T.C.
YEDITEPE UNIVERSITY
INSTITUTE OF HEALTH SCIENCES
DEPARTMENT OF PHYTOTHERAPY

EFFECT OF LYCOPENE ON CISPLATIN-INDUCED TOXICITY

MASTER OF SCIENCE THESIS

FATMA ZEHRA ERASLAN, MD

İSTANBUL - 2019

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APPROVAL

This thesis has been deemed by the jury in accordance with the relevant articles of Yeditepe University Graduate Education and Examinations Regulation and has been approved by Administrative Board of Institute with decision dated 17.05.2019 and numbered 2019/08-03

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Director of Institute of Health Sciences

DECLARATION

I hereby declare that this thesis is my own original work and that to the best of my knowledge and belief, it contains no material previously published or written by another person nor material, which has been accepted for the award of any other degree except where due acknowledgment has been made in the text.

09/05/2019

Fatma Zehra Eraslan, MD

DEDICATION

To my beloved family

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CONTENTS

APPROVAL ii

DECLARATION iii

DEDICATION iv

ACKNOWLEDGEMENT v

CONTENTS vii

TABLES x

FIGURES xi

ABSTRACT xii

ÖZET xiv

SYMBOLS / ABBREVIATIONS xvi

1. INTRODUCTION 1

2. GENERAL DESCRIPTION 3

2.1. Carotenoids 3

2.1.1. Functions of the Carotenoids in Plants 4

2.1.2. Major Carotenoids 4

2.2.Chemotherapeutics and Platinum-Based Chemotherapeutics 10

2.2.1. Effect Mechanisms of Platinum Compounds 11

2.2.2. Cisplatin and Its Action Mechanism 13

2.3. Cisplatin-Induced Toxicity (CIT) and Effect of Lycopene on CIT 15

3. MATERIALS & METHODS 19

3.1. Materials 19

3.1.1. Equipment 19

3.2. Methods 19

3.2.1. Experimental design and Animal Husbandry 19

3.2.2. Blood Parameters 20
3.2.2.1. Complete Blood Count Tests 20
3.2.2.2. Blood Biochemistry Tests 21
3.2.3. Histopathological Examinations 21
3.2.4. Statistical Analyses 22
4. RESULTS 23
4.1. Body and Tissue Weights 23
4.2 Blood Parameters 23
4.2.1. Complete Blood Count Tests 23
4.2.2. Blood Biochemistry Parameters 32
4.3. Histopathological Examinations 37
4.3.1. Kidney Histopathology 37
4.3.2. Testicular Histopathology 37
4.3.3. Liver Histopathology 38
5. DISCUSSION & CONCLUSION 42
5.1. Body and Tissue Weights 42
5.2. Complete Blood Count Tests 43
5.3. Blood Biochemistry Parameters 45
5.4. Histopathological Examinations 48
6. REFERENCES 52
7. Appendıx........ 81
7.1. Certificate of Animal Use in Experimental Research........ 81
7.2. Animal Research Ethics Committee Approval........ 82
8. Currıculum Vıtae........ 83

TABLES

Table 1. Molecular mechanisms of resistance to cisplatin (154). 15
Table 2. Equipment and manufacturer 19
Table 3. Experimental groups and drug application design. 20
Table 4. Body and tissue weights of the animals in different experimental groups. Data are mean ± SD. (n=7 animals in each group, except for the group A, n=6 animals) Data sharing the same superscript letters in different groups are significantly different from each other. 27
Table 5. Baseline (Day 0), day 7 and day 10 CBC measurements. Data are mean ± SD. (n=7 animals in each group, except for the group A and D on day 10, n=6 animals). Data sharing the same superscript letters in different groups and are significantly different from each other. 28
Table 6. Blood biochemistry on baseline (Day 0), day 7 and day 10. Data are mean ± SD. (n=7 animals in each group, except for the group A on day 10, n=6 animals). Data sharing the same superscript letters in different groups and days are significantly different from each other. 34
Table 7. Histopathological assessment of the kidneys in different experimental groups. Data are mean ± SD. (n=7 in each group, except for the group A, n=6.) Data sharing the same superscript letters in different groups are significantly different from each other. 37

FIGURES

Figure 1. Platinum (Pt^{II})-based drugs and complexes investigated in clinical studies (11). 2
Figure 2: The common (semisystemic) numbering system of the carotenoids and the structure of lycopene (4). 3
Figure 3. The major end groups of carotenoids. The common names of the carotenoids based on the end groups are indicated in brackets. (4) 3
Figure 4. Major carotenoids present in the human tissue and blood (38). 5
Figure 5. Platinating agents can form DNA monoadducts, intra- and inter-strand crosslinks and DNA-protein crosslinks (155). 12
Figure 6. After aquation, the platinum atom of cisplatin binds to the purines at the N7 position to form intra- and inter-strand crosslinks. Cisplatin-DNA adducts activate various pathways including cell cycle arrest, inhibition of replication and transcription, apoptosis and DNA repair (154). 14
Figure 7. Testicular histopathology results according to Johnsen scoring system. Data are mean ± SD. (n=7 animals in each group, except for the group A, n=6 animals) 37
Figure 8. Liver histopathology findings of Group A (A and B), Group B (C and D), Group C (E and F) and Group D (G and H). (A) Central vein dilatation and congestion (thick arrow) and perivenular dilatation (thin arrow), (B) Bile duct proliferation (arrow), (C and D) Infiltrated inflammatory cells into the capsule (thick arrow) and vacuolar degeneration in the parenchyma cells (thin arrow), (E) Focal necrosis in parenchyma, (F) Perivenular (thick arrow) and periportal (thin arrow) sinusoidal dilatation, (G) Inflammatory cells in the hilum region (star) and Parenchymal damage in a focal region around the surrounding parenchyma, (H) Inflammatory cells replacing the parenchymal cells around the focal region surrounding the hilum. 39
Figure 9. Inflammatory scores in (A) capsular, (B) portal, (C) hilum and (D) focal parenchyma regions. Data are mean ± SD. (n=7 animals in each group, except for the group A, n=6 animals) 40
Figure 10. Vacuolar degeneration scores. Data are mean ± SD. (n=7 animals in each group, except for the group A, n=6 animals, *p<0.05) 40
Figure 11. Bile duct proliferation scores. Data are mean ± SD. (n=7 animals in each group, except for the group A, n=6 animals) 41

ABSTRACT

Eraslan, F.Z. (2019). Effect Of Lycopene On Cisplatin-Induced Toxicity. Yeditepe University, Institute of Health Science, Department of Phytotherapy, MSc Thesis, Istanbul.

Liver and kidney are the major regulators of the body homeostasis. During the drug metabolism, the metabolites and the drug itself cause the liver damage and kidney damage. Chemotherapy-induced hepatotoxicity and nephrotoxicity are some major problems encountered with during the treatment. Cisplatin is a widely used platinum-based drug in clinics and has been shown to have toxic effects on various organs. Herbal active compounds, as well as the extracts, were observed to improve the drug-induced toxic conditions when applied in combination with chemotherapeutics or prophylactic. Lycopene is a carotenoid that has been previously shown to have beneficial effects on various conditions including drug-induced toxicity in studies with animals including rats. Therefore, we aimed to investigate the effect of lycopene in tomato extract on cisplatin-induced toxicity when applied prophylactically or therapeutically in rats. Animals were randomly divided into four equal groups and toxicity was induced by cisplatin administration (either on day 0 or 7). Then, the animals were administered with lycopene either prophylactically (for 7 days before cisplatin) or therapeutically (for 10 days after cisplatin), while two groups were not administered with lycopene. Hematology and biochemistry, as well as the tissue weights and histopathology were investigated. Tissue weights did not differ significantly in different groups, but adjusted heart weight of the animals treated with cisplatin at the first day of experiment was found to be significantly lower than the animals received cisplatin on day 7 and received lycopene as prophylactically. No major effects of either cisplatin or lycopene on the haematological parameters were observed in the study. Administration with lycopene as therapeutic agent but not prophylactic was observed to be contribute to improvement aspartate aminotransferase levels suggesting a hepatoprotective action. The animals in the group received cisplatin on day 7 and received lycopene as prophylactically exhibited significantly higher amylase and glucose levels than other groups. Tubular necrosis of the kidney was significantly higher in the group received cisplatin on day 0 and lycopene for 10 days. Testicular histopathology did not differ among the groups. Liver histopathology

results revealed no change in inflammation score and bile duct proliferation, but the group received cisplatin on day 7 and received lycopene as prophylactically showed higher vacuolar degeneration. In conclusion, our data suggest that prophylactic administration of lycopene on cisplatin treatment may not be suitable.

Key words: acute hepatotoxicity, acute nephrotoxicity, tomato extract, lycopene, liver histopathology, kidney histopathology

ÖZET

Eraslan, F.Z. (2018). Likopenin Sisplatin ile İndüklenen Toksisite Etkilerinin Değerlendirilmesi, Yeditepe Üniversitesi, Sağlık Bilimleri Enstitüsü, Fitoterapi Anabilim Dalı, Yüksek Lisans Tezi, İstanbul.

Karaciğer ve böbrek vücut homeostazının düzenlenmesinde görevli ana organlardandır. İlaç metabolizmasında, ilacın kedisi ve metabolitleri karaciğer ve böbrek hasarına sebep olmaktadır. Kemoterapi kaynaklı hepatotoksisite ve nefrotoksisite, tedavi sırasında karşılaşılan en önemli problemlerdendir. Sisplatin klinikte yaygın olarak kullanılan platin bazlı bir ilaç olup çeşitli organlara olan toksik etkileri gösterilmiştir. Bitkisel aktif bileşikler ve ekstrelerin profilaktik veya kemoterapötik birlikte uygulandığı durumlarda, ilaç kaynaklı toksik durumlarda iyileşme olduğu gözlemlenmiştir. Likopen, daha önce sıçanların da dahil olduğu hayvan çalışmalarında, ilaç kaynaklı toksisite de dahil olmak üzere birçok durumda yararlı olduğu gösterilmiş bir karotenoiddir. Bu sebeple, bu çalışmamızda likopen içeren domates ekstresinin sisplatin kaynaklı toksisite üzerindeki profilaktik veya terapötik etkisini sıçanlarda araştırmayı amaçladık. Hayvanlar rastgele dört eşit gruba ayrıldı ve sisplatin ile toksisite oluşturuldu (sıfırıncı gün veya yedinci gün). Ardından hayvanlara profilaktik (sisplatinden önce 7 gün) veya terapötik (sisplatinden sonra 10 gün) likopen uygulandı, geri kalan iki gruba ise likopen uygulanmadı. Hayvanların hematolojik değerleri, kan biyokimyaları, doku ağırlık ve histopatalojisi incelendi. Doku ağırlıkları gruplar arasında farklılık göstermedi, ancak düzeltilmiş kalp ağırlığı, ilk gün sisplatin uygulanan hayvanlarda yedinci günde sisplatin ve profilaktik likopen uygulanan hayvanlara kıyasla anlamlı derecede düşük bulundu. Hematolojik parametrelerde likopenin veya sisplatinin önemli bir etkisi gözlemlenmedi. Likopenin terapötik bir ajan olarak uygulanması aspartat aminotransferaz aktivitesinde iyileşmeye sebep olmuştur bu da, hepatoprotektif aktiviteyi düşündürmektedir. Sisplatinin yedinci günde ve likopenin profilaktik uygulandığı hayvanlarda amilaz ve glukoz seviyeleri diğer gruplara göre anlamlı derecede yüksek bulunmuştur. Böbreklerde tübüler nekroz, sisplatinin sıfırıncı günde uygulanıp devamında likopenin 10 gün boyunca uygulandığı grupta anlamlı şekilde yüksekti. Testis histopatolojisi gruplar arasında farklılık göstermedi. Karaciğer histopatolojisi sonuçları imflamasyon skorları veya safra kanalı proliferasyonu açısından fark göstermezken, profilaktik likopen uygulanan ve yedinci

gün sisplatin uygulanan hayvanlarda vakuoler dejenerasyon diğer gruplara kıyasla anlamlı bulundu. Sonuç olarak, yaptığımız çalışma likopenin profilaktik uygulamasının sisplatin uygulamasından önce olmasının uygun olmayabileceğini göstermektedir.

