# ROLE OF VITAMIN E (α TOCOPHEROL) TO PREVENT THE SPERMATOGONIA, SERTOLI CELL, AND LEYDIG CELL DAMAGE IN RATS TESTICLE (STRAIN WISTAR) AFTER CISPLATIN TREATMENT

**[1]Rahmad Krismantoro, [2]I Ketut Sudiana, [1]Doddy M. Soebadi, [1]Fikri Rizaldi.**

[1]Department of Urology, Faculty of Medicine/Universitas Airlangga, Soetomo General Hospital, Surabaya.
[2]Department of Pathology Anatomy, Faculty of Medicine/Universitas Airlangga, Soetomo General Hospital, Surabaya.

***ABSTRACT***

***Objective:****To determine the difference in spermatogonium cells, leydig cells and sertoli cells count in white rats testicle (wistar strains) obtained with the combination of cisplatin and vitamin E compared with that only received cisplatin.* ***Material & Methods:*** *There were 4 random groups out of a total of twenty four winstar strain rats (n=6). The control group (I) injected normal saline 0.9% intraperitoneally (i.p.) as the placebo on the 3rd week. Group (II) given cisplatin (5 mg/kgbw) injection i.p. on 3rd week, Group (III) given cisplatin injection 5 mg/kgbw i.p. on 3rd week + vitamin E 50 mg/kgbw by gavage for 7 weeks and group (IV) cisplatin injection 5mg/kgbw i.p. on 3rd week + vitamin E 200 mg/kgbw by gavage for 7 weeks. Vitamin E was given 3 weeks before up to 4 weeks after cisplatin injection (total 7 weeks). Observations by calculating the average number of spermatogonia, sertoli and leydig cells on a cross-sectional section of the seminiferous tubule with Haematoxylin-Eosin staining using a 400 x light magnification microscope.* ***Results:*** *Cisplatin decreases spermatogonia, sertoli, and leydig cells significantly against control. Vitamin E 200 mg/kgbw significantly increased the number of spermatogonium, sertoli, and leydig cells ($p<0.05$) compared to group in combination with vitamin E 50 mg/kg bw and cisplatin or cisplatin only group. Only leydig cells count was significantly increased in the combination group of vitamin E 50 mg/kgbw and cisplatin compared to the cisplatin group.* ***Conclusion:*** *Vitamin E 200 mg/kgbw provides a protective effect against decreased spermatogonia, sertoli and leydig cells due to cisplatin 5 mg/kgbw exposure which its protectivity depends on the given dose.*

***Keywords:*** *Vitamin E, spermatogonium cells, sertoli cells, leydig cells, cisplatin.*

***ABSTRAK***

***Tujuan:*** *Untuk mengetahui perbedaan jumlah sel spermatogonium, sel leydig dan sel sertoli testis pada tikus putih strain wistar yang mendapatkan kombinasi cisplatin dan vitamin E dibandingkan dengan tikus putih strain wistar yang hanya mendapatkan cisplatin.* ***Bahan & Cara:*** *Sampel penelitian dibagi menjadi 4 kelompok group secara acak (n=6). Kelompok kontrol (I) diberikan injeksi normal saline 0.9% 1cc, 1x intraperitoneal (i.p) sebagai placebo pada minggu ketiga. Kelompok kedua (II) diberikan injeksi cisplatin 5mg/kgbb, 1x, intraperitoneal (i.p) pada minggu ketiga. Kelompok ketiga (III) diberikan vitamin E 50 mg/kgbb per sonde selama 7 minggu dan injeksi cisplatin 5mg/kgbb pada minggu ketiga, 1x, intraperitoneal (i.p). Kelompok keempat (IV) diberikan vitamin E 200 mg/kgbb per sonde selama 7 minggu dan injeksi cisplatin 5mg/kgbb pada minggu ketiga, 1x, intraperitoneal (i.p). Vitamin E diberikan secara per sonde mulai 3 minggu sebelum hingga 4 minggu sesudah injeksi cisplatin. Dilakukan orkidektomi bilateral pada minggu ke tujuh dan diperiksa secara histologis. Pengamatan dengan cara menghitung rerata jumlah spermatogonium, sel Sertoli dan sel Leydig pada 10 penampang sayatan melintang dari tubulus seminiferus dengan menggunakan mikroskop cahaya perbesaran 400x dengan pewarnaan Haematoxylin Eosin.* ***Hasil:*** *Cisplatin 5mg/kgbb menurunkan jumlah spermatogonium, sel sertoli dan sel leydig signifikan terhadap kelompok kontrol ($p<0.05$). Vitamin E 200 mg/kgbb (kelompok IV) signifikan meningkatkan jumlah sel spermatogonium, sel sertoli, dan sel leydig ($p<0.05$) dibandingkan kelompok yang mendapatkan kombinasi vitamin E 50 mg/kgbb dan cisplatin (III) maupun kelompok cisplatin (II) saja. Hanya jumlah sel leydig saja yang signifikan meningkat ($p<0.05$) pada kelompok kombinasi vitamin E 50 mg/kgbb dan cisplatin dibandingkan dengan kelompok cisplatin saja.* ***Simpulan:*** *Vitamin E 200 mg/kgbb memberikan efek protektif terhadap penurunan jumlah spermatogonium, sel sertoli dan sel leydig akibat paparan cisplatin 5mg/kgbb, protektifitas vitamin E tersebut tergantung pada dosis yang diberikan.*

