The possible ameliorative effects of vitamin E against cisplatin-induced injury on adult rat liver and testes

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SUMMARY

Cisplatin is one of the most potent cytotoxic drugs used to treat cancer, but clinical use is linked to testicular and liver damage. According to a number of studies, antioxidant supplementation may have an impact on the toxicity caused by cisplatin. The purpose of the current investigation was to determine how vitamin-E supplementation protected rats from cisplatin-induced damage. Thirty laboratory adult male albino rats were divided into three groups: Group I received saline orally, once daily for 21 days. Group II received cisplatin on day 0, 7, 14 and were sacrificed on day 21. Group III received cisplatin on day 0, 7, 14 and received orally vitamin E daily, starting 5 days before the first dose of cisplatin until day 21. Liver and both testes were obtained and fixed. Sections from the liver and both testes were stained by H&E and Trichrome stain, and then examined under light microscope.

Alterations included a significant increase in malondialdehyde (MDA) level in the cisplatin group compared with the other groups (p value for comparing between control and each other group, statistically significant at $p \leq 0.05$). Histopathologically, cisplatin induced signs of hepatic injury; it also showed signs of testicular degeneration in all rats. However, the cisplatin induced disturbances significantly improved by treatment with Vitamin E. Statistical analysis showed a significant difference among the three groups in all signs of injury ($p<0.001$). According to this research, cisplatin has a toxic impact on the liver and testicles, and when it is administered along with vitamin E, a noticeable improvement is seen.

Key words: Cisplatin – Liver – Testes – Vitamin E – Oxidative stress

INTRODUCTION

One of the most powerful anticancer medications is cisplatin (cis-diammine dichloroplatinum II CDDP). It is a successful anti-cancer drug used to treat a variety of malignancies, including ovarian, gastric, pulmonary, head-and-neck, prostate, bladder, and cervical cancers, lymphoma, and osteosarcoma (Lirdi et al.,2008; Roldan-Fidalgo et al., 2016). Cisplatin is frequently used to treat cancer, either alone or in combination with other medications (Pectasides et al., 2009; Takeshita et al., 2013).

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By enabling a covalent bond between purine bases and the platinum molecule, which causes G2 cell cycle arrest and initiates death, cisplatin produces its lethal action through contact with DNA (Pil et al.,1992).

CDDP has a number of toxic side effects, including ototoxicity, nephrotoxicity, myelotoxicity, and gastrointestinal toxicity, as well as serious side effects affecting the neurologic, hematologic, and reproductive systems, despite having promising results, particularly in the treatment of testicular cancer (Beytur et al., 2012; Kaya et al., 2015). One of the cisplatin side effects that is frequently observed is long-lasting testicular dysfunction (Colpi et al., 2004). Due to the high rate of spermatogenic cell proliferation in the testis, the adverse effects of chemotherapy on the testis may be severe and irreversible, resulting in the death of spermatogenic cells during the process of spermatogenesis and changes in the sperm DNA, which may result in oligozoospermia, azoospermia, or even prolonged sterility (Ekinci Akdemir et al 2019).

When Cisplatin is used at large dosages, liver damage might ensue (Santos et al., 2007; Martins, 2008). Signs of harm are present in the abnormalities in liver function brought on by cisplatin. Cisplatin can deplete reduced glutathione (GSH) and produce reactive oxygen species (ROS), (Yilmaz et al., 2004; Wozniak et al., 2004) which can cause tissue damage through reactions with cellular macromolecules such as proteins, nucleic acids, and lipids, leading to cell injury and death, despite the paucity of information on the underlying mechanisms causing its hepatotoxic effect (Halliwell, 2006). Strengthening intracellular survival pathways is the key to protection against cisplatin-induced testicular injury. Studies have shown that antioxidant compounds protect against the damage caused by cisplatin (Kaya et al., 2015; Anand et al., 2015). Alpha-tocopherols like vitamin E have the strongest antioxidant action. It is a crucial non-enzymatic antioxidant defense system that can only be supplied by food or supplementation, and it performs a wide range of biological tasks including enzymatic activity, gene regulation, and platelet aggregation inhibition (Schneider, 2005; Villacorta et al., 2003; Atkinson et al., 2008). Due to its lipophilic action, vitamin E is found in the

membrane-rich cell components and is a membrane-specific antioxidant. It possesses anticancer, cardiovascular, neurological, anti-diabetic, osteoprotective, immunomodulatory, and gastroprotective properties (Aggarwal et al., 2010). As a very potent antioxidant, it forms the first defense line that protects unsaturated fatty acids in the structure of phospholipids from the effects of free radicals. It removes the lipid peroxyl radicals, and terminates lipid peroxidation chain reactions. Therefore, it is also known as chain-breaking antioxidant (Aggarwal et al., 2010).