Anahtar kelimeler: akut hepatotoksisite, karaciğer histopatolojisi, domates ekstresi, likopen, karaciğer histopatolojisi, böbrek histopatolojisi

SYMBOLS / ABBREVIATIONS

8-OHdG	8-hydroxy-2'-deoxyguanosine
AKI	Acute kidney injury
ALP	Alkaline phosphatase
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
BUN	Blood urea nitrogen
DNA	Deoxyribonucleic acid
GSH	Glutathione
GST	Glutathione S-transferase
HDL	High density lipoprotein
HO-1	Heme oxygenase-1
IGF-I	Insulin-like growth factor-I
IL-1β	Interleukin 1β
LDL	Low density lipoprotein
MDA	Malondialdehyde
NF-κB	Nuclear factor-kappaB
NRF-2	Nuclear factor erythroid 2-related factor 2
ROS	Reactive oxygen species
SOD	Superoxide dismutase
TC	Total cholesterol
TNFα	Tumor necrosis factor alpha

1. INTRODUCTION

Liver and kidney are one of the major organs that regulates the homeostasis of the internal environment of the body. Hepatoxicity is described as the chemical-induced liver injury and is the main reason for the drug withdrawal from the pharmaceutical market (1). Nephrotoxicity, on the other hand, is described as the disruption in the kidney function leading to unproper kidney-specific detoxification and excretion of the toxic substances (2).

Carotenoids are lipid soluble pigment molecules produced by plants, fungi and bacteria (3) and act together with the chlorophyll in the photosynthesis process (4). They also act as protectors against singlet oxygen ($^1O_2$) that is produced by the chlorophyll when it is exposed to light (5) and have role in light harvesting (6). Until now, more than 700 types of carotenoid produced by plants, fungi and algae have been identified (3, 7). Among these, around 50 of these carotenoids are absorbed and metabolized through human diet (3) while only the six of them that are α-carotene, β-carotene, lycopene, β-cryptoxanthin, lutein, and zeaxanthin are indicated to serve as the most of the carotenoids (~95 %) in the blood of the individuals (8). Within these, lycopene represents 21-43 % of the total carotenoids (9). The potential health benefits of lycopene have been investigated in various conditions including cancer and cardiovascular disease (10).

Cisplatin is platinum (Pt^{II})-based antineoplastic drug with a core platinum structure shared with other platinum-based chemotherapeutic agents (11) (Figure 1). The mode of action of cisplatin is indicated to be connected to that it crosslinks the purines in the deoxyribonucleic acid (DNA), disrupts the DNA replication and repair, leading to DNA damage and subsequently causes death of the fast replicating cells (12). It is widely used in the treatment of various types of malignancies including lung, bladder, breast, ovarian and brain cancer (12). Although it is stated to be one of the most powerful chemotherapeutic agents, development of resistance to it and causing toxicities is an important problem (12). The cisplatin toxicities include nephrotoxicity (13), hepatotoxicity (14, 15), cardiotoxicity (16-18) and testicular toxicity (19-21).

Figure 1. Platinum (Pt^{II})-based drugs and complexes investigated in clinical studies (11).

In order to overcome the drug resistance and toxicities caused by the chemotherapy, the combinatorial treatments are of interest (22). Previous studies showed that lycopene led to reduction in testicular damage (23-25) and nephrotoxicity (26, 27) induced by cisplatin and other toxicities induced by chemotherapeutic agents (28). Additionally, the schedule of the complimentary treatment (as prophylactic (prior to the chemotherapy) or combination therapy (together with chemotherapy)) were found to have efficacy in chemotherapy (29). Therefore, we investigated whether lycopene containing extract (5 %) have any prophylactic or protective effect on cisplatin-induced toxicity (CIT) in liver, kidney or testicular toxicity in rats. Lycopene was either administered prior to the CIT or after induction. Later, liver, kidney and testis tissues were sectioned and examined histopathologically. Besides, blood parameters were investigated.

2. GENERAL DESCRIPTION

2.1. Carotenoids

Carotenoids are a group of lipid-soluble pigments that are synthesized by plants and algae, as well as some fungi and bacterial species (3, 30). Most of the carotenoids are terpenoids with 40 carbons and consist of eight isoprenoid units and they are symmetrical at the middle of the compound (6) (Figure 2). The other derivatives of the carotenoids are based on this structure and depend on derivation the position of the group substitutions or hydrogenation, the derivative is stated by the prefixes or suffixes (in example *β*-carotene; Figure 3).

Figure 2: The common (semisystemic) numbering system of the carotenoids and the structure of lycopene (4).

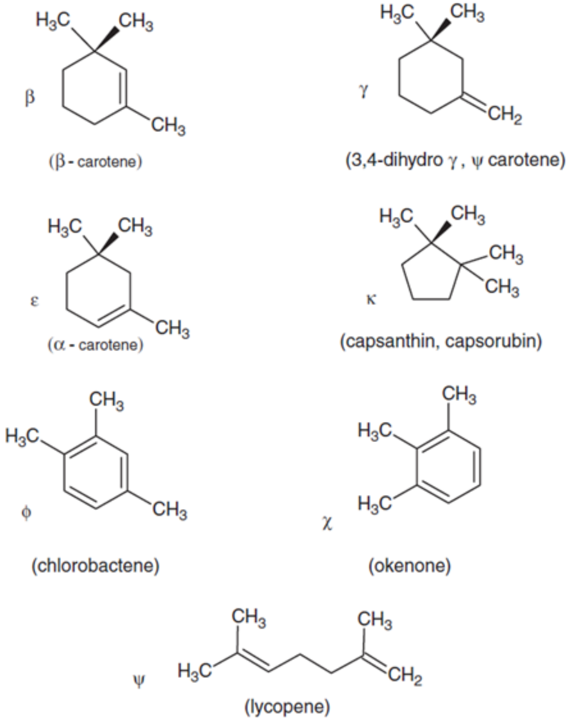


Figure 3. The major end groups of carotenoids. The common names of the carotenoids based on the end groups are indicated in brackets. (4)

2.1.1. Functions of the Carotenoids in Plants

The main function of the carotenoids in the plants are their roles in the light harvesting and protection of the plants from oxidative stress in photosynthesis (4, 5). The carotenoid pigments role in the transfer the absorbed energy from the blue-green light to the chlorophylls (4). The expansion of the light wavelength range by them led to increased efficacy in the light reactions of the photosynthetic process (4). They transfer the energy by the carotenoids and act as the accessory elements in the light harvesting process by expanding the solar spectra and assist the photosynthesis. On the other hand, during the photosynthesis, electronically excited $^1O_2$, which is toxic to the plants, is produced (31). Carotenoids deactivate the $^1O_2$ by either physical (32) or chemical (33) quenching. Additionally, carotenoids also have functions in the regulation of the membrane viscosity (34).

2.1.2. Major Carotenoids

Fifty out of more than 700 carotenoids are included in the human diet (35) and among these, only around 20 of them found in the human body (36). As abovementioned, six out of these carotenoids corresponds to 95 % of the carotenoids in the human body, and the noteworthiest carotenoids found in the human plasma include phytoene, phytofluene, ζ-carotene, neurosporene, lycopene, γ-carotene, α-carotene, β-carotene, lycopene, α-cryptoxanthin, β-cryptoxanthin, lutein and zeaxanthin (35, 37, 38) (Figure 4). The bioavailability, absorption, metabolism, transport and storage of the them are indicated to depend on various conditions such as the food matrix in which the carotenoids are taken (39), genetic factors (40, 41), age (42) and diseases of digestive tract or surgeries (43-45). Accumulation of the carotenoids, on the other hand, have been shown to be mainly in liver and adipose tissue, while they were found in different tissues such as adrenal gland, testes, retina, kidney, ovary (38) and brain (46).

Carotenoids have various potential beneficial roles as antioxidant in human health and diseases including cancer and cardiovascular conditions (38). As, among the major carotenoids, lycopene (See Figure 2 and Figure 4) is found at the concentrations between 0.22 and 1.06 μM in the blood, representing 21-43 % of the total carotenoids (9) and is one of the most abundant carotenoids present in human plasma (47), it will be the major focused of this work.

Figure 4. Major carotenoids present in the human tissue and blood (38).

2.1.2.4.1. Lycopene

Lycopene is a linear carotenoid with the formula of $C_{40}H_{56}$ (See Figure 2 and Figure 4), is the major carotenoid present in the tomato and responsible for the red pigmentation (48). It is an acyclic isomer of *β*-carotene (38) (Figure 4) and has the structure of an unsaturated hydrocarbon chain with 11 conjugated and 2 unconjugated bonds (49) (Figure 2 and Figure 4). In plants, it is dominantly present in all-*trans* form in the plants (49), while it is found in a *cis*- / *trans*-lycopene mixture with a ratio of 40:60 (50).

Lycopene is one of the most powerful antioxidants among the carotenoids (51, 52) by having the ability of quenching $^1O_2$ two and 10 times higher than β-carotene and α-tocopherol, respectively (51), and decreasing the production of reactive oxygen species (ROS) (53). Additionally, the antioxidant enzymes glutathione S-transferase (GST), superoxide dismutase (SOD) and quinone reductase are upregulated by lycopene (54) therefore the health benefits of lycopene is thought to be mainly its roles in antioxidant activity and responses.

In the next section, the health benefits of lycopene on different conditions will be discussed.

2.1.2.4.1.1. Lycopene and Cancer

Numerous studies have investigated the anticancer activities of lycopene. The main outcome is that lycopene has protective effects on cancer. In previous studies, lycopene was found to be protective against colon cancer by inhibiting N-methylnitrosourea-induced aberrant crypt foci formation (55) and colon carcinogenesis (56) , and azoxymethane-induced colon carcinogenesis via inhibition of cyclooxygenase-2 and inducible nitric oxide synthase (57) in rats. A recent meta-analysis including 15 studies revealed no relationship between the lycopene consumption and the risk of colorectal cancer (58).

An initial study, in which possible anticarcinogenic effect of lycopene against mammary tumor induced by N-methylnitrosourea in rats was investigated, failed to show any improvements (59). However, 7,12- dimethyl benz(a)anthracene-induced breast cancer models, lycopene administration either alone or in combination with melatonin (60) or genistein (61) or tocopherol (62) led to decreased tumor incidence and increased antioxidant activity, as well as abolished the mammary tumor growth in spontaneous mouse model (63). A study showed that plasma levels of total carotenoids were in a significant inverse relation with the oxidative stress marker, 8-hydroxy-2'-deoxyguanosine (8-OHdG), measured in urine samples of individuals who were previously treated for breast cancer (64). In this study, same association but not significant was found between lycopene and 8-OHdG. A study including two patient groups, first group had a history of breast cancer and the second group had a high familial risk of breast cancer, revealed the beneficial effects of lycopene on the women having high risk of breast cancer that was suggested through the modulation of insulin-like growth factor-

I (IGF-I) system (65). However, another study suggested a positive association between the baseline lycopene concentrations and risk of invasive breast cancer in postmenopausal woman (66), while another recent studies suggested higher lycopene concentrations is correlated with lower risk of breast cancer (67, 68).

