This study has obtained ethical approval with a registration number 2.KE.027.02.2019.

24 white male rats were obtained from the integrated research institute of Gadjah Mada University (UGM) Yogyakarta. Experimental animal acclimatization was carried out for two weeks before being given treatment with exposure to a dark-light irradiation cycle of 12 hours for each group. Mice are kept in cages with a size of 1600 cm² at room temperature and regular air circulation. Food in the form of pellets is given as much as 20-25 grams per day with clean water that is always available.

The process of animal grouping was randomly assigned into four treatment groups, namely: negative control group (CN) which received normal saline injection treatment 0.9% 1 cc intraperitoneal, positive control group (CP) received cisplatin injection (Cisplatin, Kalbe Farma, Indonesia) intraperitoneal with a dose of 5 mg/kg, group P1 was given vitamin E (Blackmores, Australian Catalent) 50 mg/kg per Sonde for 7 weeks combined with injection of cisplatin 5 mg/kg intraperitoneal, P2 group was given vitamin E treatment (Blackmores, Catalent Australia) 200 mg/kg per Sonde for 7 weeks combined with injection of cisplatin (Cisplatin, Kalbe Farma, Indonesia) 5 mg/kg. Cisplatin exposure in each treatment group was carried out in the third week with an intraperitoneal injection route.

At the end of the 7th week of treatment, each experimental animal was sacrificed to analyze the degree of kidney damage. Anesthesia in experimental animals was carried out using 75 mg/kg intraperitoneal ketamine. After anesthesia, the mouse was positioned supine. The mice were fixed on the fixation board for layer by layer incisions to make the kidney visible. Arteries and veins around the kidney are clamped before the action is continued with nephrectomy. The rest of experimental animals and unused organs will be destroyed.

The method for examining histological changes in this preparation is using the modified

Klopfleisch (2013) method. Observations were made on the glomerulus and proximal tubules. Observations were performed at 100 and 400 times magnification using a light microscope. At 100 times magnification, observation is focused on seeing tubular dilatation and glomerular damage lesions. Meanwhile, at 400 times magnification, the observation is focused on assessing the presence of tubular degeneration, tubular necrosis, and interstitial inflammatory cell infiltration. Kidney damage scoring in each group is the average amount of all forms of occurred lesions. All scoring examinations use the Olympus CX21 light microscope, taking pictures to use a Nikon H6001 light microscope equipped with a 300 megapixel DS Fi2 digital camera and Nikkon Image System graphics processing software.

Data obtained from observations will be quantified and calculated statistically by one-way ANOVA parametric test. If the data is not normally distributed, statistical analysis will be done using the Kruskal-Wallis non-parametric test.

RESULTS

The normality test was carried out on the score of nephron damage degree obtained in each

group. From the Shapiro Wilk normality test, the scores of renal nephron damage degree were normally distributed and each group was homogeneous with $p > 0.05$ (Table 1). Then a comparative test was performed on the mean score of the nephron damage degree using the One Way ANOVA parametric test. The results showed a significant difference in the mean between study groups with a $p < 0.05$ (Table 1). Data variability between groups was further analyzed using the Levene test, and there was no significant difference in data variance between groups with a $p > 0.05$. Therefore, the LSD post hoc test was used to determine the difference in nephron damage in each treatment group.

The result is a significant difference in nephron damage in all treatment groups when compared with the negative control group with $p < 0.05$ (Table 2). Furthermore, no significant differences in glomerular damage were found in the positive control group compared to the treatment group which received vitamin E 50 mg/kg supplementation. The treatment group is also found a significant difference in nephron damage in the group that received vitamin E supplementation of 50 mg/kg compared to the group that received vitamin E supplementation 200 mg/KgBB (Figure 1).

Table 1. The effect of vitamin E on mice kidney that received cisplatin.