Since it is a very potent antioxidant, it works as the first line of defense against the effects of free radicals for the unsaturated fatty acids contained in the structure of phospholipids. Lipid peroxidation is stopped in its tracks by the elimination of the lipid peroxyl radicals. The phrase "chain-breaking antioxidant" is another term for it (Abdel-Daim et al., 2018; Ourique et al., 2016).

In this study, we aim to investigate the role of Vitamin-E in the protection against cisplatin-induced liver and testes injury. The aim of the current study was to examine the protective effects of vitamin E against Cisplatin induced liver and testes injury in adult albino rat.

MATERIALS AND METHODS

Chemicals

Cisplatin was used in a 1 mg/ml intravenous infusion (Cisplatin Mylan Pharma 1 mg/ml solution à diluer pour perfusion). Vitamin E was purchased as soft gelatin capsules from PHARCO pharmaceuticals Company, Egypt. Each capsule contained 400mg of vitamin E.

Experiment animals

Thirty laboratory adult male albino Wistar rats, 7-9 weeks old. Each of 180-220 g average weight were obtained from the Animal house center, Faculty of Medicine, Alexandria University. The animals were allowed to acclimatize for two weeks before the experiment. The animals were maintained under standard laboratory conditions of temperature, humidity and 12 hours light/dark cycle. First injection of cisplatin is considered as day 0.

Experimental design

Male albino rats were randomly separated into three equal groups of ten each. Group I (control group) received saline (the vehicle) orally, once daily for 21 days. Group II (Cisplatin group) received cisplatin (single dose of 6.5 mg/kg intraperitoneal) on day 0, 7, 14 and were sacrificed on day 21. Group III received cisplatin (single dose of 6.5 mg/kg intraperitoneal) on day 0, 7, 14 and then received orally by gavage vitamin E at a dose of 100 mg/kg body weight daily, starting 5 days before first dose of cisplatin till day 21. They were sacrificed on day 21 (Li et al., 2020; Moneim et al., 2019; Park et al., 2012; Erdemli et al.,2019).

Estimation of malondialdehyde (MDA)

At the end of the 21-day period, the animals were then sacrificed, and the liver and testes tissues were removed. MDA levels in the liver and testes tissues homogenate were measured. The measured outcomes were presented as MDA nmol/g tissue (Biochemistry lab, El Mowasah, Faculty of Medicine, Alexandria University).

Preparation of tissue homogenates

1. Prior to dissection, perfuse tissue with a PBS (phosphate buffered saline) solution, PH 7.4 containing 0.16 mg/ ml heparin to remove any red blood cells and clots.

2. Homogenize the tissue in 5-10 ml cold buffer (i.e, 50 mM potassium phosphate, PH 7.5) per gram tissue.

3. Centrifuge at 4000r.p.m for 15 minutes.

4. Remove the supernatant for essay and store on ice. If not assaying on the same day, freeze the sample at -80C. The sample will be stable for at least one month (Satoh, 1978; Ohkawa et al., 1979).

Histopathological investigations

At the end of the experiment, the animals were sacrificed by decapitation under mild anesthesia using 50 mg/kg of sodium pentobarbital. The liver and both testes were obtained and fixed in 10% neutral buffered formalin and Bouin's solution respectively. Ascending grades of ethanol (70%, 90% and 100%) were used for dehydration, followed by clearing in xylene. Embedding was done using soft and hard paraffin. The resulting paraffin blocks were sectioned at a thickness of 5-7 micrometers, using a rotatory microtome, then cut and immersed in xylene for dewaxing, followed by descending grades of ethanol (100%, 90% and 70%) for rehydration. Sections from the liver and both testes were stained by Harris Hematoxylin & Eosin (H&E) and Masson Trichrome stain. All sections were examined under light microscope (Olympus CX23).

Sections from the liver were examined for signs of hepatic injury including feathery degeneration, lytic necrosis, portal inflammation, congestion, and cholestasis. Portal expansion and fibrosis were assessed by Masson Trichrome.

The testes were examined for signs of degeneration of the seminiferous tubules. The spermatogenesis was assessed, and the following was recorded whenever encountered: 1) Active spermatogenesis; 2) Maturation arrest; 3) Germ cell aplasia.