Investigations of the chemopreventive effects of lycopene in lung cancer models showed variable results. One study showed positive effects of lycopene on carcinogenesis (69), while another did not reveal any chemoprevention. On the other hand, a more recent study showed that lycopene metabolite, apo-10'-lycopenoic acid, inhibits the lung tumorigenesis in mice (70). Additionally, smoke-induced squamous metaplasia was prevented by lycopene supplementation in ferrets (71).

Lycopene was also studied in prostate cancer. Several studies in rats failed to show beneficial effects of lycopene on prostate cancer induced by N-methyl-N-nitrosourea and testosterone (72) and by 2'-dimethyl-4-aminobiphenol and 2-amino-1-methylimidazo[4,5-b]pyridine (73). However, experiments in mice showed that lycopene alone (74-76) or combination of it with Vitamin E (77) inhibit human prostate cancer cell growth in mice. A clinical trial showed an inverse association between the prostate lycopene concentration and the risk of development of prostate cancer in the patients with high-grade prostatic intraepithelial neoplasia (78). However, although some studies suggested beneficial effects of lycopene on prostate cancer (79-81), several other clinical trials reported no association between the lycopene supplementation and prostate cancer development and risk (82-85).

On the other hand, effect of lycopene on hepatic carcinogenesis is also controversial as lycopene was found both to attenuate (86-89) or not to reduce the hepatic carcinogenesis (90) in rodents. In renal cell carcinoma risk was found to be inversely correlated with micronutrient intake, including lycopene intake (91). Additionally, in a study in rats bearing a mutation leading to spontaneous renal cell carcinoma development, lycopene supplementation was shown to be beneficial to the prevention of renal cell carcinoma (92). A study regarding the non-Hodgkin lymphoma indicated that high serum levels of lycopene and several other carotenoids are inversely associated with risk of non-Hodgkin lymphoma (93), however, two meta-analysis studies showed no correlation between the serum lycopene levels and the risk (94, 95).

2.1.2.4.1.2. Lycopene and Cardiovascular Conditions

Lycopene was also indicated to have role in cardiovascular conditions. In a rat model of cardiotoxicity and nephrotoxicity induced by adriamycin (doxorubicin) showed that lycopene administration showed that cardiac and renal histopathology upon adriamycin administration were improved by lycopene (96). Lycopene pre-treatment improved the myocardial damage, myocardial antioxidant status and lipid peroxidation and improved the edema and cardiac muscle damage caused by myocardial ischemia-reperfusion (97). The functional heart impairment, as well as the decreased antioxidant status and increased oxidative stress caused by isoproterenol-induced myocardial infraction was improved by lycopene pre-treatment in rats (98). Another study also indicated similar changes in heart and decrease in apoptotic activity (99). In another myocardial infraction model that was established by left anterior descending coronary artery ligation, lycopene attenuated the collagen volume fraction, enhanced the cardiac function and ventricular remodeling (100). Using the same model, post-infraction application of lycopene was shown to reduce the inflammation through the inhibition of nuclear factor κB, apoptosis and ventricular remodeling (101). Also, lycopene also decreased the myocardial infract area and oxidative stress (102). A study investigated the effect of lycopene on thrombus formation that was induced by irradiation of mesenteric venules suggested the beneficial effects of lycopene supplementation against platelet aggregation (103). Besides, lycopene administration was stated to be beneficial as a chemopreventive against atrazine-induced cardiotoxicity through modulation of the transcription of ATPases and activities, thereby regulating ionic homeostasis in mice (104). Additionally, in streptozotocin-induced diabetic rat, lycopene reduced oxidative parameters while improving the antioxidative capacity and improved the endothelium-dependent vasorelaxation (105).

An intervention study showed that lycopene-rich food intake led to decreased susceptibility of low density lipoprotein (LDL) to oxidation in the individuals who are not smokers, however, this effect of it was not observed in the smokers (106). Additionally, plasma lipid-normalized lycopene was indicated to be negatively associated with the smoking index (107). On the other hand, lycopene supplementation was suggested to contribute in the alleviation of the atherosclerosis by protecting the LDL from oxidation (108). Another intervention study showed that tomato product

consumption led to decreased LDL oxidizability in positive correlation with the lycopene content (109). Lycopene-rich tomato extract supplementation was found to reduce the systolic and diastolic blood pressure significantly in the patients with grade 1 hypertension (110). Another study showed a significant negative association between systolic blood pressure and lycopene levels in the uncontrolled hypertensive patients (111). However, although tomato purée consumption led to increase in the lycopene plasma levels, no significant association between the lycopene levels and endothelial function that was determined by flow-mediated dilatation in healthy subjects (112). Tomato extract supplementation led to significant decreases in the systolic and diastolic blood pressure while elevated apolipoprotein A-I levels in the patients with type 2 diabetes (113). On the other hand, lycopene supplementation for 8 weeks led to improvements in the endothelial function, increased superoxide dismutase levels and decreased systolic blood pressure(114). Additionally, lycopene supplementation in the tomato paste led to improvements in the endothelial function by modulating the flow-mediated dilatation and oxidative stress markers in the midterm but not the short-term (115). Besides, endothelial function patients with cardiovascular disease but not in the healthy volunteers (116). In the Chinese patients with subclinical atherosclerosis, lycopene, not alone but in combination with lutein, decreased the carotid intima-media thickness (117).

Tomato products rich in lycopene was also shown to be protective against lipemia-induced postprandial oxidative stress and inflammatory responses by significantly reducing oxidized LDL and interleukin-6, respectively (118). However, a study showed that neither lycopene nor lycopene-rich tomato products were effective in attenuating the cardiovascular disease risk markers in the subjects who are moderately overweight, healthy, middle-aged (119). Additionally, lycopene-rich supplementation resulted in decrease in LDL, total cholesterol (TC) and malondialdehyde (MDA) levels and increase in high density lipoprotein (HDL) and glutathione levels in postmenopausal women and the results were comparable with the effects of hormone replacement therapy (120). Elevated serum lycopene levels were also suggested to be protective against the carotid atherosclerosis (121). A study in which male and female patients with heart failure were supplemented with lycopene-rich juice showed that serum C-reactive protein levels decreased only in females not males, suggesting a gender specific effect (122).

Additionally, lycopene was suggested to be protective in the moderately overweight middle-aged subjects by promoting the HDL functionality by which exerting its antiatherogenic properties (123). In the subjects having high risk of cardiovascular diseases, lycopene supplementation significantly reduced the levels of the inflammatory biomarkers, intracellular adhesion molecule 1 (ICAM-1) and vascular cell adhesion molecule 1 (VCAM-1) (124). However, tomato-rich diet did not alter the vascular inflammation biomarkers in volunteers (125). All in all, lycopene is suggested to be beneficial for the patients having cardiovascular conditions or subjects at high risk of cardiovascular diseases.

2.1.2.4.1.3. Lycopene and Other Conditions

Lycopene has various beneficial effects besides the abovementioned conditions. Studies in rodents showed protective effects against various conditions such as diabetes (105, 126-128), inflammation (129-133), osteoporosis (134, 135) and neurodegeneration (136-138). Clinical trials also led to promising results. For instance, endogenous lymphocyte DNA damage was significantly decreased in postmenopausal women upon lycopene supplementation (139). In type 2 diabetic patients, lycopene serum lycopene concentrations were negatively associated with serum IgG levels, while in positive association with IgM levels, suggesting that lycopene modulates the adaptive humoral immunity (140). In postmenopausal women, lycopene supplementation led to significant elevation in the total antioxidant capacity while attenuating lipid peroxidation, protein oxidation and N-telopeptide, the bone resorption marker, suggesting that lycopene may be beneficial against osteoporosis (141). Lycopene, however, did not alter the lung function and have any protective effect in the athletes having difficulty in breathing induced by exercise (142). It was previously found the lycopene alone or in combination with vitamin C supplementation decreased the total cholesterol and C-reactive protein levels (143). Additionally, lycopene supplementation led to reduction in the systemic inflammation in the overweight and obese female subjects (144).

2.2. Chemotherapeutics and Platinum-Based Chemotherapeutics

Cancer is a complex disease that can basically be characterized by abnormal cell growth, proliferation, invasion, and sometimes in some forms, spread of the abnormal cells into other tissues (145). Chemotherapy, radiotherapy and surgery comprise the main

available treatment approaches (146). There are various types of chemotherapeutic agents for the treatment (147) and more are under development process (148).

Pt^{II} -based drugs are one of the groups that are currently used in the cancer chemotherapy (149, 150). Reversible anti-proliferative effects of certain platinum compounds on Gram-negative rods were first discovered by Barnett Rosenberg in 1960s (151). Rosenberg's studies were initially focused on effects of electric or magnetic dipole fields on bacterial and mammalian cell division. However, experiments involving *Escherichia coli* revealed that electrolysis products of the platinum electrodes rather than electric field were found effective on the bacteria (151). Later, these compounds were tested for their anti-cancer activity *in vivo* on Sarcoma 180- and Leukaemia L1210-induced mouse cancer models and found effective as anti-cancer agents (152). Moreover, *in vitro* studies revealed that these compounds impair cell division on eukaryotic cells, disrupt chromosome morphology in the few cells that can divide. As a result, researchers reported that platinum compounds may form a new class of anti-cancer agents (152). Cisplatin has become the first platinum compound, which has been taken for clinical testing on 1971 by the US National Cancer Institute (NCI) and approved by the US Food and Drug Administration (FDA) on 1978. This discovery draw attention to synthesis and evaluation of other cisplatin analogues, mainly aiming to design a drug causing lesser toxicity and overcoming the tumour resistance that cells may develop upon cisplatin treatment (153).

2.2.1. Effect Mechanisms of Platinum Compounds

Platinum compounds exert their effects by becoming aquated once they enter the cell: this process involves a hydrolysis reaction causing the molecule to lose its oxalate or chloride ions due to lower intracellular ion concentration and gaining water molecules, thus, becoming more reactive, enabling the drug to interact with nucleic acids and proteins (154). DNA is considered as the main target of platinating agents, different lesions observed upon interacting with platinating agents are given at Figure 5.

