THE EFFECT OF VITAMIN E (A TOCOPHEROL) AS NEPHROPROTECTOR ON BLOOD UREA NITROGEN AND SERUM CREATININE LEVEL OF STRAIN WISTAR RATS AFTER CISPLATIN TREATMENT: IN VIVO STUDY

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ABSTRACT

Objective: To analyze the differences in kidney function of Wistar strain rats that received a combination of vitamin E and cisplatin, compared with cisplatin alone. *Material & Methods:* An experimental prospective study with post-test only control design, using male Wistar strain rats (Rattus norvegicus). Rats were randomized using the simple randomized sampling method. Treatment was given in the form of exposure to cisplatin 5 mg/kg (positive control group), with a combination of vitamin E 100 mg/kg and 200 mg/kg (treatment group), to evaluate its effect on kidney function as measured by blood urea nitrogen (BUN) and creatinine serum. *Results:* Statistical analysis of Mann Whitney showed that there were no differences in BUN levels in the positive control group (cisplatin 5 mg/kg) against each treatment group (p>0.05). Further analysis showed that there was no significant difference between treatment group 1 (Vitamin E 100 mg/kg) and treatment group 2 (Vitamin E 200 mg/kg). There was no difference in serum creatinine levels in the positive control group compared to a treatment group that received both vitamin E 100 mg/kg and the vitamin E 200 mg/kg (p>0.05). further analysis revealed no significant difference in serum creatinine levels between the group that receives vitamin E 100 mg/kg and 200 mg/kg. *Conclusion:* Vitamin E at doses of 100 mg/kg and 200 mg/kg did not have the nephroprotective feature in cisplatin-exposed Wistar rats.

Keywords: Blood urea nitrogen, cisplatin, nephroprotective, serum creatinine, vitamin E.

ABSTRAK

Tujuan: Menganalisis perbedaan fungsi ginjal tikus strain Wistar yang mendapatkan kombinasi vitamin E dan cisplatin, dibandingkan dengan cisplatin saja. *Bahan & Cara:* Studi prospektif eksperimental dengan post test only control design, menggunakan binatang coba tikus putih (Rattus norvegicus) jantan strain Wistar. Tikus dirandomisasi menggunakan metode simple ramdomized sampling. Pada binatang coba diberikan perlakuan berupa paparan cisplatin 5 mg/kg BB (kelompok kontrol positif), dengan kombinasi vitamin E 100 mg/kg BB dan 200 mg/kg BB (kelompok perlakuan), untuk melihat pengaruhnya terhadap fungsi ginjal yang diukur dengan marker blood urea nitrogen (BUN) dan serum kreatinin. *Hasil:* Tidak terdapat perbedaan kadar BUN pada kelompok kontrol positif (cisplatin 5 mg/kgBB) terhadap masing-masing kelompok perlakuan (p>0.05). Analisis lanjutan menujukan bahwa tidak ada perbedaan secara signifikan terhadap kelompok perlakuan yang mendapatkan Vitamin E 100 mg/KgBB dengan kelompok perlakuan yang mendapatkan Vitamin E 200 mg/KgBB. Salian itu, tidak didapatkan perbedaan kadar serum kreatinin pada kelompok kontrol positif dibandingkan dengan kelompok perlakuan yang mendapatkan vitamin E 100 mg/KgBB dan kelompok paparan Vitamin E 200 mg/KgBB (p>0.05). Perbedaan juga tidak ditemukan pada kadar serum kreatinin antar kelompok perlakuan yang mendapatkan vitamin E 100 mg/KgBB dan 200 mg/KgBB. *Simpulan:* Vitamin E pada dosis 100mg/KgBB dan 200mg/KgBB tidak memiliki fungsi sebagai nefroprotektor pada tikus putih Wistar yang terpapar cisplatin.

Kata Kunci: Blood urea nitrogen, cisplatin, nefroprotektor, serum kreatinin, vitamin E.