Group	n	Mean \pm SD	Normality	p value
Negative Control	4	3 \pm 0,707	0.161	0.00*
Positive Control	6	6.83 \pm 0,601	0.601	
Group 1	8	8.13 \pm 0,611	0.425	
Group 2	8	10.75 \pm 0,412	0.114	

CN: Control group

CP: Cisplatin group

P1 : Cisplatin + Vitamin E 50 mg/kgBW group

P2 : Cisplatin + Vitamin E 200 mg/kgBW group

Tabel 2. Comparison of nephron damage between groups.

Comparison of nephron damage between groups	Mean Difference	Confidence interval 95%		p value
		Lower bound	Upper bound	
CN vs CP	-3.883*	-5.79	-1.87	0.001
CN vs P1	-5.125*	-6.99	-3.26	0.000
CN vs P2	-7.750*	-9.61	-5.89	0.000
CP vs P1	-1.292	-2.93	0.35	0.117
CP vs P2	-3.917*	-5.56	-2.28	0.00
P2 vs P1	-2.625*	-1.11	-4.14	0.02

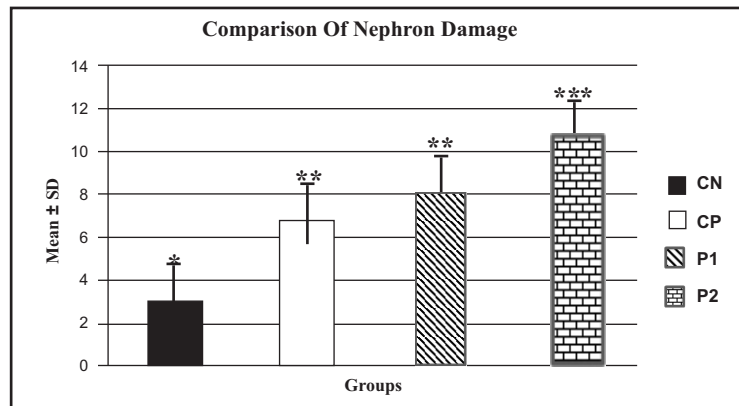


Figure 1. A Comparison of nephron damage in groups.