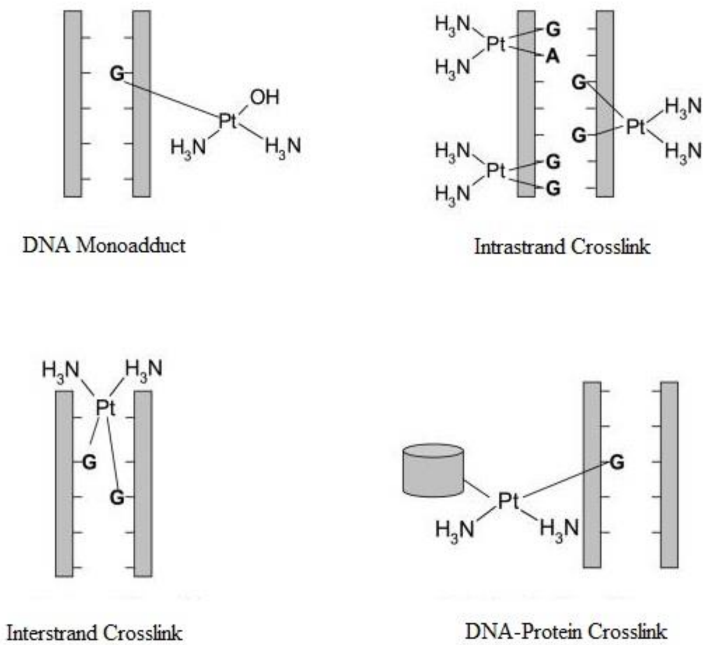


Figure 5. Platinating agents can form DNA monoadducts, intra- and inter-strand crosslinks and DNA-protein crosslinks (155).

Monoadducts are formed upon the loss of a water molecule the aquated platinating agent gained after entering the cell. More than 90 % of monoadducts later form DNA crosslinks with the majority being 1,2-d(GpG) crosslinks. Different agents are known to form different amount of crosslinks that may not affect their potency: oxaliplatin forms less crosslinks than cisplatin at the same concentration, yet is found as efficient as its counterpart by inducing similar numbers of single strand and double strand DNA breaks. All crosslinks cause DNA contortion (156). Cisplatin- and carboplatin-induced intra-strand crosslinks were shown to bend double helix structure toward the major groove at 32 - 35 °, whereas oxaliplatin has shown to bend further (157). Inter-strand lesions result in the extrusion of cytosines at crosslinked d(GpC)d(GpC) sites, causing double helix bend toward the minor groove by 20 - 40°. Adducts differ among different analogues, causing different effects in the cell (158). For example, cisplatin is closely related to carboplatin as they show similar mechanism of action and form identical DNA lesions, while oxaliplatin do not share same mechanisms (154). However, all platinum-based compounds potentially lead to cell cycle arrest and induce apoptosis (159).

2.2.2. Cisplatin and Its Action Mechanism

Cisplatin, which has been used as an anticancer agent for over 30 years, is the first platinum drug approved globally (160) and one of the most effective and commonly used chemotherapeutic agents for treating solid tumors including lung (161), head and neck (162), breast (163), cervical (164) and ovarian cancers (165).

Cisplatin is a highly polar molecule and its cellular uptake is relatively slow compared to other chemotherapy drugs (153). It was initially thought to be passive diffused into the cell, however, today, it is known that cellular uptake of cisplatin is directly linked with cellular copper and platinum concentrations: copper and cisplatin affect cellular uptake of each other and promote degradation of the copper transporter CTR1, which also takes part in cisplatin uptake (166). Loss of CTR1 has shown to be directly linked with cisplatin resistance (166).

Upon entering the cell, cisplatin gains affinity by losing one or two chloride groups by hydrolysis, enabling the molecule to bind DNA to form DNA adducts, formation of which activates signal-transduction pathways that result in cell cycle arrest, apoptosis and DNA damage recognition (167). Platinum-induced DNA adducts disable the DNA strand separation process, thus blocks replication and transcription (167). Cisplatin-induced DNA damage activates tumor suppressor gene p53: in addition to activating nucleotide excision repair (NER) machinery (168), p53 initiates apoptosis via inhibiting anti-apoptotic protein Bcl2 and activating pro-apoptotic caspases, process of which is considered as the main cisplatin-induced anti-cancer mechanism (169). Caspase activation is crucial for apoptotic process, and different caspases take part in two different types of apoptosis which are called extrinsic and intrinsic apoptotic pathways. Ligand binding to tumor necrosis factor-α (TNFα) receptors activate extrinsic pathway via caspase-8 (170). In contrary to extrinsic pathway, DNA damage activates intrinsic pathway via disrupting mitochondria integrity, causing cytochrome c release to form apoptosome structure to activate caspase-9. In addition to caspase activation, apoptosis has distinct morphological features such as cell shrinkage, chromatin condensation and translocation of phosphatidyl serine residues which are normally located at the inner side of the cell membrane to outer side (171). Schematic representation of cisplatin's effect mechanism is given at Figure 6.

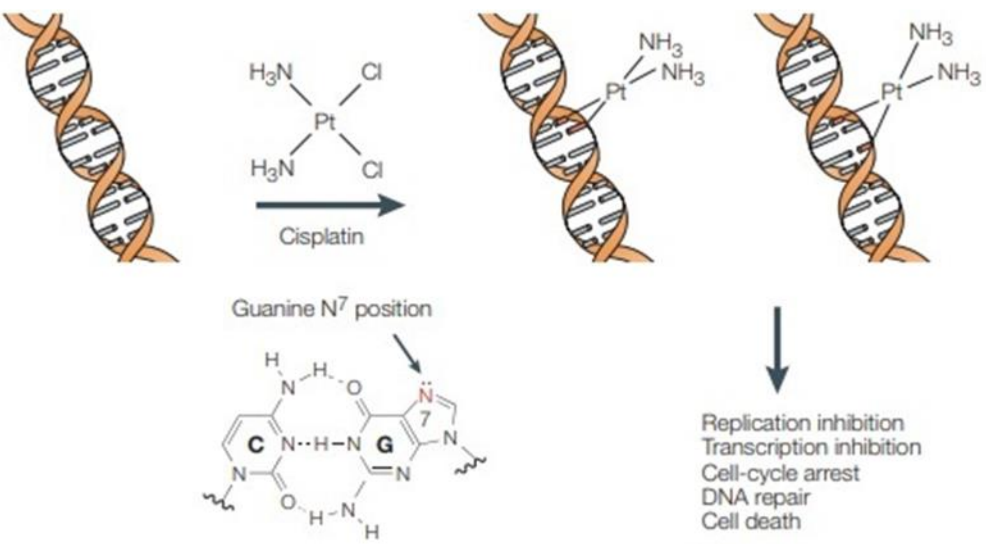


Figure 6. After aquation, the platinum atom of cisplatin binds to the purines at the N7 position to form intra- and inter-strand crosslinks. Cisplatin-DNA adducts activate various pathways including cell cycle arrest, inhibition of replication and transcription, apoptosis and DNA repair (154).

Cisplatin is a very effective against cancer and has important clinical impact, however, it should be noted that some tumors are intrinsically resistant to it and some acquire resistance during therapy limiting its applications. Studies aiming to determine cisplatin resistance revealed that this process may be mediated via two different mechanisms: one of them is the failure of the drug to reach DNA and the second one is the disruption of programmed cell death mechanisms that should be activated as a response to platin-DNA adducts (153). Major molecular mechanisms of cisplatin resistance are given on Table 1.

Cisplatin resistance due to low drug-DNA binding is reported in many studies indicating acquired resistance compared to parental cells (172). Downregulation of CTR1 because of internalization followed by degradation is shown to be linked with cisplatin resistance (173). Recently, ATPases ATP7A and ATP7B have also shown to regulate cisplatin uptake (174). Once platin-DNA adducts are formed, removal of these adducts or developing tolerance also leads to cell survival. Studies focusing tumours with cisplatin resistance indicates increased DNA-repair capacity compared to sensitive counterparts. This may occur due to increased function of various components of NER pathway, which is the major pathway responsible for removing DNA-platin adducts (175).

Table 1. Molecular mechanisms of resistance to cisplatin (154).

Molecular mechanisms	Examples of tumour type or cell line
Inactivation of cisplatin by glutathione, metallothionein or other sulphur containing molecules	Ovarian or bladder cancer, L1210 murine leukaemia or small-cell lung cancer line
Increased repair of cisplatin adducts	Ovarian cancer; non-small-cell lung cancer line
Reduced cisplatin intake due to altered uptake/efflux profile	Ovarian cancer; epidermoid carcinoma; oral squamous carcinoma
Increased resistance cisplatin adducts and failure of apoptosis	Ovarian cancer; L1210 murine leukaemia

2.3. Cisplatin-Induced Toxicity (CIT) and Effect of Lycopene on CIT

Drug-induced toxicities do not occur only as a consequence of an overdose. Even at the doses that is relevant for the treatment of the patient (176). One of the major organs that are affected is liver (1, 177) as it is a major organ that metabolizes and removes the drugs, process of which is important for the homeostatic balance (177). Even though acute liver injury caused by the drug-induced hepatotoxicity is rare, it is the major reason for the acute liver failure in Europe and USA (178-180) and the leading cause of the liver transplantation in USA (181). Up to date, more than thousands of drugs have been reported to cause hepatotoxicity (177). In addition to this, some of the pharmaceutical agents cause nephrotoxicity leading to serious conditions such as acute kidney injury (AKI) (182). Prospective studies conducted on adults indicated that nearly 16 - 24 % of the nephrotoxicity are the acute kidney injury cases (183-185). On the other hand, adverse effects of various groups of drugs that causing toxicity in other sites in the body such as cardiovascular system (186), pulmonary system (187) and nervous system (188, 189).

2.3.1. Cisplatin-Induced Toxicity (CIT)

Antineoplastic agents are intentionally designed as the toxic compounds in order to treat cancer, and due to their toxic effects, the adverse and toxic effects to other organs are unavoidable (190). Cisplatin, like other antineoplastic agents, have toxicities on various organs. Nephrotoxicity is one of the most stated side effects of cisplatin and it is the main dose-limiting factor (191). AKI is the major and of the common manifestations of cisplatin nephrotoxicity and is observed in nearly one third of the patients (191).

Cisplatin is taken up by the kidney at a higher concentration than other organs (192), mainly due to its high affinity to CTR1 (193) and organic cation transporter 2 (OCT2) (194), as well as the highest expression of OCT2 in kidney compared to other organs (195), and probably due to the gamma-glutamyl transpeptidase that have a role in the uptake of cisplatin by cleaving glutathione-cisplatin conjugate leading to uptake of cisplatin by the proximal tubule of kidney (196). Cisplatin-induced nephrotoxicity have various other manifestations including hypomagnesemia, Fanconi-like syndrome and chronic renal failure (191).

Although the main organ affected by the cisplatin toxicity is kidney, hepatotoxic effects have also been reported in the clinics (197-203), but to a lesser extent than nephrotoxicity. It was stated that cisplatin treatment rarely causes hepatotoxicity when the standard doses are applied, however, at the higher doses, abnormal tests results for alanine aminotransferase (ALT) and aspartate transaminase (AST) were noted, suggesting hepatotoxicity of cisplatin is dose-dependent (204).

Studies indicated several mechanisms that play role in the cisplatin-induced nephrotoxicity and hepatotoxicity. Cisplatin is mainly taken and accumulated in the liver and the kidney cells (205) and induces mitochondrial dysfunction and induction of ROS production in the renal epithelial cells (206). Reduction in glutathione (GSH) levels and antioxidant enzyme activities in the kidneys activating the mitogen-activated protein kinase (MAPK) signaling pathway causes the renal cell death (207). Induction of ROS production via cytochrome P450 E1 also plays a role in the renal injury (208). A recent study showed that cisplatin induced oxidative stress leads to the processing of membrane bound death activator Fas ligand (mFasL) to soluble FasL (sFasL), and this results in the activation of the apoptotic cascade and eventually tubular cell death (209). On the other hand, cisplatin also induces the oxidative stress in the liver by increasing the MDA, nitric oxide (210), nuclear factor erythroid 2-related factor 2 (NRF-2) and glutathione peroxidase levels (211) and decreasing the GSH levels (210, 211), inhibiting the inflammation(210) and activation of the MAPK pathway (211) and causes liver damage and hepatic cell death. Another study also indicated that cisplatin alters the activity of electron transport chain enzymes in the isolated liver mitochondria (212). Additionally, cisplatin also disrupts the levels of liver enzymes including ALT, AST (210, 211, 213, 214) and alkaline phosphatase (ALP) (210) and serum lipid parameters including TG and

TC (211, 214). Additionally, cardiotoxicity associated with the cisplatin treatment was reported by several studies (215-217), as well as testicular (218, 219) and ovarian toxicities (220, 221).