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INTRODUCTION

Cisplatin is an antitumor agent that is used as a therapy for various malignancies, for example in the field of urology such as the bladder, testicular and penile cancers.[1] Cisplatin (DDP, cis-diamminedichloroplatinum II) is an effective antitumor agent with a wide variety of activities in dealing with solid

tumors.[2] However, cisplatin has nephrotoxic side effects in patients because it causes necrosis of the proximal tubule and apoptosis from the distal nephron.[3]

The prevalence of cisplatin nephrotoxicity is quite high with one-third of patients undergoing cisplatin therapy will find a decrease in glomerular filtration function, an increase in serum creatinine and a decrease in serum magnesium and potassium levels.[4] Significant increases in serum creatinine and non-significant increases in Blood Urea Nitrogen (BUN) after cisplatin administration was found to be five times greater than before treatment.[5]

The mechanism of nephrotoxicity by cisplatin is complex and is a manifestation of various multifactorial such as inflammation, production of Reactive Oxygen Species (ROS), Reactive Nitrogen Species (RNS), and cellular apoptosis.[3] Besides cisplatin induces a decrease in antioxidant levels in plasma, this causes the phenomena of the failure of the antioxidant defense mechanism against oxidative damage induced by antitumor drugs.[6]

Vitamin E consists of 8 fat-soluble groups and alpha-tocopherol has the highest biological activity among other molecules. Vitamin E is obtained from food intake and has various functions such as enzymatic activity, gene regulation, and inhibition of platelet aggregation. However, the most important function of vitamin E is its ability as an antioxidant and this ability makes vitamin E have an important role in the antioxidant defense system in cells.[7] Vitamin E has been widely studied as an antioxidant agent in the event of oxidative stress. The use of vitamin E in chemotherapy based on the use of cisplatin is still very limited. Besides, the relationship between kidney damage markers such as BUN and serum creatinine against oxidative stress is rarely investigated. So researchers want to further evaluate the role of vitamin E in inducing a reduction in oxidative stress and nephrotoxicity in the kidneys and its relationship with BUN and serum creatinine in mice.

OBJECTIVE

To analyze the differences in kidney function of Wistar strain rats based on BUN levels and serum creatinine that received a combination of vitamin E and cisplatin compared to cisplatin alone.

MATERIAL & METHODS

This research is an experimental study that uses male Wistar strain (Rattus norvegicus) white mouse. In animals, treatment was given in the form of cisplatin administration with a combination of vitamin E to see its effect on kidney function as measured by BUN markers and creatinine serum

The design of this research study is an experimental laboratory with a post-test only control group design, with the evaluation of kidney function as measured using BUN and creatinine serum after the experimental animals are treated. The grouping of experimental animals was carried out by randomization, with the repetition of 6 experimental animals in each group and there was a control group as a comparison (positive control and negative control).8 This study was approved by the ethical committee of the Faculty of Veterinary Medicine, Airlangga University, with the number 2.KE.149.07.2019.

Samples are grouped into 4 groups randomly, with the sample size in each group (r) needed is 6 rats. In this study there was a possibility that the sample had dropped out, therefore it was decided to take 7 samples per group so that the overall sample in this study were 28 male white rats (Rattus norvegicus) strain of Wistar.

Samples of male white rats (Rattus norvegicus) strain of Wistar were only obtained from the Department of Biochemistry of the Faculty of Medicine, Airlangga University, Surabaya, beginning with the process of adaptation in a cage/research environment for 2 weeks with a cycle of 12 hours of light, 12 hours of dark. The control group in this study included a negative control group (CN), which was given 1cc intraperitoneal 0.9% normal saline injection on day 7 as a placebo, then on day 10, this group would take blood samples. The positive control group (CP), which was given cisplatin treatment (Tokyo chemical industries Ltd, Japan) at a dose of 5 mg/kg intraperitoneal 1 time on day 7, then blood was taken on the day 10 for further measurement of BUN parameters and serum creatinine.

The sample of Wistar strain white rat (Rattus norvegicus) which newly obtained from the Department of Biochemistry of the Faculty of Medicine Airlangga Surabaya, begins with the process of adaptation in a cage/research environment for 2 weeks with a cycle of 12 hours of light, 12 hours of dark. There are two treatment groups, group P1, the group given the treatment of vitamin E (Blackmores, Catalent Australia) 100 mg/kg bodyweights orally for 2 days (figure 1) before and 2 days after cisplatin (Tokyo chemical industries Ltd, Jepang) 5 mg/kg of bodyweights intraperitoneal on

day 7, combined with cisplatin 5 mg/kg body weight intraperitoneal 1 time on day 7, then blood samples were taken on day 10 to measure BUN parameters and serum creatinine with an automatic analyzer. The second treatment group P2, the group treated with vitamin E 200 mg/kg bodyweight for 9 days, then blood samples were taken on day 10 to measure BUN and serum creatinine parameters with an automatic analyzer.