Statistical analysis of the data

Data were uploaded to the computer and analyzed using IBM SPSS software package, version 20.0. (Armonk, NY: IBM Corp). For continuous data, they were tested for normality by the Shapiro-Wilk test. Quantitative data were expressed as range (minimum and maximum), mean, standard deviation and median. ANOVA was used for comparing the three studied groups, followed by Post Hoc test (Tukey) for pairwise comparison. Significance of the obtained results was judged at the 5% level.

RESULTS

MDA Measurement

The changes in the MDA are shown in Table 1, Fig. 1. The MDA concentrations in the tissues were used as an index of lipid peroxidation. The MDA levels in the liver and testes tissue were significantly higher in the cisplatin-treated group when compared to control group. On the other hand, it was observed that the MDA levels in liver and testes tissue significantly decreased in cisplatin and Vitamin E-treated rats compared to the cisplatin group. These data were consistent with the histopathological findings.

Table 1. Comparison between the three studied groups according to MDA in tissue homogenate.

MDA in Tissue homogenate (nmol/g)	Group I (n = 10)	Group II (n = 10)	Group III (n = 10)	F (p)	Significance between groups
Liver					
Mean ± SD	57.8 ± 10.3	207 ± 42.7	113 ± 13.3	80.925* (<0.001*)	$p_1<0.001^*$, $p_2<0.001^*$, $p_3<0.001^*$
Median (Min – Max)	56 (44 – 76)	197 (155 – 287)	112 (95 – 131)		
Testes					
Mean ± SD	57.5 ± 11.4	170 ± 37.8	75 ± 17.1	59.461* (<0.001*)	$p_1<0.001^*$, $p_2=0.274$, $p_3<0.001^*$
Median (Min – Max)	57.5 (40 – 78)	170 (112 – 222)	75 (51 – 101)		

SD: **Standard deviation**
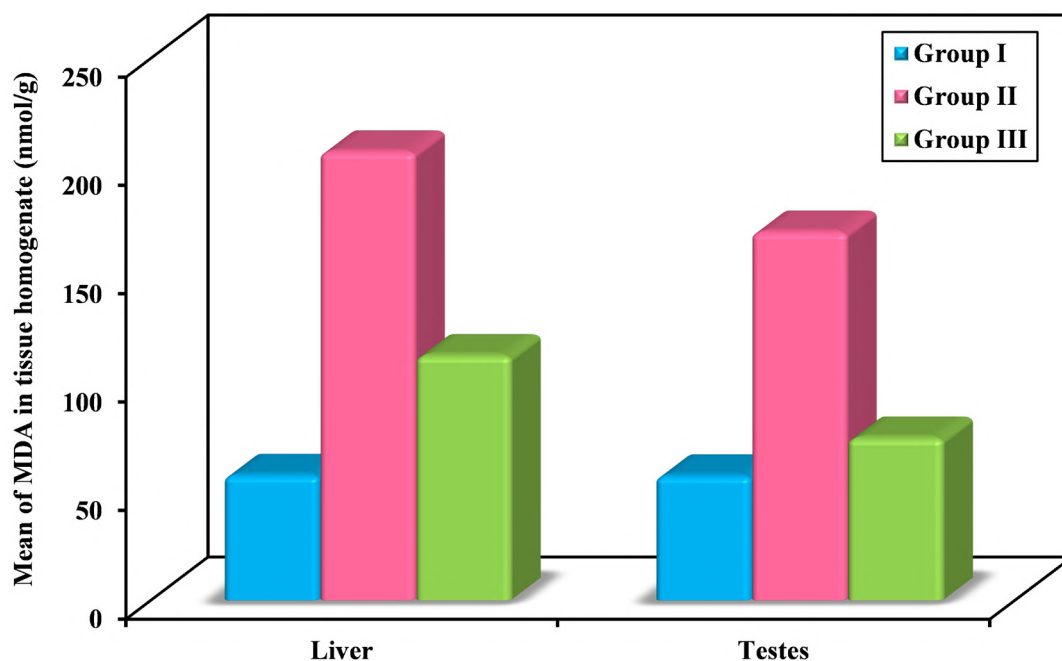F: **F for One way ANOVA test**, Pairwise comparison between each 2 groups was done using **Post Hoc Test (Tukey)**
p: p value for comparing between the three studied groups
p_1: p value for comparing between **Control** and **Cisplatin**
p_2: p value for comparing between **Control** and **Vitamin E**
p_3: p value for comparing between **Cisplatin** and **Vitamin E**
*: Statistically significant at $p \leq 0.05$

Fig. 1.- Comparison between the three studied groups according to MDA in tissue homogenate.