***Kata Kunci:*** *Vitamin E, sel spermatogonium, sel sertoli, sel leydig, cisplatin.*

Correspondence: Fikri Rizaldi; c/o: Department of Urology, Faculty of Medicine/Universitas Airlangga, Soetomo General Hospital, Surabaya. Jl. Mayjen. Prof. Dr. Moestopo 6-8 Surabaya 60286. Phone: +62315501318; Fax: +62315024971. Mobile phone: +6282132031975. Email: fikririz@gmail.com.

## INTRODUCTION

Increased incidence rate of cancer per year reaches 6% in the general population. According to the Dutch National Cancer Registry, over last 20 years, the number of cancer survivors has improved from 366.000 to 692.000. In children, we found an increased number of cancers per year by 1% and in adolescents 1.5%.[1] In adolescent, one of the most common malignancies affecting in men is testicular cancer.[2] There are 83% testicular cancer survivors after undergoing cisplatin-based chemotherapy combined with surgery and/or radiotherapy.[3,4] However, an undesirable effect of anti-cancer drugs is often. It has been reported that cisplatin can cause damage to the liver, kidneys and testes.[5]

Most of the patients who received chemotherapy cisplatin were in reproductive years, and many of them became azoospermia for a long time, and some even became permanently infertile.[5] Research by Drasga reported that 96% of men with TGCT became azoospermia in a 2-month postoperative evaluation chemotherapy.[6] Cisplatin dose 400 mg/m$^2$ has been reported as threshold for cisplatin-related infertility.[7,8] If the dose is beyond the threshold, there may an irreversible suppression of sperm production.[8] Hansen et al., Report male infertility after received 6 cisplatin, vinblastine, and bleomycin chemotherapy regimens. Azoospermia was found in 28% of patients at 2 years, and 27% of them last up to 64 months.[9]

Reactive oxygen species (ROS) production increase, including free radicals and oxidative damage to bimolecular precess, is associated with the cisplatin cytotoxicity mechanism.[5] Free radicals that produced as a product chemical processes in the body, are atoms or molecules have one or more unpaired electrons. They can oxidize fats, amino acids, carbohydrates, and cause damage to deoxyribonucleic acid (DNA).[10] The conditions when there is an inequal among free radicals product and antioxidant defense capacity, especially ROS causes oxidative stress.[10] The excess amount of ROS can cause testicular cell and spermatozoa DNA damage.[11] Testicular tissue (spermatogonia, sertoli, and leydig cells) is sensitive to ROS, by lipid peroxidation process at cell membrane, it will trigger both apoptotic and necrotic of the cells.[12]

Testicular disintegration, cells apoptosis, and defect in leydig cells has been proven induced by cisplatin exposure in mice.[13-15] Significant lipid peroxidation and malondialdehyde (MDA) level enhancement also induced by cisplatin.[16] Oxidative stress causes infertility through its negative effects on spermatozoa such as increased motility loss, increased membrane damage, morphological depletion, viability, and spermatozoa ability.[16] Continuous ROS exposure may cause cellular dysfunction, apoptosis and necrosis. One way to protect the sperm cell wall from oxidative damage is by increasing the antioxidant intake.[16]

Vitamin E is one of the fat-soluble vitamins. It is easy to obtain and the price is relatively cheap in the market. Vitamin E is known to have strong antioxidant activity. Vitamin E can provide a reduction or prevent oxidative stress as protective effect to cells damage. Vitamin E works to prevent lipid peroxidase reactions in cell membranes in reactions caused by ROS which is known as non-enzymatic antioxidants.[17] Vitamin E has also been shown to improve infertility through in vitro studies in patients with non-obstructive infertility. By neutralizing the effects of free radicals due to the administration of cisplatin, it is expected that free radicals product will decrease.[17] The decreasing of free radical will be followed by the decrease of oxidative stress in tissue or cell to minimize damage in testes tissues or cells, nerves or other body tissues. Finally, the number of spermatogonium, Leydig cells, and Sertoli cells will increase, followed by an increase in the amount of testosterone and improvement of spermatogenesis.

## OBJECTIVE

To determine the difference in spermatogonium cells, leydig cells and sertoli cells count in white rats testicle (wistar strains) obtained with the combination of cisplatin and vitamin E compared with that only received cisplatin

## MATERIAL & METHODS

This was a laboratory experimental research with post-test only control group design, using white male rats (Rattus norwegicus) wistar strain. Ethical eligibility obtained from medical research ethics committee, Faculty of Medicine, Airlangga University.