Data on kidney function marked with BUN and serum creatinine will be used in statistical analysis. The normality test is considered significant if p>0.05. One way Annova parametric test was used as a parametric test on data with the normal distribution. Kruskal-Wallis nonparametric test is used if the data do not meet the requirements for parametric tests. The comparative test in this study is meaningful if the p-value <0.05.9 All data

processing techniques are computerized analyzed using statistical software and service solution 20 for windows (SPSS 20).

Figure 1. Vitamine E administration on Rats for treatment groups.

RESULTS

Table 1. Rats body weight distribution.

Group	(Mean $\pm$ SD).	Normality	P-value
Negative Control	202.4 $\pm$2.9	0.160	0.506
Positive Control	201.5 $\pm$ 4.1	0.173	
Vitamin E 100 mg/KgBB	199.8 $\pm$ 2.4	0.210	
Vitamin E 200 mg/KgBB	201.1 $\pm$ 2.9	0.224	

Table 2. The comparison of BUN level on research subjects.

Group	(Median $\pm$ SE).	Lower Bound -Upper Bound	Normality	P-value
Control Negative	12 $\pm$ 0 71	10.53 – 14.03	0.120	0.001*
Control Positive	104 $\pm$ 12.34	66.21 – 126.63	0.200*	
Exposure of vitamin E 100 mg/KgBB	117 $\pm$ 8.41	103.54 – 144.7	0.026	
Exposure of vitamin E 200 mg/KgBB	107 $\pm$ 16.4	50.36 – 130.78	0.200*	

In this study, the research subjects were randomized to reduce research bias. Rats were randomized using the simple randomized sampling method. To assess the success of randomization, the Kolmogorov-Smirnov homogeneity test was performed. The results of normality weight data test subjects showed normal body weight of rats (p>0.05). Further analysis was carried out to assess differences in mean body weight of rats between groups using the Oneway Anova parametric test (Table 1). The results found no mean difference between treatment groups (p>0.05). From this analysis, it can be concluded that the randomization of research subjects has been successfully carried out.

In this study, the normality test was carried out using the Kolmogorov-Smirnov test, the result is the data distribution was abnormally distributed (p<0.05), hence a non-parametric Kruskal-Wallis test was performed. Kruskal Wallis nonparametric test results showed that there were significant mean differences between groups with p<0.05. Average data between groups were further analyzed to determine the difference in mean BUN levels between treatment groups with the Mann Whitney test (Table 2).

The statistical analysis results of the Mann Whitney test found that there were differences in the mean BUN levels in the negative control group to the

positive control group and each treatment group with p-value <0.05. On the other hand, there were no significant differences in BUN levels in the positive control group for the treatment group and between treatment groups with p>0.05. Further analysis showed that there was no significant difference between treatment group 1 and treatment group 2 (Table 3).

The sample in this study was the blood serum of rats obtained from research subjects. The analysis was performed on the value of serum creatinine levels in each study subject. The mean value of serum creatinine was then tested for normality using the Kolmogorov Smirnov test. Mean serum creatinine data between groups were normally distributed (p>0.05). The analysis was followed by a comparison of the average creatinine serum levels in each group using the one way ANOVA parametric test. The analysis showed that there were differences in mean

serum creatinine levels between treatment groups (p <0.05). Furthermore, Levene's test variability analysis is performed to determine the post hoc test to be used. Data on serum creatinine levels showed variability between groups (p <0.05). To prove further mean differences between groups, the post hoc Games Howell test was conducted (Table 4).

From the results of post hoc Annova Games Howell statistical analysis, it was found that there were differences in mean between the study groups when compared with the negative control group. Furthermore, differences in serum creatinine levels were not found in the positive control group compared to the treatment group who received vitamin E 100 mg/KgBB and the exposure group for Vitamin E 200 mg/KgBB. In addition, data on serum creatinine levels between treatment groups receiving vitamin E 100 mg/KgBB and 200 mg/KgBB also did not show significant differences (Table 5).

Table 3. Comparison of BUN level between each treatment group.

Group	P-Value
Control negative Vs Control Positive	0.002*
Control negative Vs Vitamin E 100 mg/KgBB	0.002*
Control negative Vs Vitamin E 200 mg/KgBB	0.002*
Control PositiveVs Vitamin E 100 mg/KgBB	0.180
Control Positive Vs Vitamin E 200 mg/KgBB	0.949
Vitamin E 100 mg/KgBB Vs Vitamin E 200 mg/KgBB	0.277

Table 4. Comparison of creatinine serum on research subject.