Histological findings

Liver:

Group I showed preservation of the hepatic architecture without detectable fibrosis or inflammation. Neither cholestasis nor congestion was observed (Fig. 2a, b).

Group II showed signs of hepatic injury including congestion in 4/10 rats (40%), feathery degeneration in 7/10 rats (70%), lytic necrosis in 5/10 rats (50%), cholestasis in 5/10 rats (50%), and portal inflammation in 3/10 rats (30%). Tri-chrome stain revealed portal expansion in 4/10 rats (40%) and bridging fibrosis in 4/10 rats (40%) (Fig. 2c-g).

Group III showed a remarkable improvement whereby neither lytic necrosis nor cholestasis were detected in any of the rats (0%). Trichrome stain revealed neither portal nor bridging fibrosis in all rats (0%). However, congestion and feathery degeneration were detected in 7/10 rats (70%) and portal inflammation was detected in 6/10 rats (60%) (Fig. 2h, i).

Statistical analysis showed a significant difference among the three groups according to signs of liver injury (Table 2, Fig. 3).

Testis:

Group I The seminiferous tubules showed normal morphology with active spermatogenesis (Fig. 4a).
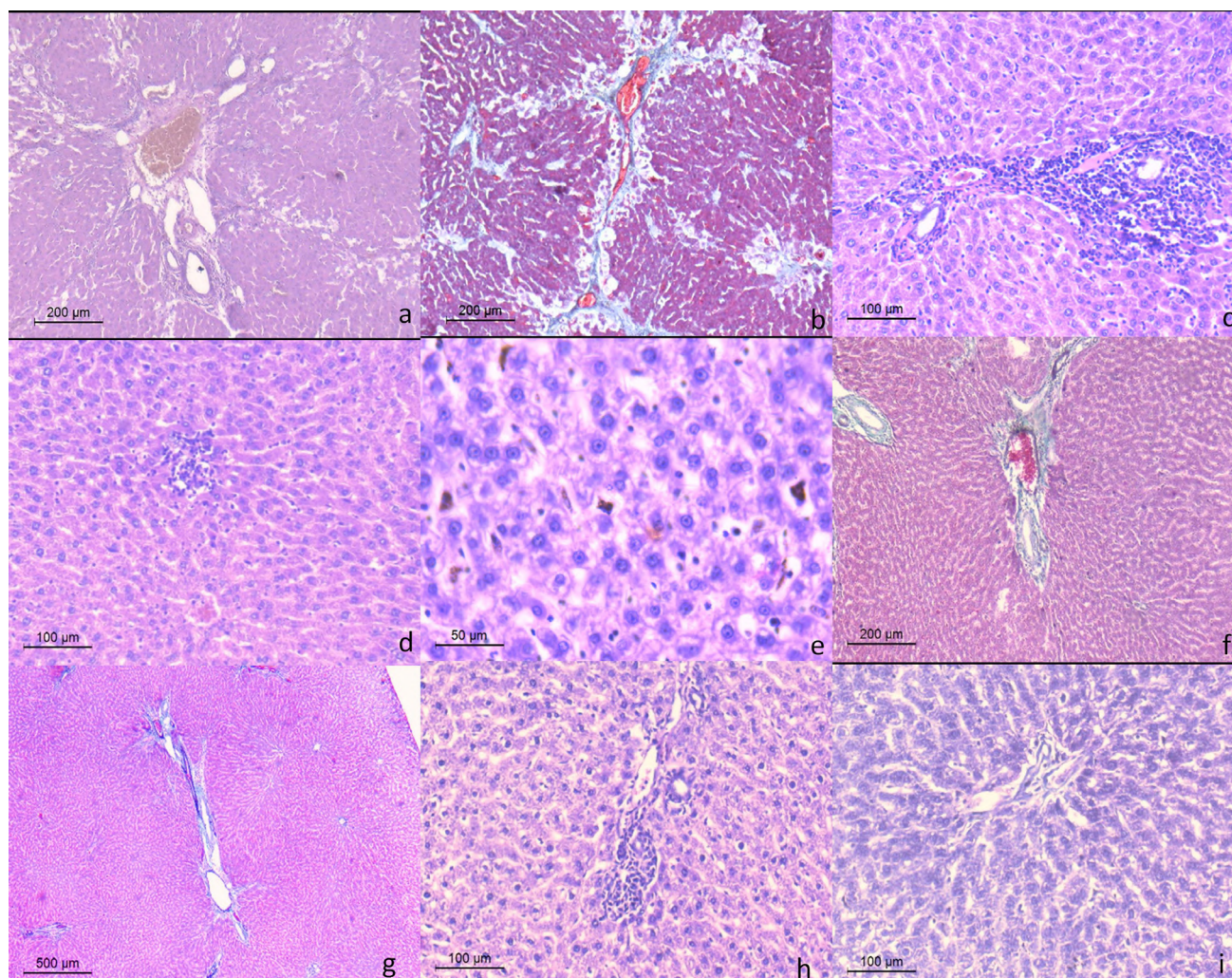
Fig. 2.- Group I (control rats) shows preserved hepatic architecture with no signs of injury (**a:** H&E) and no fibrosis (**b:** Trichrome). Group II (rats receiving cisplatin) shows portal inflammation (**c:** H&E), lytic necrosis (**d:** H&E), feathery degeneration and cholestasis (**e:** H&E), portal expansion and fibrosis (**f:** trichrome) and bridging fibrosis (**g:** Trichrome). Group III (rats receiving Cisplatin + vitamin E) shows portal inflammation and feathery degeneration (**h:** H&E) but no portal fibrosis (**i:** trichrome). p: p value for comparing between the studied groups; *: Statistically significant at p ≤ 0.05. Scale bars: a, b, f = 200 µm; c, d, h, i = 100 µm; e = 50 µm; g = 500 µm.