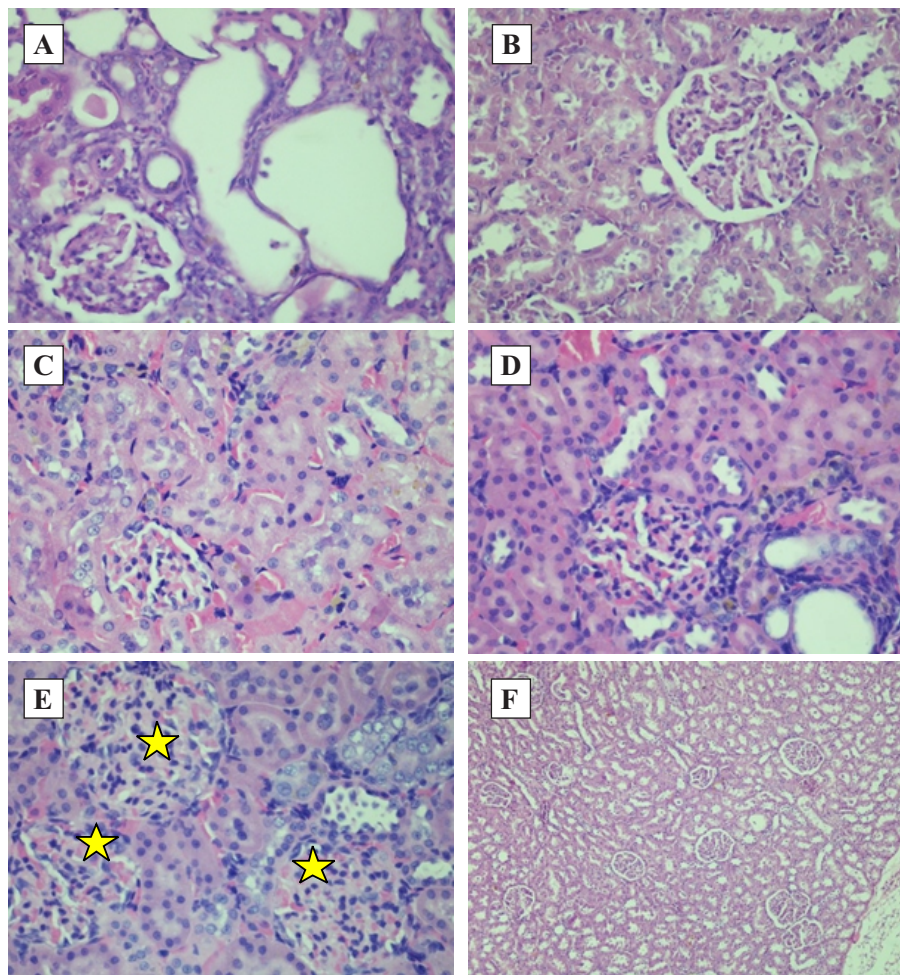


Figure 2. The results of histopathological examination using Hematoxylin Eosin (HE) staining. A and B. Microscopic images of dilated kidney tubules, lumen tubules enlargement is marked with star symbols (400X): C. Microscopic images of tubular cell degeneration, enlarged tubular cells, narrowed/full tubular lumen of 400X magnification: D. Microscopic images of tubular cells necrosis (the core appears to be picnosis), 400X magnification: E. Microscopic images of glomerular enlargement, the glomerulus appear large and full, marked with star symbols of 400X magnification; F. Microscopic images of kidneys at 200x.

DISCUSSION

Cisplatin or commonly known as Peyronie chloride first synthesized in 1845 by Michele Peyrone. This drug was finally used against ovarian and testicular cancer in 1978 by the license from the Food and Drug Administration. Subsequently, cisplatin (dichlorodiamino platinum) became an inorganic platinum-based chemotherapy agent that has widely used in the treatment of various malignant solid tumors.¹⁰ Until now, cisplatin has been used in the treatment of cancer of the testis, ovary, bladder, head and neck, esophagus, lung, breast, cervix, stomach, prostate cancer, Hodgkin and non-Hodgkin's lymphoma, neuroblastoma, sarcoma, multiple myeloma, melanoma and mesothelioma. Although it is famous for its mechanism of action damaging DNA, cisplatin also causes dysfunction of cytoplasmic organelles especially the endoplasmic reticulum and mitochondria. Cisplatin also activates the apoptotic pathway and causing cell damage through oxidative and inflammatory stress.^{10,11}

By the first time usage of cisplatin within certain doses (50-100mg/m²), about one-third of patients experienced nephrotoxicity. The main target of toxic effects by various chemical agents is the kidney, so drug-induced AKI (Acute Kidney Injury) has often found in clinical medicine. The incidence of nephrotoxic AKI is difficult to predict because of the variability in population and the criteria for AKI. However, nephrotoxicity has already been reported to suggest that cisplatin induce hospital-acquired AKI by 8-16%.¹²⁻¹⁴ Renal vascular resistance, decreased renal plasma flow, and glomerular filtration (GFR) makes cisplatin nephrotoxicity an ideal model for studying the mechanism that induces all types of AKI.¹⁵

The use of cisplatin in this study has shown significant glomerular damage compared to the control group. These results are in accordance with the results of previous studies, which showed that cisplatin has the effect of nephrotoxicity. Like the antineoplastic effect, cisplatin has other nephrotoxic effects on various parts of the nephron. The nephrotoxic effects are tubular damage, prolong inflammation and vascular injury.⁵ Cisplatin enters kidney cells with a passive mechanism and/or facilitated channel. Tubular cell exposure to cisplatin initiates some pathways of cell death (MAPK, p53, ROS, etc.) or cytoprotective (p21). Cisplatin also known induces TNF- α expression in renal tubular

cells, subsequently promotes inflammatory response, which in turn contributes to tubular cell injury and death. Cisplatin also causes injury to the renal arteries, causing ischemic tubular cell death and a decrease in glomerular filtration rate. Acute kidney failure could take place by the stimulation of cisplatin. There are four mechanisms of cisplatin-induced AKI: (1) proximal tubular injury, (2) reactive oxidative stress (ROS), (3) inflammation and the last one is kidney vascular injury. Proximal tubular injury divide into different mechanisms including apoptosis, autophagy, cell cycle protein dysregulation, activation of mitogen-activated protein signaling pathways (MAPK), renal tubular epithelial cells direct damage, DNA damage response, and mitochondrial impairment.¹⁴