2.3.2. Effect of Lycopene on CIT

Natural products have been of interest in order to prevent the drug-induced organ toxicity for a long time. In recent years, several studies investigated the possible preventive and protective effects of lycopene.

One of the initial studies reported that the lycopene was ineffective in reducing the bleomycin-induced DNA damage in human lymphocyte (222). On the other hand, lycopene partially rescued degree of the pulmonary fibrosis induced by bleomycin in rats by improving oxidative stress and decreasing the plasma and lung levels of TNFα (223).

Adriamycin-induced cardiotoxicity and nephrotoxicity were prevented and oxidative markers, as well as the serum creatinine levels, were improved by the lycopene pre- and post-treatment (96). Another study also showed that antioxidant enzyme levels and activities that were disrupted by adriamycin-induced nephrotoxicity were improved and renal damage was reduced in mice (224). Additionally, lycopene pre-treatment improved the testicular damage and oxidative stress induced by adriamycin in rats (25). Adriamycin-induced DNA damage in the cardiomyocytes were prevented by lycopene (225) and tomato-oleoresin (226), however, lycopene were not improve the cardiac dysfunction caused by adriamycin (225).

Cyclophosphamide-induced increase in the lipid peroxidation, morphological abnormalities in sperm and testicular histopathology were improved by lycopene administration, but not the catalase and SOD activities (227, 228).

In the methotrexate-induced nephrotoxicity model of rats, lycopene significantly reduced TNFα and interleukin 1-beta (IL-1β) levels, while improving the renal damage (229). Lycopene also reduced the histopathological damage and IL-1β levels, total oxidative status and oxidative stress index that were induced by methotrexate (230) and improved the liver injury in rats (231).

A study investigated whether pre- or post-treatment with lycopene on cisplatin-induced testicular damage and oxidative stress and reported that both pre- and post-

treatment with lycopene significantly improved sperm quality, oxidative stress, while the post-treatment exhibited better activity than the pre-treatment (24). Acute or subacute lycopene treatment led to reduction in DNA damage induced by cisplatin in rat bone marrow cells (232). In rat model of cisplatin-induced nephrotoxicity, lycopene was suggested to prevent the toxicity via improving the NRF-2 and heme oxygenase-1 (HO-1) levels downregulated by cisplatin and decreasing the nuclear factor-kappaB p65 (NFκB p65) expression induced by cisplatin (27). Lycopene treatment either alone or in combination with selenium counteracted the decrease in the testes weight, length and thickness, as well as the epididymis weight reduction, induced by cisplatin, and improved the epididymal sperm counts and abnormal sperm rate (23). In the same study, it was also shown that testicular oxidative stress was improved by lycopene and its combination with selenium and decreased testosterone levels were partially rescued by the treatments (23). Lycopene was also suggested to be possibly protective against cisplatin-induced ototoxicity (233).

As abovementioned, lycopene has beneficial effects on the chemotherapy-induced toxicity. In this study, it was aimed to be investigated that whether lycopene has prophylactic or therapeutic activity against the toxic effects of cisplatin in kidney, liver, testes and spleen. For this purpose, animals were administered with lycopene intraperitoneally either prior to or on the same day with the cisplatin administration. Blood was tested for biochemical, liver and kidney function parameters, and histopathological examinations were conducted on heart, aorta, liver, kidneys and testes.

3. MATERIALS & METHODS

3.1. Materials

3.1.1. Equipment

Equipment is indicated in Table 2.

Table 2. Equipment and manufacturer

Equipment	Manufacturer
Micropipette	Rainin, USA
Micropipette tips	Rainin, USA
Centrifuge tubes	Isolab, Turkey
Refrigerator	Arcelik, Turkey
Blood collection tubes	BD Biosciences, USA
Injectors	Set Medikal San. ve Tic. A.Ş., Turkey
Chemistry analyser	Beckman Coulter, USA
Haematology analyser	New Life, USA
Centrifuge	Nüve, Turkey

3.2. Methods

3.2.1. Experimental design and Animal Husbandry

CIT experiments was conducted in Sprague Dawley rats. Rats were obtained from the Yeditepe University Medical School Experimental Research Center (YUDETAM) and housed under controlled environmental conditions (temperature of 21 ± 1 °C, 12:12 h light-dark cycle in polypropylene cages with bedding). The experimental protocol was approved by the Ethic Committee of Yeditepe University. Adult male rats (8 - 10 - week old) were randomly assigned into four groups including seven animals in each group. The experimental groups and administrations are indicated in

Table *3*.

Table 3. Experimental groups and drug application design.

Experimental group	Cisplatin administration	Lycopene administration
Group A	On day 0, single dose of 5 mg/kg intraperitoneally	Lycopene (5 % lycopene (w/w) in tomato extract; 200 mg/day) administration for 10 days via oral gavage
Group B	On day 7, single dose of 5 mg/kg intraperitoneally	Lycopene (5 % lycopene (w/w) in tomato extract; 200 mg/day) administration for 10 days via oral gavage
Group C	On day 0, single dose of 5 mg/kg intraperitoneally	Not administered
Group D	On day 7, single dose of 5 mg/kg intraperitoneally	Not administered

The cisplatin was applied to induce toxicity in rats on day zero or five as indicated in

Table *3*. Lycopene in tomato extract (5 % w/w; 200 mg/day) was administered to rats in Group A and B for 10 consecutive days. At the 10th day, rats were euthanized by carbon dioxide, and heart, aorta, liver, kidney and testes were dissected and collected for further analyses.

3.2.2. Blood Parameters

800 µL blood was withdrawn via vena jugularis externa under isoflurane anesthesia (2-4 %) on day zero, seven and 10 and divided into two for the complete blood count and blood biochemistry tests.

3.2.2.1. Complete Blood Count Tests

400 µL blood samples were taken from the rats on day zero, seven and 10 and placed in a blood collection tube (SST™II Advance Plus Blood Collection Tube, BD Biosciences) and ran on a hematology analyzer (Prokan, PE6800) for the complete blood count tests.

3.2.2.2. Blood Biochemistry Tests

400 µL blood samples were taken from the rats on day zero, seven and 10 and placed in a blood collection tube (K2E Plus Blood Collection Tube, BD Biosciences), centrifuged at 4100 RPM in a centrifuge (NF 800R, Nüve) and ran on a chemistry analyzer (AU480, Beckman Coulter) for blood biochemistry tests.

3.2.3. Histopathological Examinations

After sacrificing the animals, the tissues collected were fixed in 10 % formaldehyde solution and stored at 4°C. Then, the tissue samples were washed with water for around 2 hours and dehydrated in alcohol series (70%, 90%, 96% and 100%, respectively). The tissues were gotten transparent with toluene and embedded in paraffin at 60 °C overnight in the oven.

3.2.3.1. Kidney Histopathology

Kidneys were embedded in paraffin and sliced with a microtome at 3 µm thickness longitudinally including the regions cortex, medulla and renal pelvis. Then,

each slice was stained with Hematoxylin-Eosin (H&E) dye to make histopathologic scoring. Each slice was assessed in terms of necrosis (0: no change, 1: necrosis in some areas, mild to moderate 2: confluent necrosis, severe), inflammation (0: absent, 1: present), glomerular pathology (0: absent, 1: present), and congestion (0: absent, 1: present). H&E stained tissue slices were investigated under the light microscopy (BX53, Olympus).

3.2.3.2. Testicular Histopathology

Testes were embedded in paraffin and sliced with a microtome at 3 μm thickness longitudinal axis. Then, each slice was stained with Hematoxylin-Eosin (H&E) dye to make histopathologic scoring. Histopathology of 20 tubules in each sample were assessed according to Johnsen scoring system (234). (1: No cells present in the tubules, 2: No germ cells present in the tubules but Sertoli cells are present, 3: Spermatogonia is present as germ cell, 4: Spermatocyte count is less than 5, spermatid/spermatozoa is not present, 5: No spermatid/spermatozoa is present, abundant of spermatocyte is present, 6: No spermatozoa is present, number of spermatids is less than 5-10, 7: No spermatozoa is present, abundant of spermatid is present, 8: Number of spermatozoa is less than 5-10, 9: Abundant of spermatozoa is present, germinal epithelium is disorganized and lumen is obliterated, 10: Abundant of spermatozoa with a complete spermatogenesis.). H&E stained tissue slices were investigated under the light microscopy (BX53, Olympus).

3.2.3.3. Liver Histopathology

Liver tissue were embedded in paraffin and sliced with a microtome at 3 μm thickness. Then, each slice was stained with Hematoxylin-Eosin (H&E) dye to make histopathologic assessment. Each slice was assessed in terms of dilatation of central vein, presence of central vein thrombosis, perivenular and periportal sinusoidal dilatation, inflammation, vacuolar degeneration, proliferation of bile ducts. The density of the predominantly mononuclear inflammatory cells was evaluated (1: minimal or mild, 2: moderate, 3: severe). Vacuolar degeneration was evaluated and scored as follows: 0: no degeneration, 1: minimal or mild, 2: moderate, 3: severe. Bile duct proliferation was evaluated and scored as follows: 0: no proliferation, 1: focal proliferation, 2: disseminated proliferation.

3.2.4. Statistical Analyses

Statistical analyses were conducted by using GraphPad Prism 7. Data are expressed as mean ± standard deviation (SD). Body and tissue weights were compared by using One-way ANOVA followed by Tukey's multiple comparison test. Ordinary two-way ANOVA followed by Tukey's multiple comparison test by comparing the columns (Groups) within each row (CBC parameter / blood biochemistry parameter) was used for the intergroup comparisons. Intragroup analyses for each individual CBC parameter or blood biochemistry parameter were conducted by ordinary two-way ANOVA followed by Tukey's multiple comparison test by comparing the rows (Days) within each column (Groups). Intergroup histological assessments were compared by using One-way ANOVA followed by Tukey's multiple comparison test for kidneys, testicles and liver.

4. RESULTS

4.1. Body and Tissue Weights

Animals were weighed before sacrificing and kidneys (right and left), testicles (right and left), heart and liver were dissected and weighed. Body weight (BW) was significantly higher in group D compared to other groups (Table 4), but there was no significant difference between the groups with regards to tissue weight. However, when the tissue weights were adjusted to body weights, right kidney weight of the animals in group D was found significantly lower than in Group D ($p < 0.001$; Table 4). Additionally, BW-adjusted heart weight of group C was significantly lower than group B ($p < 0.01$) and group D ($p < 0.05$; Table 4). Nevertheless, BW-adjusted weights of other tissues were not significantly different between the groups.

4.2. Blood Parameters

Complete blood count (CBC) test, blood biochemistry and the levels of the enzymes showing the liver and kidney function were determined.