Twenty-four Wistar strain rats obtained from laboratory animals Tropical Disease Center (TDC), Airlangga University Surabaya (weight 150-200 g, age 10-12 weeks). Environment adaptation process was done by giving 12 hours light cycle for

two weeks in long. The rats were kept in a cage about 1500 $cm^2$ for a group of 4 rats. Each group was placed in a separated cage, insulated between each mouse, and maintained in such a way to prevent the interaction among the rat. Foods and drinks are provided ad libitum. Types of food provided were commercial pellets "hi pro vite 593, 20-25 grams/day and drinking enough water.

The samples were divided randomly into 4 groups (n=6). The control group (I) performed only normal saline 0.9% injection intraperitoneally (i.p.) as the placebo on the 3rd week. Group (II) given cisplatin (5 mg/kg bw) injection i.p. on 3rd week. Group (III) injected cisplatin 5 mg/kg bw i.p. on 3rd week + vitamin E 50 mg /kg bw by gavage and group (IV) injected cisplatin 5 mg/kg bw i.p. on 3rd week + vitamin E 200 mg/kg bw by gavage. Vitamin E is given 3 weeks before up to 4 weeks after cisplatin injection (total 7 weeks).

All samples will be doing the bilateral orchidectomy on the 7th week of vitamin E treatment. All procedures were performed under sterile conditions. The rats were anesthetized with ketamine 75mg/kg BW intraperitoneally (i.p).

Formalin buffer 4% is used to fix the testicular tissue sample. Slide in made by standard technique using paraffin block. 5µm testis tissue was cut in, and staining was done using Hematoxylin and Eosin (HE). Evaluation on histopathological changes were investigated using 400x magnification microscope.

Observations by calculating the average number of spermatogonia, sertoli and leydig cells on a cross-sectional section of the seminiferous tubule in ten fields of vision, 8 edge cross-section, and two central area, using a 400x light magnification microscope with Haematoxylin-Eosin staining.

The result of the calculation of sertoli, leydig, and spermatogonium cells was ratio data. The normality test and variance test was done before the hypothesis test. If the data distribution was normal, then the hypothesis was tested using one-way ANOVA. But if the data were not distributed normally and variance were not same, then the hypothesis was tested using the alternative Kruskal-Wallis test.[18]

The hypothesis was determined based on the significance value (p). The results were significant if the p value <0.05. Furthermore, multiple comparison tests or Post hoc test LCD (if the variant was same) or Post hoc Test Tamhane (if the variant was not same) is done, if the significance value of one-way ANOVA was <0.05. If the p value of Kruskal-Wallis test is <0.05, then the next step to do is the Mean-Whitney test. The results were significantly different if $p<0.05$.[18] All the technical data processing was analyzed by computer using statistical software product and service solution version 20 for Windows (SPSS ver 20).

## RESULTS

Shapiro-Wilk statistic test showed that the number of spermatogonium cells distribution in each group is normal ($p>0.05$). One-way ANOVA test showed that there was significantly different of spermatogonium cell count in each group ($p<0.05$) (Table 1).

Based on variance homogeneity test, it is known that the data variant is not homogeneous with the p-value <0.05. Tamhane Post Hoc data analysis showed there was a significantly different in the number of spermatogonium cells between control group and cisplatin group. This indicates that cisplatin can significantly decrease the number of spermatogonium cells. Treatment of vitamin E is expected to prevent the decrease in spermatogonium cell.

The group that receives cisplatin and vitamin E 50 mg/kgbw had higher spermatogonium cell counts than the group only receiving cisplatin but not statistically significant ($p>0.05$). This condition indicates that administration of vitamin E 50 kgbw did not give significant protective effect to spermatogonium cell count from cisplatin exposure.

In contrast to the previous group, the group that receives cisplatin and vitamin E 200 mg/kgbw had higher spermatogonium cell counts than the group receiving cisplatin with a significant difference ($p<0.05$). Furthermore, there was a significantly different between spermatogonium count in the cisplatin and vitamin E 200 mg/kgbw group compared with the cisplatin and vitamin E 50 mg/kg bw group ($p<0.05$). This indicates that the administration of Vitamin E 200 mg/kgbw had a significant effect in preventing the decreasing of the spermatogonium cell count in the group that receive cisplatin injection and its effectiveness superior compared with vitamin E 50 mg/kgbw.

The number of sertoli cells distribution in each group was normal with a homogeneous variant ($p>0.05$). One-way ANOVA test analysis showed there was significantly different of sertoli cell count in each group ($p<0.05$) (Table 2).