Table 2- Comparison between the three studied groups according to signs of liver injury.

Parameter	Group I (n=10)	Group II (n=10)	Group III (n=10)	χ^2	^{MC}p
Congestion	0 (0.0%)	4 (40.0%)	7 (70.0%)	11.101*	0.006*
Feathery degeneration	0 (0.0%)	7 (70.0%)	7 (70.0%)	14.040*	0.001*
Lytic necrosis	0 (0.0%)	5 (50.0%)	0 (0.0%)	9.441*	0.006*
Cholestasis	0 (0.0%)	5 (50.0%)	0 (0.0%)	9.441*	0.006*
Portal inflammation	0 (0.0%)	3 (30.0%)	6 (60.0%)	8.622*	0.017*
Portal expansion (Tri)	0 (0.0%)	4 (40.0%)	0 (0.0%)	6.876*	0.022*
Bridging fibrosis (Tri)	0 (0.0%)	4 (40.0%)	0 (0.0%)	6.876*	0.022*

χ^2: **Chi square test**; MC: **Monte Carlo**
p: p value for comparing between the studied groups
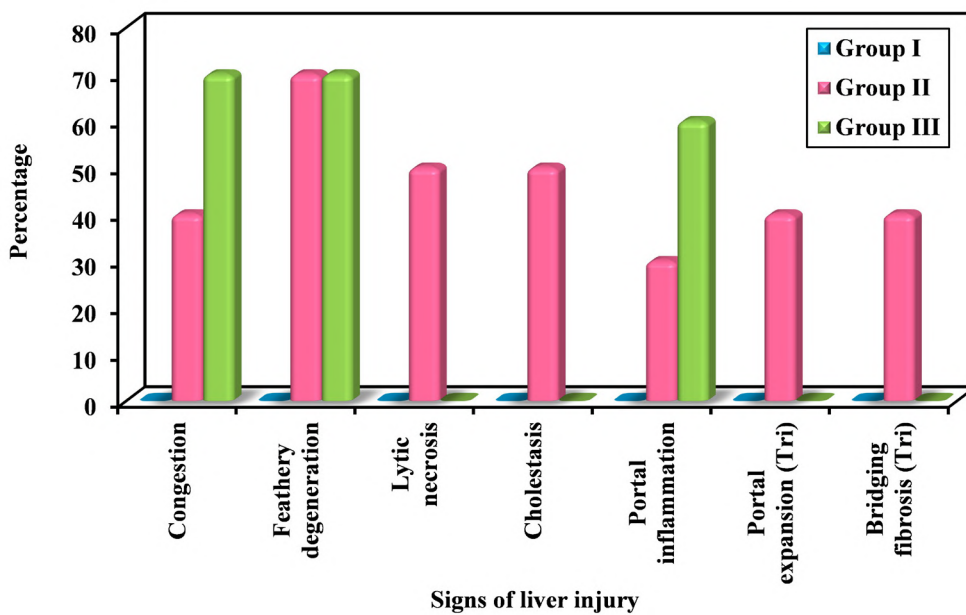*: Statistically significant at p ≤ 0.05