4.2.1. Complete Blood Count Tests

Complete blood count tests did not differ significantly between the groups at the baseline except for the platelet (PLT) counts differed among the groups significantly. Group A had the lowest number of platelet counts, group D had the highest number of platelets at the baseline (Table 5). Group C had higher number of platelets compared to group B (Table 5). On day 7 and 10, group A had significantly lower PLT counts compared to group C and D, but not group B. Group C had the highest PLT counts among all groups ($p < 0.05$; Table 5). There was no significant difference between group A and B ($p>0.05$)

The white blood cell (WBC) counts showed that WBC counts increased significantly either seven days or two days after cisplatin administration in all groups except group D (Table 5). On the other hand, the increased WBC counts decreased to the levels at which there was no significant differences between the day 10 and baseline and between the day 10 and day 7 measurements (Table 5). However, the counts decreased significantly to the basal levels on day 10 in group C ($p < 0.0001$) and even below the basal levels in group D ($p < 0.001$ vs. baseline and $p < 0.0001$ vs. day 10).

In group A, the percentage of lymphocytes (LYM (%)) were found increased, but not significantly, on day 7 compared to day 0 (Table 5). This increase was significantly decreased on day 10 ($p < 0.0001$). However, there was no difference in the measurements of LYM counts (LYM #) on different days (Table 5). In group B, significant increase in LYM (%) was observed on day 7 ($p < 0.01$), and this was counteracted on day 10 ($p < 0.0001$) and decreased below baseline levels ($p < 0.01$; Table 5). LYM # was also found similar to the LYM (%), except the counts on day 10 were not below the baseline (Table 5). LYM (%) did not change significantly in group C and D on day 7, however, was decreased below the baseline levels on day 10 (Group C: $p < 0.01$ and Group D: $p < 0.001$). LYM # was also found in the similar pattern with the LYM (%) over time, except the increase in the LYM # on day 7 was significantly different than day 0 in group B ($p < 0.0001$).

Percent of the mid-range absolute count (MID (%)) was found significantly decreased on day 7 ($p < 0.01$) but normalized on day 10 in group A (Table 5). On the other hand, no significant changes were observed in MID #. In group B, MID (%) was significantly decreased on day 7 ($p < 0.001$) and did not change on day 10 compared to day 7 (Table 5). However, there was no change in MID #. In group C, MID (%) was unchanged on day 7 compared to day 0 but significantly lower than on day 10 ($p < 0.05$), while MID # significantly increased on day 7 ($p < 0.001$) and it decreased to the basal levels on day 10 (Table 5). MID (%) was found unchanged over time in Group D, while MID # was significantly reduced on day 10 compared to day 0 ($p < 0.05$) and day 7 ($p < 0.001$).

In all groups, percentage of granulocytes (GRAN (%)) were found decreased, but not significantly, on day 7 and significantly higher on day 10 compared to day 0 and day 7 ($p < 0.05$, Table 5). On the other hand, GRAN # was found unchanged in group A, while it was significantly higher in group B on day 10 compared to day 0 ($p < 0.05$). Additionally, GRAN # was significantly higher on day 7 compared to day 0 in group C ($p < 0.05$), and although a decrease was observed on day 10, this decrease was not significant compared to day 0 and 10 (Table 5). In group D, GRAN # was significantly lower on day 10 than on day 7 ($p < 0.01$) and on day 0 ($p < 0.05$).

The non-significant decrease in the red blood cell (RBC) counts on day 7 than on day 7 were significantly increased on day 10 in group A ($p < 0.001$; Table 5). In group

B, baseline RBC counts were found significantly decreased on day 7 ($p < 0.01$) and that was counteracted on day 10. In group C, RBC remained at the same level with day 0 and was significantly increased on day 10 ($p < 0.001$ vs. day 0 and $p < 0.0001$ vs. day 7), while group D exhibited significantly lower RBC counts on day 7 than on day 0 ($p < 0.05$; Table 5) and it was significantly increased on day 10 ($p < 0.0001$).

Haemoglobin (HGB) levels were non-significantly increased on day 7 compared to the levels on day 0 and it was significantly higher on day 10 than on day 7 ($p < 0.05$) in group A and B (Table 5). Group C, on the other hand, exhibited significantly higher HGB levels on day 10 compared to the levels on day 0 ($p < 0.01$) and day 7 ($p < 0.05$). In group D, however, no significant differences were observed on different days although the HGB levels increased (Table 5). Mean corpuscular haemoglobin (MCH) was found unchanged in group A (Table 5). On the other hand, significant increase in the MCH levels in group B, C and D was decreased to the basal levels (Table 5). Additionally, the increase in the mean corpuscular haemoglobin concentration (MCHC) levels in group A, B and C was downregulated to the basal levels measured on day 0 (Table 5).

Haematocrit (HCT) levels were found significantly decreased on day 7 compared to the levels on day 0 in only Group B ($p < 0.05$) and it was significantly higher on day 10 than on day 0 and 7 ($p < 0.05$; Table 5). In group A, C and D, HCT levels were non-significantly decreased and the levels on day 10 was significantly higher than the levels on both day 7 and 0 (Table 5).

Mean cell volume (MCV) was not changed during the experimental period in group A (Table 5). On the other hand, MCV was found increased non-significantly in group B and significantly in group C ($p < 0.01$) and D ($p < 0.001$), and this increase was found to be reversed on day 10 (Table 5).

There was no difference in the red cell distribution width-standard deviation (RDW-SD) in group A and B over time (Table 5). A small but significant increase was observed in group C on day 7 compared to the value on day 0 ($p < 0.05$). On the other hand, increase in the RDW-SD in group D on day 7 ($p < 0.01$) was also observed on day 10 ($p < 0.05$) compared to the RDW-SD on day 0 (Table 5). On the other hand, red cell distribution-coefficient of variation (RDW-CV) value did not change in group C and D (Table 5) over time, while there was a significant difference between the values on day 7

and day 10 in group B ($p < 0.01$). In group C, RDW-CV was significantly higher on group D than on day 0 ($p < 0.001$) and day 7 ($p < 0.05$).

Platelet counts (PLT) did not change significantly in group A, B and D (Table 5), however, group C exhibited significantly lower PLT on day 0 than day 7 and day 10 ($p < 0.05$). On the other hand, platelet distribution width in percent (PDW (%)) value was significantly higher in group D on day 7 than on it was day 0 and 10 ($p < 0.01$), but did not change in other groups over time in other groups (Table 5).

In addition to these, mean platelet volume (MPV), procalcitonin levels in percent (PCT (%)) and platelet large cell ratio (P-LCR (%)) were not changed over time in any groups significantly (Table 5).

Table 4. Body and tissue weights of the animals in different experimental groups. Data are mean ± SD. (n=7 animals in each group, except for the group A, n=6 animals) Data sharing the same superscript letters in different groups are significantly different from each other.

		Groups			
		A	B	C	D
BW (g)		269.18 ± 22.97 [c]	300.86 ± 54.98 [a]	313.14 ± 64.05 [e]	384.86 ± 28.33 [a, e, c]
Kidney (mg)	Right	1802.67 ± 479.53	1524.43 ± 175.13	1653.00 ± 215.70	1812.43 ± 285.66
	Left	1659.83 ± 395.28	1598.71 ± 160.45	1581.43 ± 269.30	1899.29 ± 255.15
Testicle (mg)	Right	1066.67 ± 248.60	1107.86 ± 413.92	1143.14 ± 353.03	1540.29 ± 102.29
	Left	1027.67 ± 317.46	1113.86 ± 380.75	1133.71 ± 359.61	1537.71 ± 82.22
Liver (mg)		7239.50 ± 2299.48	10256.29 ± 1478.82	10848.14 ± 3199.20	10163.71 ± 4032.86
Heart (mg)		1067.00 ± 134.78	1352.43 ± 131.03	1036.43 ± 150.72	1598.14 ± 222.12
BW-adjusted tissue weights (mg tissue/g animal)					
		Groups			
		A	B	C	D
Kidney (mg/g)	Right	6.73 ± 1.84 [c]	5.14 ± 0.59	5.39 ± 0.89	4.69 ± 0.48 [c]
	Left	6.19 ± 1.51	5.41 ± 0.68	5.14 ± 0.80	4.93 ± 0.44
Testicle (mg/g)	Right	3.96 ± 0.93	3.60 ± 1.03	3.64 ± 1.01	4.01 ± 0.12
	Left	3.81 ± 1.19	3.63 ± 0.91	3.60 ± 1.04	4.00 ± 0.15
Liver (mg/g)		26.92 ± 8.19	34.63 ± 5.48	34.16 ± 3.51	26.71 ± 10.65
Heart (mg/g)		3.97 ± 0.50	4.57 ± 0.55 [b]	3.37 ± 0.50 [a, b]	4.16 ± 0.52 [a]

$^{a\text{ and }e}$ $p < 0.05$, ^{b} $p < 0.01$, ^{c} $p < 0.001$ and ^{d} $p < 0.0001$

Table 5. Baseline (Day 0), day 7 and day 10 CBC measurements. Data are mean ± SD. (n=7 animals in each group, except for the group A and D on day 10, n=6 animals). Data sharing the same superscript letters in different groups and are significantly different from each other.

	Groups / Days	A	B	C	D
WBC (10^3 cells/µL)	**0**	19.00 ± 8.28 ^{k}	18.10 ± 5.26 ^{l}	17.00 ± 6.76 ^{n}	41.90 ± 23.64 ^{r}
	7	40.40 ± 6.17 ^{k}	40.90 ± 9.61 ^{l}	52.20 ± 23.20 $^{n, s}$	52.00 ± 23.20 ^{s}
	10	27.40 ± 15.50	24.40 ± 13.40	18.40 ± 4.62 ^{s}	10.40 ± 4.11 $^{r, s}$
LYM (%)	**0**	56.50 ± 3.66 ^{p}	53.90 ± 4.30 $^{q, x}$	60.80 ± 4.06 ^{r}	60.97 ± 7.38 ^{r}
	7	67.50 ± 5.22 ^{s}	71.20 ± 5.99 $^{q, s}$	66.30 ± 10.50 ^{q}	62.40 ± 12.20 ^{y}
	10	41.00 ± 15.80 $^{p, s}$	34.70 ± 21.70 $^{x, s}$	43.70 ± 7.03 $^{q, r}$	37.40 ± 11.70 $^{r, y}$
MID (%)	**0**	13.50 ± 1.71 ^{l}	14.50 ± 1.30 $^{m, r}$	10.40 ± 1.45	10.56 ± 3.47
	7	9.29 ± 0.93 $^{k, l}$	9.26 ± 1.97 ^{m}	9.70 ± 2.28 ^{k}	10.00 ± 2.64
	10	13.30 ± 4.16 ^{k}	8.69 ± 4.55 ^{r}	13.00 ± 1.11 ^{k}	13.20 ± 1.68
GRAN (%)	**0**	30.00 ± 2.75 ^{k}	31.60 ± 3.57 ^{n}	28.80 ± 2.95 ^{k}	28.47 ± 4.63 ^{m}
	7	23.20 ± 5.09 ^{m}	19.60 ± 5.00 ^{s}	23.60 ± 8.69 ^{l}	27.50 ± 9.85 ^{r}
	10	45.70 ± 12.70 $^{k, m}$	56.60 ± 24.20 $^{n, s}$	43.30 ± 6.14 $^{k, l}$	49.40 ± 11.10 $^{m, r}$
LYM # (10^3 cells/µL)	**0**	11.40 ± 5.30	9.87 ± 3.35 ^{l}	10.40 ± 4.24 ^{n}	25.83 ± 14.47 ^{m}
	7	24.10 ± 9.93	29.50 ± 8.36 $^{l, q}$	35.20 ± 15.60 $^{n, s}$	33.20 ± 16.30 ^{n}
	10	13.10 ± 13.10	9.14 ± 11.30 ^{q}	8.21 ± 3.05 ^{s}	3.63 ± 1.40 $^{m, n}$