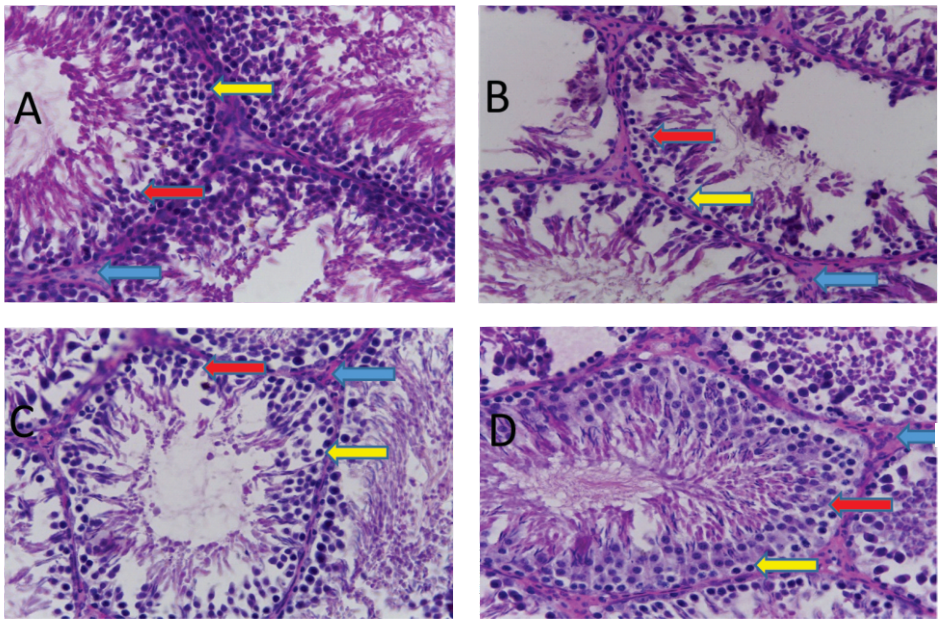
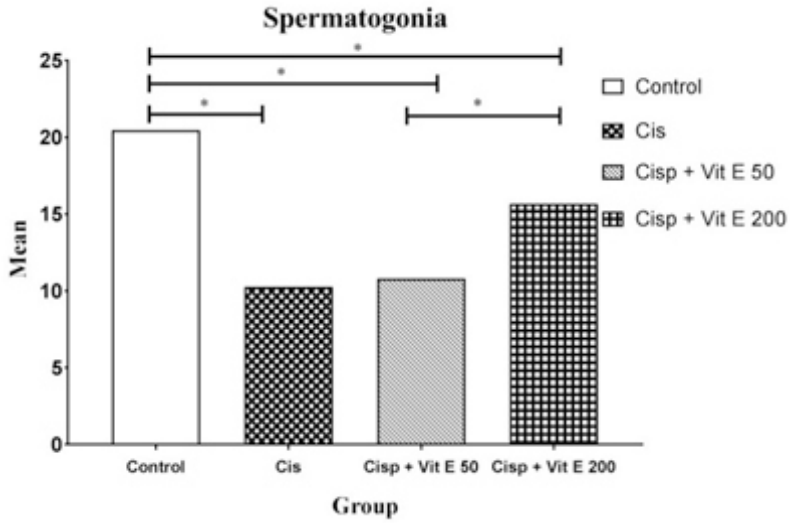



**Figure 1.** Histopathology of the research result. Microscopic cross-section of seminiferous tubules in rats' groups: A. Negative Control, B. Cisplatin Group 5mg/kgbw, C. Vitamin E Group 50 mg/kgbw + Cisplatin 5 mg/kgbw, D. Vitamin E Group 200 mg/kgbw + Cisplatin 5 mg/kgbw.
Description: Blue arrow shows Leydig cell. The yellow arrow shows the spermatogonium cell. The red arrow shows Sertoli cells. Nikon eclipse e-100 Microscope. Optilab Viewer 2.2. 400x.

**Table 1.** Comparison of the number of spermatogonium cells in each group.

| Group | n | Mean ± SD | p value |
|---|---|---|---|
| Control | 6 | 20.47 ± 0.28 | 0.00* |
| Cisplatin injection | 6 | 10.27 ± 0.87 | |
| Cisplatin injection + Vitamin E 50 mg/kgBB | 6 | 10.80 ± 2.65 | |
| Cisplatin injection + Vitamin E 200 mg/kgBB | 6 | 15.67 ± 1.90 | |

* = statistically significant

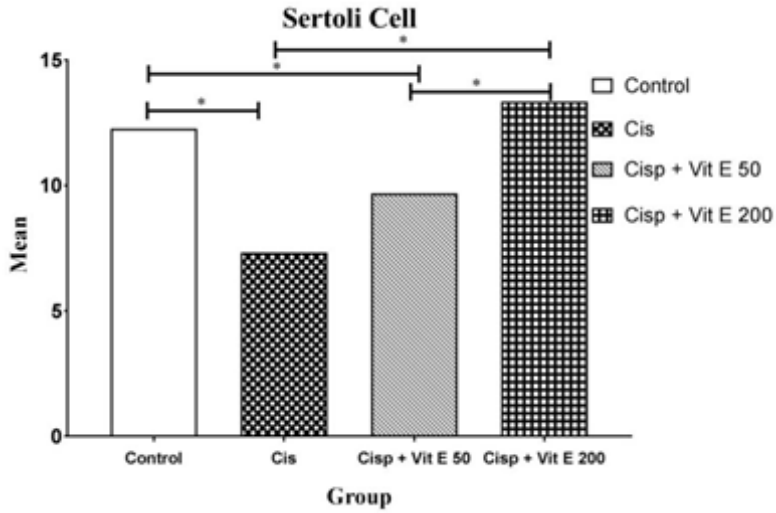



**Graph 1.** Comparison of the mean of spermatogonium cells per group. The asterisk sign (*) means that there was a significant comparison between each group.