Fig. 3.- Comparison between the three studied groups according to signs of liver injury.
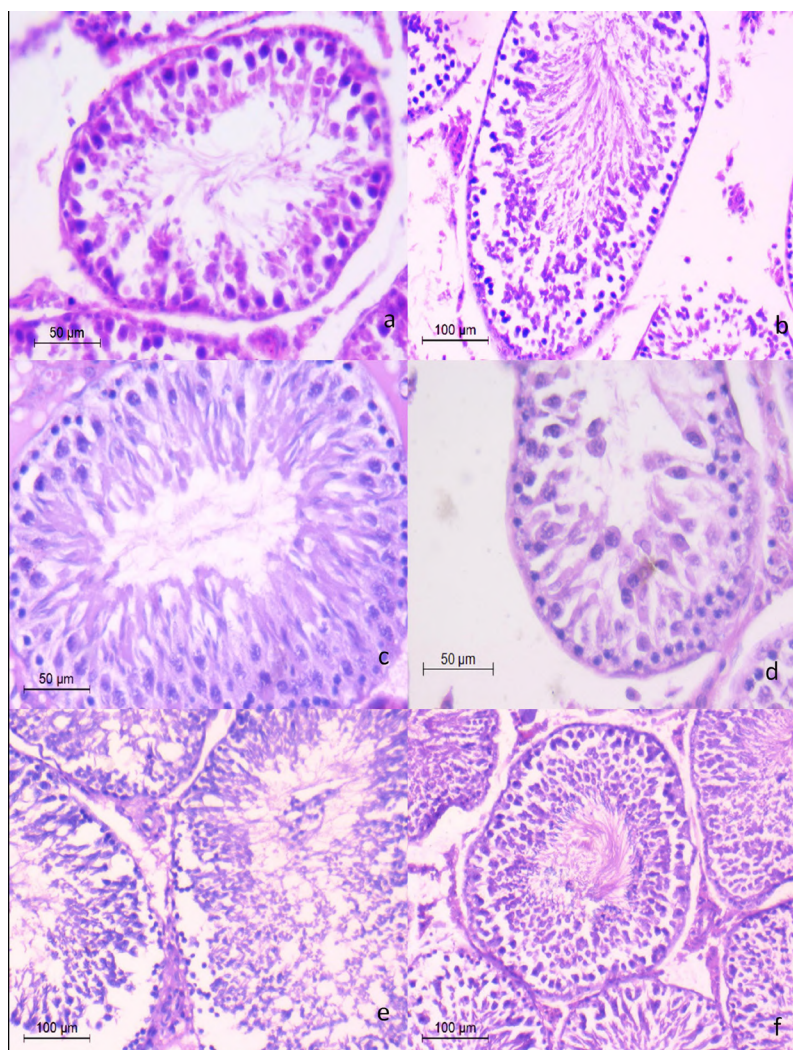

Fig. 4.- Group I (control rats) shows active spermatogenesis without degeneration (**a:** H&E). Group II (rats receiving cisplatin) shows degeneration (**b**), germ cell aplasia with only Sertoli cells and no spermatogonia (**c**), maturation arrest with spermatogonia, primary spermatocytes and Sertoli cells (**d**) and active spermatogenesis (**e**). Group III (rats receiving Cisplatin + vitamin E) showing active spermatogenesis without degeneration (**f**: H&E). p: p value for comparing between the studied groups; *: Statistically significant at p ≤ 0.05. Scale bars: a, c, d = 50 μm; b, e, f = 100 μm.
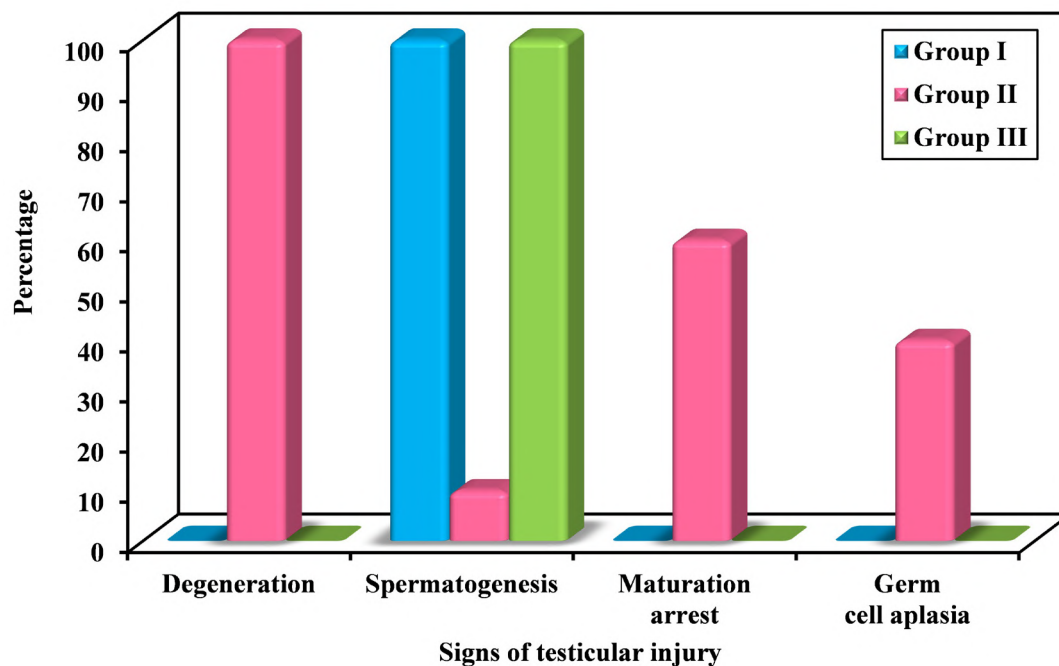
Table 3.- Comparison between the three studied groups according to signs of testicular injury.