MID # (10^3 cells/µL)	**0**	2.61 ± 0.87	2.63 ± 0.71	1.76 ± 0.70 [m]	4.04 ± 2.04 [k]
	7	4.59 ± 2.22	3.77 ± 1.10	5.14 ± 2.73 [m, l]	5.14 ± 2.46 [m]
	10	3.35 ± 1.26	1.96 ± 1.45	2.44 ± 0.59 [l]	1.38 ± 0.54 [k, m]
GRAN # (10^3 cells/µL)	**0**	5.87 ± 2.24	5.57 ± 1.43 [k]	4.89 ± 2.02 [k]	12.03 ± 7.47 [k]
	7	8.50 ± 4.08	7.71 ± 1.78	11.80 ± 6.62 [k]	13.70 ± 7.24 [l]
	10	11.00 ± 1.76	13.30 ± 9.27 [k]	7.84 ± 1.53	5.33 ± 2.55 [k, l]
RBC (10^6 cells/µL)	**0**	7.00 ± 1.62	8.05 ± 1.21 [l]	6.93 ± 2.40 [m]	7.21 ± 2.14 [k, p]
	7	4.79 ± 1.49 [m]	4.02 ± 3.09 [l, n]	5.62 ± 2.97 [n]	4.33 ± 3.63 [k, n]
	10	9.44 ± 1.33 [m]	10.50 ± 1.26 [n]	11.30 ± 0.30 [m, n]	10.30 ± 1.76 [p, n]
HGB (g/dL)	**0**	18.6 ± 5.32	21.4 ± 4.02	19.3 ± 6.68 [l]	24.84 ± 3.30
	7	17.60 ± 4.61 [k]	16.30 ± 7.44 [k]	21.70 ± 4.40 [k]	18.80 ± 8.66
	10	25.40 ± 4.41 [k]	26.30 ± 1.25 [k]	30.50 ± 0.82 [k, l]	26.30 ± 5.84
HCT (%)	**0**	39.00 ± 8.92 [k]	44.40 ± 7.73 [k, p]	39.20 ± 13.70 [m]	43.27 ± 9.95 [l]
	7	30.30 ± 8.64 [m]	26.30 ± 16.00 [k, n]	36.00 ± 14.70 [s]	30.10 ± 19.10 [n]
	10	57.00 ± 8.25 [k, m]	62.80 ± 7.42 [p, n]	66.30 ± 2.00 [m, s]	63.20 ± 10.10 [l, n]
MCV (fL)	**0**	55.90 ± 2.16	55.00 ± 2.25 [m]	56.70 ± 1.79 [l]	61.01 ± 5.64 [m]
	7	64.30 ± 6.98	71.70 ± 12.80 [k, m]	69.60 ± 14.50 [k, l]	78.40 ± 14.10 [m, r]
	10	60.50 ± 1.85	59.60 ± 1.78 [k]	58.70 ± 1.41 [k]	61.40 ± 1.17 [r]
MCH (pg)	**0**	26.10 ± 2.25	26.50 ± 1.22 [m]	27.70 ± 2.25 [l]	35.71 ± 5.92 [l]
	7	37.40 ± 4.16	49.20 ± 17.10 [l, m]	46.70 ± 21.40 [l, q]	53.30 ± 15.7 [l, n]

	10	26.70 ± 1.77	27.60 ± 2.18 ^{l}	27.40 ± 0.70 ^{q}	25.90 ± 1.36 ^{n}
MCHC (g/dL)	**0**	46.80 ± 4.05 ^{k}	48.20 ± 2.39 ^{m}	49.10 ± 5.50 ^{l}	58.44 ± 6.67 ^{l}
	7	58.40 ± 5.48 $^{k, p}$	67.30 ± 14.30 $^{m, r}$	66.00 ± 17.30 $^{l, q}$	67.00 ± 10.40 ^{n}
	10	44.20 ± 1.87 ^{p}	46.00 ± 4.93 ^{r}	46.30 ± 0.65 ^{q}	42.10 ± 2.75 $^{l, n}$
RDW-SD (fL)	**0**	33.20 ± 3.11	31.60 ± 3.41	34.80 ± 2.55 ^{k}	30.79 ± 2.37 $^{k, l}$
	7	38.70 ± 6.73	34.50 ± 2.11	43.50 ± 15.90 ^{k}	41.70 ± 11.60 ^{l}
	10	39.70 ± 4.33	38.00 ± 2.37	36.60 ± 2.80	40.60 ± 1.82 ^{k}
RDW-CV (%)	**0**	17.20 ± 1.17	16.40 ± 1.41	17.90 ± 1.18	14.93 ± 1.20 ^{m}
	7	17.90 ± 2.12	14.90 ± 2.45 ^{l}	18.70 ± 3.92	16.40 ± 3.37 ^{k}
	10	19.20 ± 1.60	18.70 ± 0.75 ^{l}	18.30 ± 0.98	19.40 ± 0.86 $^{k, m}$
PLT (10^3 cells/µL)	**0**	312.00 ± 114.00 $^{a, c}$	376.00 ± 124.00 $^{a, b, c}$	468.00 ± 312.00 $^{b, c, k, p}$	707.70 ± 140.90 ^{c}
	7	464.00 ± 114 ^{a}	496.00 ± 213.00 ^{b}	827.00 ± 487.00 $^{a, b, k}$	775.00 ± 283.00 $^{a, b}$
	10	564.00 ± 210.00 $^{a, b}$	605.00 ± 274.00 $^{c, d}$	839.00 ± 292.00 $^{a, c, e, p}$	701.00 ± 263.00 $^{b, d, e}$
MPV (fL)	**0**	7.43 ± 0.28	8.39 ± 1.28	8.79 ± 1.31	8.77 ± 1.27
	7	7.79 ± 0.54	8.13 ± 0.72	8.63 ± 1.84	9.76 ± 1.48
	10	8.50 ± 0.99	8.57 ± 1.32	9.40 ± 1.12	8.95 ± 0.83
PDW (%)	**0**	8.43 ± 0.75	8.79 ± 1.11	9.39 ± 1.05	9.24 ± 0.69 ^{l}
	7	9.30 ± 1.49	9.77 ± 2.08	10.30 ± 3.91	13.00 ± 3.57 ^{l}
	10	9.47 ± 1.52	8.64 ± 1.05	9.33 ± 1.12	10.70 ± 3.33
PCT (%)	**0**	0.23 ± 0.09	0.32 ± 0.14	0.60 ± 0.44	0.61 ± 0.11

	7	0.38 ± 0.09	0.41 ± 0.21	0.73 ± 0.65	0.78 ± 0.36
	10	0.46 ± 0.15	0.54 ± 0.32	0.80 ± 0.33	0.62 ± 0.21
	0	9.31 ± 2.20	12.70 ± 2.34	12.40 ± 4.59	12.14 ± 6.57
P-LCR (%)	**7**	7.79 ± 3.56	6.57 ± 3.97 [k]	8.69 ± 3.10 [l]	8.93 ± 8.26
	10	10.70 ± 4.52	13.60 ± 3.78 [k]	16.90 ± 4.50 [l]	13.70 ± 8.52

Intergroup analyses: Baseline (Day 0): [a] $p < 0.05$, [b] $p < 0.001$ and [c] $p < 0.0001$; Day 7: [a and b] $p < 0.0001$; Day 10: [a, c and e] $p < 0.0001$, [b] $p < 0.001$, [d] $p < 0.05$.

Intragroup analyses: [k and p] $p < 0.05$, [l, q and x] $p < 0.01$, [m, r and y] $p < 0.001$ and [n and s] $p < 0.0001$.

GRAN, granulocytes; HCT, haematocrit; HGB, haemoglobin; LYM, lymphocyte; MCH, mean corpuscular haemoglobin; MCHC, mean corpuscular haemoglobin concentration; MCV, mean cell volume; MID, mid-range absolute count; MPV, mean platelet volume; PCT, procalcitonin; PDW, platelet distribution width; P-LCR, platelet large cell ratio; PLT, platelet; RBC, red blood cells; RDW-CV, red cell distribution-coefficient of variation; RDW-SD; red cell distribution width-standard deviation; WBC, white blood cell.

4.2.2. Blood Biochemistry Parameters

Aspartate aminotransferase (AST), alanine aminotransferase (ALT), total and direct bilirubin were measured as the indicators of liver function (Table 6). No significant differences between the groups were observed when the measurements different days were compared. On the other hand, AST was significantly decreased compared to baseline and day 7 in group A ($p < 0.01$), and on day 10 compared to day 7 ($p < 0.01$) but not to baseline in group C. There was no change in AST in group B, while group D exhibited significantly increased AST levels on day 10 compared to day 7 ($p < 0.05$; Table 6). ALT levels, on the other hand, were significantly decreased in all groups on day 10 in all groups compared to the baseline and day 7 (Table 6). However, there were no differences in total and direct bilirubin levels in groups A, C and D on different days, but only a slight but significant increase in total bilirubin levels on day 10 compared to day 7 was observed ($p < 0.05$).

Creatinine, creatine kinase and urea levels were tested to determine the kidney function. Creatinine was found significantly increased on day 7 compared to day 0 in group A ($p < 0.001$) that was decreased significantly but not normalized on day 10 ($p < 0.05$), while it increased gradually in group D but this increase was found significant on day 10 compared to baseline ($p < 0.01$; Table 6). However, creatinine did not significantly differ in group C and D (Table 6).

On the other hand, creatine kinase (CK) levels were found significantly higher in group A compared to other groups at the baseline ($p < 0.0001$; Table 6). On day 7, CK levels in group C was significantly higher compared to group D ($p < 0.01$), while on day 10, group A exhibited the highest levels of CK compared to the other groups ($p < 0.0001$; Table 6). In group A, creatine kinase levels were found to decrease on day 7 compared to day 0 but this decrease was not significant (Table 6), however, it increased significantly on day 10 compared to day 7 ($p < 0.01$). In group B and D, there were no significant changes were observed in CK levels, on the other hand, a significant decrease was observed on day 10 compared to day 7 ($p < 0.05$; Table 6).

Blood urea levels were significantly higher in group A compared to group C and D on day 7 ($p < 0.05$ and $p < 0.01$, respectively; Table 6), while there was no significant differences between the groups on other days. On the other hand, group A exhibited

significantly higher blood urea on day 7 compared to day 0 ($p < 0.01$) and group D had significantly higher blood urea on day 10 compared to day 0 ($p < 0.01$).

Amylase levels were detected significantly lower in group D compared to all other groups on day 0 ($p < 0.0001$; Table 6). On the other hand, group C exhibited the highest amylase levels on day 7 compared to other groups, while group B had higher amylase levels compared to group A and D ($p < 0.05$ and $p < 0.0001$, respectively; Table 6). However, on day 10, group B exhibited the highest amylase levels (Table 6), amylase levels of group D were significantly higher than group A and C ($p < 0.0001$). Additionally, amylase levels did not significantly differ in group A and B, while it was found significantly decreased and increased in group C and D, respectively ($p < 0.01$ and $p < 0.05$, respectively), on day 10 compared to day 7 (Table 6).