**Table 2.** Comparison of the number of sertoli cells in each group.

| Group | n | Mean ± SD | p value |
|---|---|---|---|
| Control | 6 | 12.27 ± 2.95 | 0.00* |
| Cisplatin injection | 6 | 7.32 ± 1.11 | |
| Cisplatin injection + Vitamin E 50 mg/kgBB | 6 | 9.68 ± 1.38 | |
| Cisplatin injection + Vitamin E 200 mg/kgBB | 6 | 13.35 ± 1.83 | |

* = statistically significant




**Graph 2.** Comparison of the mean of spermatogonium cells per group. The asterisk sign (*) means that there was a significant comparison between each group.

**Table 3.** Comparison of the number of leydig cells in each group.

| Group | n | Mean ± SD | p value |
|---|---|---|---|
| Control | 6 | 7.10 ± 0.87 | 0.00* |
| Cisplatin injection | 6 | 5.42 ± 1.36 | |
| Cisplatin injection + Vitamin E 50 mg/kgBB | 6 | 6.60 ± 0.67 | |
| Cisplatin injection + Vitamin E 200 mg/kgBB | 6 | 7.77 ± 0.63 | |

* = statistically significant

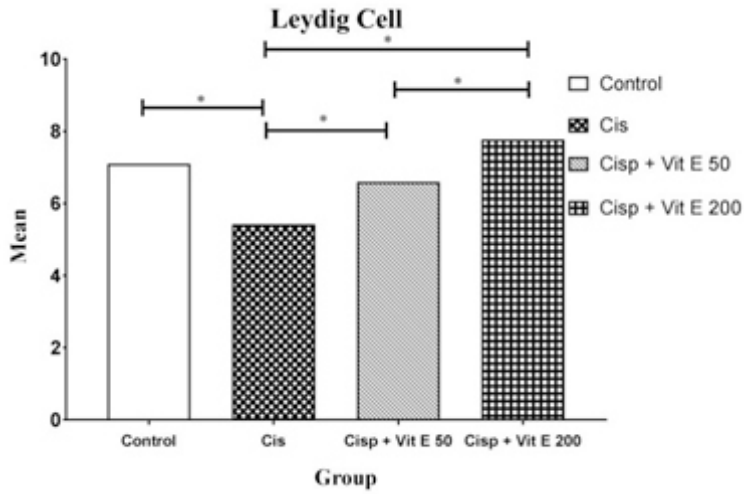
Based on the analysis of Post Hoc LSD data, there were significantly different in leydig cells count between control group and cisplatin group ($p<0.05$). This indicates that cisplatin significantly decreases the number of leydig cells compared to control.

The group that receives cisplatin and vitamin E 50 mg/kgbw had higher leydig cell counts than the group receiving cisplatin with a significantly different ($p<0.05$). Similarly in groups receiving cisplatin and vitamin E 200 mg/kgbw.

The cisplatin and vitamin E 200 mg/kgbw group had higher leydig cells counts than the cisplatin and vitamin E 50 mg/kgbw group with a significantly different ($p<0.05$). This indicates that vitamin E 50 mg/kgbw and vitamin E 200 mg/kgbw administration give a significant effect in preventing the number of leydig cells reduction in the cisplatin injection group, but the effect in vitamin E 200 mg/kgbw dose is superior than the vitamin E 50mg/kgbw dose.

The number of leydig cells distribution in each group was normal and homogeneous variants ($p>0.05$). One-way ANOVA test indicate significant differences between groups ($p<0.05$) (Table 3).

Based on Post Hoc LSD analysis data, it was found that the number of leydig cells was significantly different between the control group and




**Graph 3.** Comparison of the mean of leydig cells per group. The asterisk sign (*) means that there was a significant comparison between each group.

the cisplatin group ($p<0.05$). This indicates that cisplatin significantly decreases leydig cells count compared to controls.

Cisplatin and vitamin E 50 mg/kgbw group had higher leydig cell counts than the group receiving cisplatin with a significantly different ($p<0.05$). Similarly in groups receiving cisplatin and vitamin E 200 mg/kgbw

Cisplatin and vitamin E 200 mg/kgbw group had higher leydig cell counts than the cisplatin and vitamin E 50 mg/kgbw group with a significantly ($p<0.05$). This indicates that vitamin E 50 mg/kgbw and vitamin E 200 mg/kgbw administration provide significant effect in preventing the number of leydig cells reduction due to cisplatin exposure, but the effect in vitamin E 200 mg/kgbw dose is superior than the vitamin E 50 mg/kgbw dose.