Parameter	Group I (n=10)	Group II (n=10)	Group III (n=10)	χ^2	^{MC}p
Degeneration	0 (0.0%)	10 (100.0%)	0 (0.0%)	30.262*	<0.001*
Spermatogenesis	10 (100.0)	1 (10.0%)	10 (100.0%)	24.286*	<0.001*
Maturation arrest	0 (0.0%)	6 (60.0%)	0 (0.0%)	12.377*	0.001*
Germ cell aplasia	0 (0.0%)	4 (40.0%)	0 (0.0%)	6.876*	0.022*

χ^2: **Chi square test**; MC: **Monte Carlo**
p: p value for comparing between the studied groups
*: Statistically significant at $p \leq 0.05$

Fig. 5.- Comparison between the three studied groups according to signs of testicular injury.

Group II showed signs of degeneration in all rats (100%). Active spermatogenesis was detected in only one rat (10%), maturation arrest was seen in 6 rats (60%) while germ cell aplasia with only Sertoli cells seen lining the seminiferous tubules was found in 4 rats (40%) (Fig. 4b-e).

Group III showed a remarkable histological improvement without any signs of degeneration in any of the 10 rats (0%) and active spermatogenesis in all 10 rats (100%) (Fig. 4f).

Statistical analysis showed a significant difference among the three groups with degeneration and active spermatogenesis showing the highest significance (p<0.001, Monte Carlo significant test) (Table 3, Fig. 5).

DISCUSSION

One of the very efficient chemotherapy medications used to treat many cancer types is cisplatin. However, due to its numerous severe side effects, its clinical application is limited. [1] Sertoli cells, interstitial Leydig cells, seminiferous tubule epithelium, and notably germ cells are the main targets of its toxic effects on the male reproductive system and liver (Boekelheide, 2005). Because of this, avoiding cisplatin side effects is crucial, and there is ongoing discussion on how to do so when using the drug in clinical settings.

Cisplatin has been especially interesting since it has shown anticancer activity in a variety of tumors. It was discovered to have cytotoxic prop-

erties in the 1960s, and by the end of the 1970s it had earned a place as the key ingredient in the systemic treatment of germ cell cancers. Among many chemotherapy drugs that are widely used for cancer, Cisplatin is one of the most compelling ones. It was the first FDA-approved platinum compound for cancer treatment in 1978 (Kelland, 2007). This has led to interest in platinum (II) - and other metal-containing compounds as potential anticancer drugs (Frezza et al., 2010).

As compared with gemcitabine alone, cisplatin plus gemcitabine was associated with a significant survival advantage without the addition of substantial toxicity. Cisplatin plus gemcitabine is an appropriate option for the treatment of patients with advanced biliary cancer (Valle et al., 2010).

In this study, it has been detected that cisplatin-induced signs of testicular degeneration, maturation arrest, germ cell aplasia with only Sertoli cells and no spermatogonia. Also cisplatin-induced liver changes such as portal inflammation, feathery degeneration and cholestasis, which matched the results of many other studies (Beytur et al,. 2012; Ateşşahin et al., 2006b; Custódio et al., 2009; Iraz et al., 2006).