One of the mechanisms of AKI induced by cisplatin is oxidative stress. Oxidative stress defined as an imbalance between free radicals production and elimination. Subsequent generation of reactive oxygen species (ROS), accumulation of kidney lipid peroxidation residual, and the lack of antioxidant is considered to be the main factor of Cisplatin-induced AKI.^{16,17} Three mechanisms have been proposed that can produce ROS. First, its interaction with glutathione during activation into stronger nephrotoxins depletes powerful antioxidant molecules cell such as glutathione. In cells, cisplatin is converted into a very reactive form that get rapidly reacted with antioxidant molecules containing tool such as glutathione. As a result, thinning of glutathione causes an increase in oxidative stress in cells. Second, cisplatin also can cause mitochondrial dysfunction and advance further ROS production through disturbed respiratory chains. The third is cisplatin can induce ROS expression through the cytochrome P450 (CYP) system.¹⁴

Vitamin E was first discovered in 1922 by Evan and Bishop. Vitamin E deficiency was observed in pregnant mice who had syndrome deficiency, then impaired absorption in mice fetuses improved when given vegetable oil and salads. Discoveries about vitamin E provide greater space for structural exploration and function of vitamin E.^{18,19} There are 2 subclassifications of Vitamin E, namely tocopherol and tocotrienol. Each subclass consists of α , β , δ , and σ . The biological and antioxidant activities of each subclass of vitamin E are not a coincidence, for example, Tocopherol which is the most partition in the human diet has an antioxidant capacity 50% smaller than α -Tocopherol. Until now, α -Tocopherol is the most potent form of vitamin E as an antioxidant.²⁰

Vitamin E has hydrophobic and lipophilic properties, so it is also known as potent lipid soluble antioxidant. The distribution of vitamin E in the body starts from the absorption process by kilomicon in the small intestine. Kilomicon will circulate in the lymph vessels that lead to the thoracic duct and join the circulation of Subclavian veins in the blood vessels. Release Vitamin E in the free form of kilomicon is mediated by the LPL enzyme (lipoprotein lipase). Remnants from kilomicon which have undergone release of vitamin E will be taken to the liver to be recycled into very low density lipoprotein (VLDL) or low density lipoprotein (LDL). Free vitamin E and its isoforms can act as chain-breaking antioxidants and as a deterrent to the formation of free radicals in the cellular environment. Free-form of residual vitamin E is usually found in very low concentrations in cell membranes (1:1000), but this molecule is still considered to play lipid soluble antioxidants in the body.²¹

Free radicals are basically natural biological processes that occur in every organism. Electron transport that occurs between the cytochrome chains at each cellular respiration cycle will result in electron emissions acting as free radicals.¹⁸ Unstable free radical molecules have an affinity for destabilizing stable molecules by electronic gradient. One free radical chain that may occur at the cellular level is destabilization of Polyunsaturated Fatty Acid (PUFA). Free radical reacts with PUFA will result in the formation of PUFA, which will be oxidized to form Polyunsaturated lipid peroxyl radical (PUFA-OO). The affinity of PUFA-OO for hydrogen, which is higher than other stable molecules will result in the chain transfer of hydrogen and electron called "free radical chain reaction".²¹