## DISCUSSION

Prolonged testicular defect is one of the most disrupt adverse effects of cisplatin, despite its favorable results primarily as a testicular malignancy therapy.[19] By Increasing curative response, post-therapy cisplatin reproductive function began more thoughtfulness. That spermatogenesis could be disrupted reversibly and irreversible after chemotherapy is already known, depending on duration, and chemotherapy dose. It is important to minimize the spermatogenic damaged.[20]

Adverse effects after cisplatin therapy are primarily related to spermatogenic damage. There has been reported decreased spermatogenesis, and germ cell apoptosis after cisplatin exposure.[21] Testicular disintegration, germ cell apoptosis, and Leydig cells abnormalities has been proven induced by Cisplatin exposure in mice.[13-15]

In this study, cisplatin 5mg/kgbw injection intraperitoneal significantly reduced spermatogonium, sertoli and leydig cell counts compared with the control group ($p<0.05$). It is consistent with studies conducted by Ciftci et al., which suggest that cisplatin causes reproductive-toxicity through oxidative stress, histological defect, serum testosterone reduction and changes in sperm profile.[21]

Reactive oxygen species (ROS) production enhancement, including free radicals and oxidative damage to bimolecular process, is associated with the cisplatin cytotoxicity mechanism.[5] When endogenous and exogenous agents react with organism cells, it will produce (ROS) such as hydrogen peroxide, superoxide anions, hydroxyl radicals and singlet oxygen.[10] Free radicals have high levels electron affinity in oxidation-reduction reaction, they can oxidize fats, amino acids, carbohydrates, and cause damage to deoxyribonucleic acid (DNA).[10,22]

The amount of free radicals, especially unbalanced ROS with antioxidant defense capacity, results in oxidative stress.[10] Oxidative stress causes damage to the cellular elements of the testes and DNA spermatozoa damage.[11] Some research have shown that in cisplatin induced cytotoxicity, oxidative stress occupies an substantial role. It causes decrease GSH intracellular concentration, reduce antioxidant enzyme activity and enhance ROS production.[23] Atasayar et al., showed that a

combination of vitamin C and E with cisplatin administration was able to reduce histopathologic changes induced by cisplatin in the kidney.[24]

Testicular tissue is sensitive to free radical ROS. Lipid peroxidation occurs when ROS binds to cell membranes in the testes such as leydig, spermatogonia, and sertoli membrane cell which in turn will induce both apoptotic and necrotic process of these cells.[12] The damage of testicular cellular element produced by the chemotherapeutic agents may be known from decreasing sperm parameters, sertoli and leydig cells in seminiferous tubules. This is suitable with a research by Ganga et al., which stated there were decreased superoxide dismutase and catalase, induced lipid peroxidation, increased levels of malondialdehyde in testicular mice exposed by cisplatin.[25]

It also showed that based on descriptive data it was found that the group receiving cisplatin and vitamin E 200 mg/kgbw had higher spermatogonium, sertoli and leydig cell counts than those receiving cisplatin injection and there was significantly different based on statistical analysis ($p<0.05$).

The protection to testicular damage induced by cisplatin is basically based on the strengthening of intracellular resistance is the basic of protection from testicular damage induced by cisplatin. Cisplatin generate adducts to cross-linked DNA and afterward activate the pos point pathway and induce apoptosis.[26] Intrastrand cross-links adducted between two neighbouring guanines and between adenine and guanine are the main DNA adducts. In addition, there are also monofunctional adducts, cross-link DNA-proteins and crosslink DNA-interstrand in small numbers.[26] Some research have shown the protective effect of antioxidants on cisplatin toxicity.[21,27] Another mechanism that is very important in the development of genotoxicity induced by cisplatin can be the formation of free radicals.[28,29]

Vitamin E serves as a protector against genotoxicity based on its break off radical chain reactions ability. The electron derivation of vitamin E, a tocopheroxyl radical, is a main piece of vitamin E antioxidant activity. Alpha-tocopherol in biological membranes, as fat-soluble antioxidants, reacts with many oxidant molecules. Through an oxidation-reduction process, the OH group of tocopherol will give one of its hydrogen atoms to the radical peroxyl molecule so as to form stable tocopherol radicals.[30] By the binding of peroxyl radicals, vitamin E protects the cell membranes from lipid peroxidation processes. This reaction produces stable tocopheroxyl radicals that do not increase the radical chain. Electron donors, such as vitamin C, can regenerate these toxic radicals into alpha-tocopherols, thereby providing longer antioxidant cell protection.[30] Therefore, through the reduction-oxidation (redox) reaction, vitamin E-acetate in minimizing DNA damage by cisplatin by stabilizing free radicals and inhibit the formation of free radicals.