Group	n	Mean ± SD	Normality	P-value
Control negative	7	0.42 ± 0.03	0.200	0.000*
Control Positif	7	2.4 ± 0.78	0.200	
Exposure vitamin E 100 mg/KgBB	7	2.4 ± 0.57	0.200	
Exposure vitamin E 200 mg/KgBB	7	1.8 ± 0.7	0.200	

Table 5. Comparison of creatinine serum in every research subject for every group.

| Comparison of creatinine serum level in research group | Mean Difference | Confidence interval 95% | | P-Value |
		Lower bound	Upper bound	
Control negative Vs Control Positive	-2.03	-3.09	-1.01	0.020*
Control Negatif Vs Vitamin E 100 mg/KgBB	-2.11	-2.86	-1.36	0.000*
Control Negative Vs Vitamin E 200 mg/KgBB	-1.44	-2.461	-0.43	0.010*
Control positive Vs Vitamin E 100 mg/KgBB	-0.07	-1.18	1.02	0.997
Control positive Vs Vitamin E 200 mg/KgBB	0.07	0.36	0.99	0.515
Vitamin E 100 mg/KgBB Vs Vitamin E 200 mg/KgBB	0.66	0.99	-1.02	0.312

DISCUSSION

Cisplatin or Peyronie chloride was synthesized in 1845 by Michele Peyrone. Furthermore, cisplatin (dichlorodiamino platinum) became an inorganic platinum-based chemotherapy agent that is widely used in the treatment of various malignant solid tumors.[10] Cisplatin is used for the treatment of testicular, ovarian, bladder, head and neck cancer, esophagus, lungs, breast, cervix, stomach, prostate cancer, Hodgkin's and non-Hodgkin's lymphoma, melanoma and mesothelioma. Cisplatin can activate the apoptotic pathway and cause cell damage through oxidative stress and inflammation.[10-11]

After a single dose of cisplatin (50-100mg/m^2), one-third of patients experience nephrotoxicity. However, nephrotoxicity has been reported to contribute about 8-60% of AKI hospital-acquired cases.[12-14] Giving cisplatin in solid tumors starts from a dose of 40-50 mg/m^2 body surface area with a gap between doses for at least three weeks. The total dose reaches 100-120 mg/m^2 either as a single drug or in combination with other chemotherapy. Protection strategies in cisplatin therapy are to increase drug elimination by intravenous hydration; selection in using osmotic diuretics and reducing the use of nephrotoxic drugs.[15]

The use of cisplatin in this study has shown that kidney damage is marked by significantly increased serum BUN and creatinine levels compared to controls. These results are consistent with the results of previous studies which showed that cisplatin has a nephrotoxicity effect. Cisplatin can cause tubular damage, inflammatory damage in the interstitial, and vascular injury.[16] Exposure to cisplatin to tubular cells activates the activation of signaling pathways that cause cell death (MAPK, p53, ROS, etc.) or cytoprotective (p21).[17] In addition, cisplatin can increase TNF-a production, which will cause a strong inflammatory response, which in turn contributes to tubular cell injury and death. Cisplatin can also cause damage to kidney blood vessels, which results in ischemia of the kidney's tubular cells and as a result will decrease glomerular filtration rate (GFR). The pathophysiology of AKI cisplatin-induced involves 4 main mechanisms: (1) proximal tubular injury, (2) oxidative stress, (3) inflammation, and (4) injury to blood vessels in the kidneys.[14]

One mechanism of AKI that is induced by cisplatin is oxidative stress which is an imbalance between free radical production and consumption.

AKI caused by Cisplatin due to 3 things, namely, the formation of reactive oxygen species (ROS), accumulation of lipid peroxidation products in the kidney, and decreased antioxidant systems.[17-18] This study measures the level of creatinine and BUN 3 days after cisplatin injection and results as cisplatin increases both BUN and creatinine levels of the rats. Similar in some studies, cisplatin induces several kidney function alteration in day[3-7.1]

Deficiency of Vitamin E was observed in pregnant mice with deficiency syndromes and impaired absorption in the fetal rat which improved when given vegetable oil and salad.[19-20] There are 2 subclassifications of Vitamin E, namely: tocopherol and tocoretinol. Each subclass consists of α, β, δ, dan σ. Until now, α Tocopherol is a form of vitamin E which has the most potent activity as an antioxidant.[21]