Vitamin E in the form of T-Tocopherol (TocH) acts as a substance that reacts with PUFA-OO with the end result of hydroperoxide polyunsaturated fatty acids (PUFA-OOH) which is not a form of free radicals. The presence of the phospholipase A2 enzyme located in the cell's phospholipid membrane results in the formation of free-PUFA-OOH which is released into the cytosol. Activation of the enzymatic chain consisting of the cascade of superoxide dismutase, catalase, and glutathione peroxidase converts changes in PUFA-OOH to hydroxy polyunsaturated fatty acid (PUFA-OH). Free radical conversion to a stable molecule showed that Vitamin E has an antioxidant effect.²²

Besides being able to act as an antioxidant, Vitamin E derivatives such as α -Tocopherol also have pro-oxidant effects. Because every time the anti-oxidative mechanism occurs, α -Tocopherol radical will also be formed (α -Toc). Basically the pro-oxidative effect that occurs will be suppressed by other reactions involving α -Toc and vitamin C (L-Ascorbic Acid, Asa). The reduction that involves the enzyme Glutathione (GSH) in the cytosol and Ubiquinol (UQH2) in the cell membrane acts as the next non-oxidative molecule formation. The pro-oxidant effect on vitamin E provides a new picture of the metabolic properties of Vitamin E which turns out to have an antagonistic effect with the main working of this molecule itself.¹⁷ In certain conditions such as increased consumption of vitamin E or the presence of defects in GSH and UQH2 enzymes, it is possible to increase oxidative reactions which lead to an increase in free radical reactions in the body.

The results of this study, exposure to low doses of vitamin E did not provide a statistically significant difference compared to the group exposed to cisplatin alone. However, the level of damage to the nephrons that occurred between the two groups was seen to be greater in the treatment group that received cisplatin and a lower dose of Vitamin E. In addition, exposure to higher levels of vitamin E resulted in a significant degree of nephron damage compared to the control group and treatment group that exposed to low dose of vitamin E. It indicates that exposure to vitamin E in the damaged mice kidney will initiate a pro-oxidative mechanism and lead to increased progression of kidney damage. This might occur because of a disruption of the mechanism of transformation residual vitamin E in the form of radical α -Tocopherol. Previously administered cisplatin exposure might have an effect in decreasing the expression of essential cellular regulator proteins. The decrease in this protein can also occur in GSH and UBQ2 proteins which act as reductor α -Toc agents. The result of the previous event is the accumulation of -Toc which results in the formation of large amounts of free radicals at the cellular level.

In addition, in this study the parameters used were the changing of kidney tissue assessed from histopathological results. The degree of kidney damage assessed in this study was observed based on the acute and chronic inflammatory responses that occur in the tubules and glomerular kidneys. Cisplatin administration has been known to cause

nephrotoxicity. This has been proven by observation made in this study, which showed an increase in the score of kidney damage after exposure of cisplatin. Changing in renal histopathological in this study also serve as a reference for the effects of vitamin E on mice nephrons. The reference of the formation free radical in this study is inflammation caused by changing of free radical cell. One of the derivatives of vitamin E is α -Tocopherol, which is able to induce the decreasing free radical activity in cells by stimulating the formation of hydroxy polyunsaturated fatty acid (PUFA-OH). The solubility of very low PUFA-OH therefore the phospholipase A2 (PLA2) enzyme is needed.

The increasing expression of PUFA-OH will automatically result in increasing of PLA2 expression. In addition to increasing the solubility of PUFA-OH, PLA2 also play a role in initiating the inflammatory cascade. PLA2 also causes the formation of arachidonic acid which later will play an important role in the inflammatory response. The administration of vitamin E basically will increase PUFA-OH and PLA 2 enzymes. This may explain the microscopy picture of inflammatory in the administration of Vitamin E will result in chronic inflammation of the tubules and kidneys.²³

CONCLUSION

There was no significant increase in the degree of glomerular and tubular damage in mice that received cisplatin and vitamin E exposure at a dose of 50 mg/kg compared to glomerular and tubular mice that received cisplatin exposure alone, but there was an increased degree of glomerular and tubular damage in mice that received cisplatin and vitamin E exposure at dose of 200 mg/kg compared to glomerular and tubular mice that received to cisplatin and vitamin E exposure at dose of 50 mg/kg.

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