In this study, it was seen that the group receiving cisplatin and vitamin E 200 mg/kgbw had higher spermatogonia, sertoli and leydig cells compared with the group receiving cisplatin and vitamin E 50 mg/kgbw and the group receiving cisplatin alone with significant difference ($p<0.05$). This indicates that the administration of vitamin E 200 mg/kgbw has a significant effect in preventing the decrease of spermatogonium, sertoli and leydig cells due to cisplatin injection and its effectiveness superior compared with vitamin E 50 mg/kgbw. These data suggest that there is an emphasis on the effects of cisplatin-dependent sperm toxicity with vitamin E dose supplementation. Vitamin e protection against oxidative stress induced by cisplatin administration is demonstrated by a linear trend of increasing spermatogonium, sertoli and leydig cells.

Alpha-tocopherol (vitamin E) is an lipophilic antioxidant which its activity especially in cell membranes.[31] As a powerful antioxidant, Vitamin E act as the first line of defense that protects unsaturated fatty acids in phospholipid structure from oxidative process. Vitamin E terminating lipid peroxidation chain reaction and deactivating peroxil radicals to maintain membrane integrity.[31]

In intracellular, monohydrate complex (MHC) and ROS in mitochondria will cause mitochondrial stress that will activate proteins pro-apoptotic ie Bax and Bak. This will lead to increased permeability of mitochondrial permeability transition pore (MPTP) in the mitochondria, which is lays between the outer matrix membrane (OMM) with the inner matrix membrane (IMM).[25] Some study report that when there is apoptotic stimulation, pro apoptotic proteins (Bak and Bax) are translocated to the mithocondria. Afterward, cytochrome C is released from the mitochondria. In conjunction with apoptotic inducing factor (AIF) and dATP, cytochrome C alter procaspase-9 to its active form caspase-9. Caspase 9 will initiate

caspase 7 and caspase 3 caspase which is responsible for many apoptotic biochemical processes including poly ribo polyepathic ADP cleavage (PARP) and DNA fragmentation in Apoptosis Cell.[25]

This is consistent with the study by Gevrek et al., indicating that cisplatin also causes testicular apoptosis through the apoptotic protein pathway of Cas-3 and Bax.[32] Cisplatin increases the expression of the apoptotic pathway protein, Caspase-3, Bax and reducing anti-apoptotic protein Bcl-2 generate testicular apoptosis. Vitamin E administration provide a protection effect by decreasing expression of an apoptotic protein (Cas-3, Bax) and decreasing Bax and Bcl-2 ratios.[32] By reduced Bax expression and Bax/bcl-2 ratios, both spermatogonia, sertoli and leydig cell apoptosis decreased. As we know the function of type spermatogonia serves as the stem cell for the germinal epithelium and produces other Spermatogonia. with more spermatogonia, sertoli and leydig cells that survive the effects of cisplatin toxicity, the faster recovery of the testes leads to a normal process of spermatogenesis.

## CONCLUSION

Vitamin E 200 mg/kgbw provides a protective effect against decreased spermatogonia, sertoli and leydig cells due to cisplatin 5 mg/kgbw exposure which its protectiveness depends on the given dose.

## REFERENCES


1. Steliarova-Foucher E, Stiller C, Kaatsch P, Berrino F, Coebergh JW, ACCIS Scientific Committee. Geographical patterns and time trends of cancer incidence and survival among children and adolescents in Europe since the 1970[s] (the ACCIS project): an epidemiological study. Lancet. 2004; 364: 2097–105.
2. Bieber AM, Marcon L, Hales BF, Robaire B. Effects of chemotherapeutic agents for testicular cancer on the male rat reproductive system, spermatozoa, and fertility. Journal of Andrology. 2006; 2(27): 189-200.
3. Bower M, Newlands ES, Holden L, Rustin GJ, Begent RH. Treatment of men with metastatic non-seminomatous germ cell tumours with cyclical POMB/ACE chemotherapy. Ann Oncol. 1997; 8: 477–83.
4. Williams SD, Birch R, Einhorn LH, Irwin L, Greco FA, Loehrer PJ. Treatment of disseminated germ-cell tumors with cisplatin, bleomycin and either vinblastine or etoposide. N Engl J Med. 1987; 316: 1435–40.
5. Attesahin A, Karahan I, Turk G, Gur S, Yilmaz S, Ceribasi AO. Protective role of lycopene on cisplatin-induced changes in sperm characteristics, testicular damage, and oxidative stress in rats. Reproductive Toxicology. 2006; 21: 42-47.
6. Drasga R, Einhorn L, Williams S, Patel D, Stevens E. Fertility after chemotherapy for testicular cancer. Journal of Clinical Oncology. 1983; 1(3): 179-83.
7. Pont J, Albrecht W. Fertility after chemotherapy for testicular germ cell cancer. Fertil Steril. 1997; 68: 1–5.
8. DeSantis M, Albrecht W, Holtl W, Pont J. Impact of cytotoxic treatment on long-term fertility in patients with germ-cell cancer. Int J Cancer. 1999; 83: 864– 5.
9. Hansen SW, Berthelsen JG, von der Masse H. Long-term fertility and leydig cell function in patients treated for germ cell cancer with cisplatin, vinblastine, and bleomycin versus surveillance. J Clin Oncol. 1990; 8: 1695–8.
10. Halliwell B, Gutteridge JMC. Free radicals in biology and medicine, 4th Ed. Oxford University Press. New York: USA; 2007.
11. Agarwal A, Makker K, Sharma R. Clinical relevance of oxidative stress in male factor infertility: an update. Am J Reprod Immunol. 2008; 59: 2–11.
12. Agarwal A, Said TM. Oxidative stress, DNS damage, and apoptosis in male infertility. BJU International. 2005: 95
13. Cherry SM, Hunt PA, Hassold TJ. Cisplatin disrupts mammalian spermatogenesis, but does not affect recombination or chromosome segregation. Mutat Res. 2004; 564(2): 115–28.
14. Meistrich ML, Finch M, da Cunha MF, Hacker U, Au WW. Damaging effects of fourteen chemotherapeutic drugs on mouse testis cells. Cancer Res. 1982; 42(1): 122–31.
15. Martins NM, Santos NA, Curti C, Bianchi ML, Santos AC. Cisplatin induces mitochondrial oxidative stress with resultant energetic metabolism impairment, membrane rigidification and apoptosis in rat liver. J Appl Toxicol. 2008; 28(3): 337–44.
16. Soni KK, Kim HK, Choi BR, Karna KK, You JH, Cha JS, et al. Dose-dependent effects of cisplatin on the severity of testicular injury in Sprague Dawley rats: reactive oxygen species and endoplasmic reticulum stress. Drug Des Devel Ther. 2016; 10: 3959–68.
17. Uzunhisarcikli M, Kalender Y. Protective effects of vitamins C and E against hepatotoxicity induced by methyl parathion in rats. Ecotoxicol Environ Saf. 2011; 74: 2112-8.
18. Dahlan S. Statistik untuk kedokteran dan kesehatan:uji hipotesis. Jakarta: Bina Mitra Press; 2006.
19. Colpi GM, Contalbi GF, Nerva F, Sagone P, Piediferro G. Testicular function following chemo-radiotherapy. Eur J Obstet Gynecol Reprod Biol. 2004; 113 (Suppl 1): S2-6.