Vitamin E is hydrophobic and lipophilic, this vitamin is also known as a potent lipid-soluble antioxidant.[21] Intracellular, vitamin E acts as a chain-breaking antioxidant and prevents the formation of free radicals. Residual vitamin E in free form is usually found in very low concentrations in cell membranes (1: 1000), but this molecule is still considered a main lipid-soluble antioxidant in the body.[22]

Vitamin E in the form of α-Tocopherol (TocH) acts as a substrate that reacts with PUFA-OO with the result hydroperoxide polyunsaturated fatty acid (PUFA-OOH) which is not a free radical form. The presence of the phospholipase A2 enzyme located in the cell phospholipid membrane results in the formation of free-PUFA-OOH which is released into the cytosol. Activation of the enzymatic chain consisting of a cascade of superoxide dismutase, catalase, and glutathione peroxidase converts changes in PUFA-OOH to hydroxy polyunsaturated fatty acids (PUFA-OH). The antioxidant effect of vitamin E is to convert free radicals into stable molecules.[23]

In this study, exposure to vitamin E at doses of 100 mg/kg and 200 mg/kg did not provide a statistically significant difference compared to the group exposed to cisplatin. However, the mean BUN and serum creatinine levels showed better values in the group with the 200 mg/kg dose given. This shows the effect of vitamin E as nephroprotective is affected by the dose (dose-dependent). This is consistent with studies conducted by Dillioglugil et al. Which showed a significant decrease in MDA levels at higher vitamin doses of 200 and 400 mg/kg.[24]

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The study found that there was an increase in MDA and NO levels in kidney tissue exposed to cisplatin. Cell membrane components released MDA due to damage caused by free radicals.[25] The study found that there was an increase in MDA and NO levels in kidney tissue exposed to cisplatin. MDA is released by cell membrane components due to damage by free radicals.[25] In another study using vitamin C as a nephroprotective agent, serum creatinine levels were obtained in the administration of vitamin C doses of 100 mg/KgBB and 200 mg/KgBB significantly better than in the cisplatin group alone, but rat blood sampling was carried out on the seventh day after administration of cisplatin.[26]

For 20 years, both fundamental and clinical studies on vitamin E have a role in preventing AKI, developed from a single dose into a combination therapy. However, vitamin E supplementation has not been successful in providing consistent results in the AKI model. For this reason, the development of a new model of therapy in which vitamin E combination therapy with other factors in the form of vitamins, cells, drugs, or amino acids needs to be done. The provision of multidose vitamins C and E can provide more protection compared to vitamin E alone.[7]

In this study, there was no examination of serum BUN and creatinine levels before treatment, so it cannot be known about the kidney function of each subject. But in this study, the research subjects were obtained from the same provider with the same age, weight, and type. Then each research subject is entered into a group with blind randomization. So it can be concluded that it can represent the randomization of each subject in each group.

CONCLUSION

Although exposure to vitamin E did not provide a statistically significant difference compared to the group exposed to cisplatin, the mean BUN and serum creatinine levels showed better values in the group with the 200 mg/kg dose given. This shows the effect of vitamin E as nephroprotective is affected by the dose (dose-dependent). The development of a new model of therapy in which vitamin E combination therapy with other factors in the form of vitamins, cells, drugs, or amino acids needs to be done.