Oxidative stress is brought on by reactive oxygen species, which can form naturally as a consequence of metabolism, or more commonly as a result of ischemia situations, radiation, inflammation, ageing, chemicals, medicines, and exposure to electromagnetic fields. Oxidative stress targets DNA double-bond bases, as well as protein and lipid double-bond groups that contain double-bonds. As a result, macromolecules such as intracellular lipid, protein, and DNA are harmed, and cellular injury-induced apoptosis takes place (Yan, 2014). Testes are one among the organs that oxidative stress targets, since they have higher levels of polyunsaturated membrane lipids than other tissues (Beytur et al., 2012). Therefore, oxidative chemicals like cisplatin commonly affect the testes. However, due to their lower circulatory supply and low oxygen pressures, testes may still protect themselves from oxidative damage, albeit to a lesser extent. The testes are given the capacity to resist oxidative stress because of this situation (Aitken and Roman, 2008).

After developing oxidative damage from a variety of causes, such as radiation therapy, varicocele, chemotherapy, torsion, infections, and smoking, testicular tissue and spermatogenesis are hindered. Numerous investigations have shown that cisplatin causes harmful alterations in testicular tissue. Additionally, in our investigation, testes from the rats that received cisplatin showed signs of histological damage: this was consistent with a number of investigations (Fazile et al., 2019; Ateşşahin et al., 2006a; Alves Favareto et al., 2011; Schweyer et al., 2004). Additionally, it has been shown that cisplatin treatment causes alterations in the liver; this was consistent with a number of investigations (Custódio et al., 2009; Iraz et al., 2006; Nazıroglu et al., 2004).

These findings revealed that lipid peroxidation and free radical production were involved. Cisplatin causes lipid peroxidation and produces ROS, such such superoxide anion and hydroxyl radicals (Halliwell and Gutteridge, 1999). It is accepted that both are related to oxidative stress and lead to an imbalance between the capacity of the organism's antioxidant system and the production of oxygen-derived radicals. The administration of cisplatin has been linked in several studies to enhanced free radical production and significant oxidative stress (Antunes et al., 2001; Mora et al., 2003). The natural equilibrium between radical creation and defence against them in cells is upset as a result of an increase in free radical formation in cisplatin-induced toxicity (Conklin, 2000). As a result, proteins, lipids, and nucleic acids will sustain oxidative damage (Packer et al., 1990).

Various methods have been suggested to reduce the toxicity caused by cisplatin. The development of treatments to stop the production of free radicals may affect the development of oxidative renal damage as well as the emergence of acute renal damage brought on by cisplatin. Supplementing with natural vitamin E makes it simple and safe to enhance its levels in tissues. In this study, we looked at how vitamin E affected cisplatin-treated rats (Packer et al., 1990).

The present study has indicated that treatment with vitamin E may have protective effects against cisplatin-induced testicular degeneration. This condition was demonstrated by remarkable improvement by

analyses of general histological images of hematoxylin-eosin stained sections of testicular tissue.

According to reports, vitamin E includes tocopherol, tocotrienol, and free radical scavengers and reduces the toxicity of endothelial cells and nephrotoxicity caused by cisplatin (Aggarwal et al., 2010; Paksoy et al., 2011). In addition, vitamin E serves as a potential inhibitor of lipid peroxidation reactions and guards against free radical damage to the fatty acids that make up unsaturated phospholipid membranes (Paksoy et al., 2011; Villani et al., 2016). Kalkanis et al. (2004) found that vitamin-E supplementation reduced the toxicity of cisplatin in rats. Our study's findings are corroborated by a previous study that found dexamethasone and vitamin E supplements to be particularly beneficial in reducing the negative effects of cisplatin therapy (Paksoy et al., 2011).

In our study, where we investigated protective effects of vitamin E in cisplatin-induced toxicity, we detected that these antioxidant molecules alleviated cellular injuries induced by cisplatin administration, which was similar to the results of other studies (Soyalıç et al., 2016; De Freitas et al., 2009). Based on this literature information, we can say that vitamin E may ameliorate damage caused by cisplatin thanks to its antioxidant, free-radical scavenger and similar activities.

CONCLUSION

Based on biochemical and histological analyses, the overall results of this study demonstrated that CDDP caused damage to the liver and testicles. Vitamin E supplementation provided excellent adverse effect prevention in addition to concurrent treatment for CDDP. In order to stop CPPD-induced damage and oxidative stress from continuing, vitamin E may act as an antioxidant by scavenging free radicals.

Ethical approval

The present study was approved by the Ethical guidelines of Alexandria University on laboratory animals and the national institute for the care and use of laboratory animals. Further the Alexandria Faculty of Medicine ethical committee approval was obtained.

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