20. Anand H, Misro MM, Sharma SB, Prakash S. Protective effects of Eugenia jambolana extract versus N-acetyl cysteine against cisplatin induced damage in rat testis. Andrologia. 2015; 47: 194-208.
21. Ciftci O, Beytur A, Cakir O, Gurbuz N, Vardi N. Comparison of reproductive toxicity caused by cisplatin and Novel Platinum-N-Heterocyclic carbene complex in male rats. Basic & Clinical Pharmacology & Toxicology. 2011; 109: 328–33.
22. Sanocka D, Kurpisz M. Reactive oxygen species and sperm cells. Reproductive Biology and Endocrinology. 2004; 2: 12.
23. Huang Q, Dunn RT, Jayadev S, DiSorbo O, Pack FD, Farr SB, et al. Assessment of cisplatin-induced nephro-toxicity by microarray technology. Toxicol Sci. 2001; 63: 196–207.
24. Atasayar S, Gürer-Orhan H, Orhan H, Gurel B, Girgin G, Ozgunes H. Preventive effect of aminoguanidine compared to vitamin E and C on cisplatin-induced nephrotoxicity in Rats. Exp Toxicol Pathol. 2009; 61: 23–32.
25. Ganga UK, Kishori B, Reddy PS. Cisplatin and/or etoposide induces oxidative stress in testicular, hepatic and kidney tissues in male albino mice. Journal of Biology and Earth Sciences. 2013; 3(2): B249-54.
26. Kaminski R, Darbinyan A, Merabova N, Deshmane SL, White MK, Khalili K. Protective role of Purα to Cisplatin. Cancer Biol Ther. 2008; 7: 1926–35.
27. Kaya K, Ciftci O, Cetin A, Dogan H, Basak N. Hesperidin protects testicular and spermatological damages induced by cisplatin in rats. Andrologia. 2015; 47: 793-800.
28. Satoh M, Kashihara N, Fujimoto S, Horike H, Tokura T, Namikoshi T, et al. A novel free radical scavenger, edarabone, protects against cisplatin induced acute renal damage in vitro and in vivo. J Pharmacol Exp Ther. 2003; 305: 1183-90.
29. Brozovic A, Ambriović-Ristov A, Osmak M. The relationship between cisplatin induced reactive oxygen species, glutathione and BCL-2 and resistance to cisplatin. Crit Rev Toxicology. 2010; 40: 347-59.
30. Gunawan SG. Farmakologi dan Terapi, 5th Ed. Jakarta: FKUI. 2007; 786-787.
31. Aggarwal BB, Sundaram C, Prasad S, Kannappan R. Tocotrienols, the vitamin E of the 21st century: its potential against cancer and other chronic diseases. Biochem Pharmacol. 2010; 80: 1613-31.
32. Gevrek F, Erdemir F. Investigation of the effects of curcumin, Vit-E and their combination in cisplatin-induced testicular apoptosis using immuno-histochemical technique. Turk J Urol. 2018; 44(1): 16-23.