REFERENCES

1. Perše M, Večerič-Haler Ž. Cisplatin-induced rodent model of kidney injury: Characteristics and challenges. Biomed Res Int. 2018.
2. Rosenberg B, Charles F. Kettring prize. Fundamental studies with cisplatin. Cancer. 1985 May; 55(10): 2303-16.
3. Darwish MA, Abo-Youssef AM, Khalaf MM, Abo-Saif AA, Saleh IG, Abdelghany TM. Vitamin E mitigates cisplatin-induced nephrotoxicity due to reversal of oxidative/nitrosative stress, suppression of inflammation and reduction of total renal platinum accumulation. J Biochem Mol Toxicol. 2017; 31(1): e21833.
4. Arany I, Safirstein RL. Cisplatin nephrotoxicity. Semin Nephrol. 2003; 23(5): 460-4.
5. Arunkumar P, Viswanatha G, Radheshyam N, Mukund H, Belliyappa M. Science behind cisplatin-induced nephrotoxicity in humans: A clinical study. Asian Pac J Trop Biomed. 2012; 2(8): 640-4.
6. Weijl NI, Wipkink-Bakker A, Lentjes EGWM, Berger HM, Cleton FJ, Osanto S. Cisplatin combination chemotherapy induces a fall in plasma antioxidants of cancer patients. Ann Oncol. 1998; 9(12): 1331-7.
7. Liu P, Feng Y, Wang Y, Zhou Y, Zhao L. Protective effect of vitamin E against acute kidney injury. Liu F, Lee D-H, Lagoa R, Kumar S, editors. Biomed Mater Eng. 2015; 26(s1): S2133-44.
8. Zainuddin M. Metodologi Penelitian. Jakarta: Sagung Seto; 1995. 62-66 p.
9. Dahlan MS. Statistik untuk kedokteran dan kesehatan: uji hipotesis. Jakarta: Bina Mitra Press; 2006.
10. Manohar S, Leung N. Cisplatin nephrotoxicity: a review of the literature. J Nephrol. 2017.
11. Kunze D, Erdmann K, Froehner M, Wirth MP, Fuessel S. siRNA-mediated Inhibition of Antiapoptotic Genes Enhances Chemotherapy Efficacy in Bladder Cancer Cells. 2012; 4318: 4313-8.
12. Ozkok A, Edelstein CL. Pathophysiology of Cisplatin-Induced Acute Kidney Injury. 2014.
13. Volarevic V, Djokovic B, Jankovic MG, Harrell CR, Fellabaum C, Djonov V, et al. Molecular mechanisms of cisplatin-induced nephrotoxicity?: a balance on the knife edge between renoprotection and tumor toxicity. 2019; 9: 1-14.
14. Tchounwou PB, Dasari S. Cisplatin in cancer therapy?: molecular mechanisms of action. 2015; 364-78.
15. Tsang RY, Al-Fayea T, Au HJ. Cisplatin overdose: Toxicities and management. Drug Saf. 2009; 32(12): 1109-22.
16. Siddik ZH. Mechanisms of Action of Cancer Chemotherapeutic Agents?: DNA-Interactive Alkylating Agents and Antitumour Platinum-Based Drugs. 2002.
17. Miller RP, Tadagavadi RK, Ramesh G, Reeves WB. Mechanisms of Cisplatin Nephrotoxicity. 2010; 2: 2490-518.
18. Darwish MA, Saleh IG, Abo-youssef AM, Abdelghany TM, Khalaf MM, Abo-saif AA. Vitamin E mitigates cisplatin-induced nephrotoxicity due to

reversal of oxidative / nitrosative stress , suppression of inflammation and reduction of total renal platinum accumulation. 2016; 1-9.

19. Kontush A, Finckh B, Karten B, Kohlschutter A, Beisiegel U. Antioxidant and prooxidant activity of a-tocopherol in human plasma and low density lipoprotein. 1996; 37: 1436-48.

20. Herrera E, Barbas C. Vitamin E: action , metabolism and perspectives. 2001; 57(1): 43-56.

21. Pearson P, Lewis SA, Britton J, Young IS, Fogarty A. The Pro-Oxidant Activity of High-Dose Vitamin E Supplements in Vivo. 2006; 20(5): 271-3.

22. Tafazoli S, Wright JS, Brien PJO. Prooxidant and Antioxidant Activity of Vitamin E Analogues and Troglitazone. 2005; 1567-74.

23. Rizvi S, Raza ST, Ahmed F, Ahmad A, Abbas S, Mahdi F. The role of vitamin E in Human Health And Some Disease. 2014; 14: 157-65.

24. Dillioglugil MO, Maral Kir H, Gulkac MD, Özon Kanli A, Ozdogan HK, Acar O, et al. Protective Effects of Increasing Vitamin E and A Doses on Cisplatin-Induced Oxidative Damage to Kidney Tissue in Rats. Urol Int. 2005; 75(4): 340-4.

25. Yildirim Z. Oral erdosteine administration attenuates cisplatin-induced renal tubular damage in rats. Pharmacol Res. 2003; 47(2): 149-56.

26. Antunes G, Darin LM, Bianchi J M de LP. Protective Effects Of Vitamin C Against Cisplatin-Induced Nephrotoxicity And Lipid Peroxidation In Adult Rats: A Dose-Dependent Study. Pharmacol Res. 2000; 41(4): 405-11.