Blood electrolyte levels (Na^+, K^+, Ca^{2+} and Cl^-) were not significantly different between the groups on different days (Table 6). On the other hand, Na^+ concentrations were significantly higher on day 10 compared to day 0 and 7 in Group B ($p < 0.001$), group C ($p < 0.0001$) and group D ($p < 0.0001$). K^+ concentrations were significantly increased on day 10 compared day 0 and 7 in all groups ($p < 0.05$; Table 6). Ca^{2+} levels, on the other hand, were found significantly higher in Group A on day 7 compared to day 0 and in group C on day 10 compared to day 0 ($p < 0.01$). Additionally, Ca^{2+} concentrations were significantly higher in group B and D on day 10 compared to both day 0 and 7 concentrations ($p < 0.05$; Table 6). Cl^- concentrations, however, were found unaffected (Table 6).

Blood glucose levels, on the other hand, were significantly higher in group B compared to other groups on day 10 ($p < 0.05$), but, it was not significantly different among the groups on day 0 and 10 (Table 6). Additionally, group B had significantly higher blood glucose on day 10 compared to day 0 and 7 ($p < 0.0001$). There was no significant difference between the days of measurements among other groups (Table 6).

Table 6. Blood biochemistry on baseline (Day 0), day 7 and day 10. Data are mean ± SD. (n=7 animals in each group, except for the group A on day 10, n=6 animals). Data sharing the same superscript letters in different groups and days are significantly different from each other.

	Days \ Groups	A	B	C	D
AST (U/L)	**0**	121.30 ± 18.22 ^{l}	108.00 ± 16.61	110.00 ± 8.35	96.57 ± 21.05
	7	126.90 ± 31.76 ^{q}	102.60 ± 11.06	120.90 ± 11.61 ^{l}	93.86 ± 18.34 ^{k}
	10	83.00 ± 10.06 $^{l, q}$	113.00 ± 41.68	83.14 ± 10.87 ^{l}	123.30 ± 32.56 ^{k}
ALT (U/L)	**0**	76.43 ± 7.93 ^{m}	72.00 ± 12.25 ^{n}	72.57 ± 7.21 ^{l}	68.57 ± 9.14 ^{l}
	7	76.00 ± 15.17 ^{r}	66.86 ± 15.66 ^{l}	76.43 ± 14.74 ^{m}	63 ± 8.74 ^{k}
	10	50.00 ± 8.88 $^{m, r}$	44.71 ± 13.28 $^{l, n}$	50.14 ± 8.24 $^{l, m}$	46.71 ± 9.81 $^{k, l}$
Total bilirubin (mg/dL)	**0**	0.12 ± 0.03	0.15 ± 0.02	0.12 ± 0.02	0.13 ± 0.02
	7	0.14 ± 0.05	0.12 ± 0.06 ^{k}	0.13 ± 0.03	0.14 ± 0.02
	10	0.13 ± 0.03	0.18 ± 0.11 ^{k}	0.14 ± 0.05	0.15 ± 0.03
Direct bilirubin (mg/dL)	**0**	0.03 ± 0.01	0.02 ± 0.02	0.01 ± 0.01	0.01 ± 0.01
	7	0.01 ± 0.01	0.01 ± 0.01	0.02 ± 0.01	0.02 ± 0.01
	10	0.02 ± 0.01	0.03 ± 0.05	0.02 ± 0.01	0.01 ± 0.00
Amylase (U/L)	**0**	2741.00 ± 104.40 ^{a}	2847.00 ± 122.10 ^{b}	2783.00 ± 196.70 ^{c}	2510.00 ± 192.90 $^{a, b, c}$
	7	2593.00 ± 399.40 $^{a, c}$	2790.00 ± 253.60 $^{a, b, d}$	3040.00 ± 272.20 $^{b, c, e, l}$	2444.00 ± 321.20 $^{d, e, k}$
	10	2338.00 ± 322.90 $^{b, c}$	3161.00 ± 602.60 $^{a, b, d}$	2402.00 ± 383.60 $^{d, e, l}$	2869.00 ± 409.70 $^{a, c, e, k}$

Creatinine (mg/dL)	**0**	0.30 ± 0.02 [m]	0.30 ± 0.03 [l]	0.32 ± 0.02	0.31 ± 0.04
	7	3.59 ± 3.42 [k, m]	1.86 ± 2.11	1.21 ± 1.05	0.38 ± 0.04
	10	1.13 ± 0.74 [k]	3.00 ± 2.70 [l]	0.71 ± 0.46	1.45 ± 0.49
Creatine kinase (U/L)	**0**	974.90 ± 349.60 [a, b, c]	500.70 ± 128.40 [b]	577.20 ± 129.00 [c]	554.00 ± 273.10 [a]
	7	544.40 ± 287.30 [l]	567.60 ± 426.80	710.30 ± 312.80 [b, k]	446.70 ± 119.50 [b]
	10	1162.00 ± 978.30 [b, c, d, l]	419.30 ± 350.10 [b]	169.40 ± 54.89 [c, k]	317.10 ± 235.70 [d]
BUN (mg/dL)	**0**	45.64 ± 4.61 [l]	48.93 ± 4.63 [l]	47.25 ± 4.07	44.11 ± 3.68
	7	286.60 ± 308.00 [a, b, l]	166.20 ± 175.10	98.07 ± 73.21 [a]	40.50 ± 3.38 [b]
	10	111.30 ± 93.20	287.60 ± 270.40 [l]	70.07 ± 47.94	138.70 ± 53.95
Na^+ (mmol/L)	**0**	143.50 ± 1.03	144.40 ± 1.11 [m]	144.50 ± 1.66 [n]	143.50 ± 1.42 [n]
	7	146.50 ± 4.74	144.60 ± 0.86 [r]	145.20 ± 1.37 [s]	145.60 ± 1.20 [s]
	10	144.10 ± 4.49	150.60 ± 4.15 [m, r]	153.40 ± 2.15 [n, s]	155.20 ± 4.33 [n, s]
K^+ (mmol/L)	**0**	4.94 ± 0.27 [k]	4.70 ± 0.28 [n]	4.86 ± 0.17 [n]	4.98 ± 0.29 [n]
	7	4.17 ± 0.47 [k, l]	4.55 ± 0.45 [s]	4.64 ± 0.49 [s]	4.78 ± 0.20 [s]
	10	5.21 ± 0.18 [l]	5.96 ± 1.12 [n, s]	6.04 ± 0.47 [n, s]	6.18 ± 0.69 [n, s]
Ca^{2+} (mg/dL)	**0**	10.37 ± 0.18 [l]	10.90 ± 0.23 [m]	10.70 ± 0.17 [l]	10.43 ± 0.33 [l]
	7	11.90 ± 1.94 [l]	11.41 ± 0.59 [l]	11.03 ± 0.33	10.89 ± 0.32 [k]
	10	11.20 ± 1.06	12.83 ± 1.61 [l, m]	12.09 ± 0.25 [l]	12.04 ± 0.44 [k, l]
Cl^- (mmol/L)	**0**	100.20 ± 1.04	99.32 ± 0.98	100.10 ± 1.45	100.00 ± 1.38
	7	99.26 ± 12.17	97.26 ± 4.16	99.84 ± 2.56	101.50 ± 0.72

	10	99.15 ± 4.89	96.37 ± 4.01	98.07 ± 2.645	101.6 ± 3.44
Glucose (mg/dL)	**0**	125.90 ± 9.44	129.70 ± 11.37 [n]	140.00 ± 15.31	136.40 ± 4.08
	7	126.40 ± 42.06	152.10 ± 25.15 [s]	147.40 ± 10.86	133.90 ± 11.38
	10	149.00 ± 21.74 [f]	502.10 ± 360.20 [a, f, g, n, s]	157.00 ± 24.19 [g]	171.3 ± 40.73 [a]

Intergroup analyses: Baseline (Day 0): [a, b and c] $p < 0.0001$; Day 7: [a] $p < 0.05$, [b] $p < 0.01$ [c, d and e] $p < 0.0001$; Day 10: [a] $p < 0.05$, [f and g] $p < 0.01$, [b, c, d and e] $p < 0.0001$.

Intragroup analyses: [k] $p < 0.05$, [l and q] $p < 0.01$, [m and r] $p < 0.001$ and [n and s] $p < 0.0001$.

ALT, alanine aminotransferase; AST, Aspartate aminotransferase; BUN, Blood urea nitrogen; CK, Creatine kinase

4.3. Histopathological Examinations

4.3.1. Kidney Histopathology

Tubular necrosis was significantly higher in Group A compared to all other groups, while there was no significant difference between other groups (Table 7). There were no statistically significant differences between the groups. None of the animals showed glomerular pathology.

Table 7. Histopathological assessment of the kidneys in different experimental groups. Data are mean ± SD. (n=7 in each group, except for the group A, n=6.) Data sharing the same superscript letters in different groups are significantly different from each other.

	Groups			
	A	**B**	**C**	**D**
Tubular Necrosis	$1.83 \pm 0.39^{a, b, c}$	0.71 ± 0.73^{a}	0.79 ± 0.89^{b}	0.50 ± 0.52^{c}
Inflammation	0.33 ± 0.49	0.14 ± 0.36	0 ± 0	0.07 ± 0.27
Glomerular pathology	0 ± 0	0 ± 0	0 ± 0	0 ± 0

$^{a \text{ and } b}$ $p < 0.001$ and ^{c} $p < 0.0001$

4.3.2. Testicular Histopathology

There was no histopathological difference between the groups according to Johnsen scoring (Figure 7).

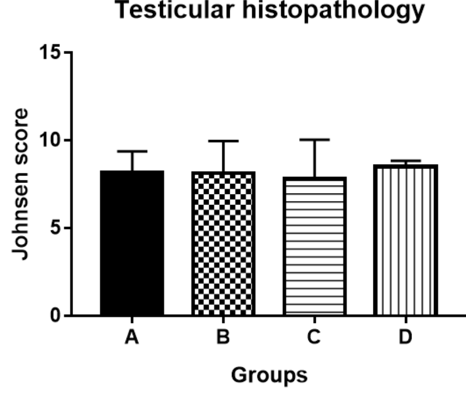


Figure 7. Testicular histopathology results according to Johnsen scoring system. Data are mean ± SD. (n=7 animals in each group, except for the group A, n=6 animals)

4.3.3. Liver Histopathology

In none of the samples, central vein thrombosis was observed. Central vein dilatation and congestion, periportal and pericentral sinusoidal dilatation was observed in all samples (Figure 8A and F). Mild proliferation of bile ducts was observed at the focal regions (Figure 8B). The density of the infiltrated inflammatory cells was variable among the samples from the same liver. In general, inflammation was mild in the portal area and the parenchyma. Focal necrosis was mainly observed in the subcapsular region. Increase in the inflammatory cell density was mild in the hilum. On the other hand, denser inflammatory cell populations were observed in a region around the parenchyma around the hilum and parenchyma damage was observed (Figure 8C, D, E, G and H). Inflammation was milder in the regions further than the hilum in all samples from different groups. Vacuolar degeneration was more obvious in the capsular regions surrounding the subcapsular region (Figure 8C and D).

There was no significant difference in the inflammatory scores in different regions of the kidney between the groups (Figure 9A-D). Vacuolar degeneration score of the Group B was significantly higher than other groups (Figure 10, $p<0.05$). There were no significant differences between the groups when the bile duct proliferation scores were compared.

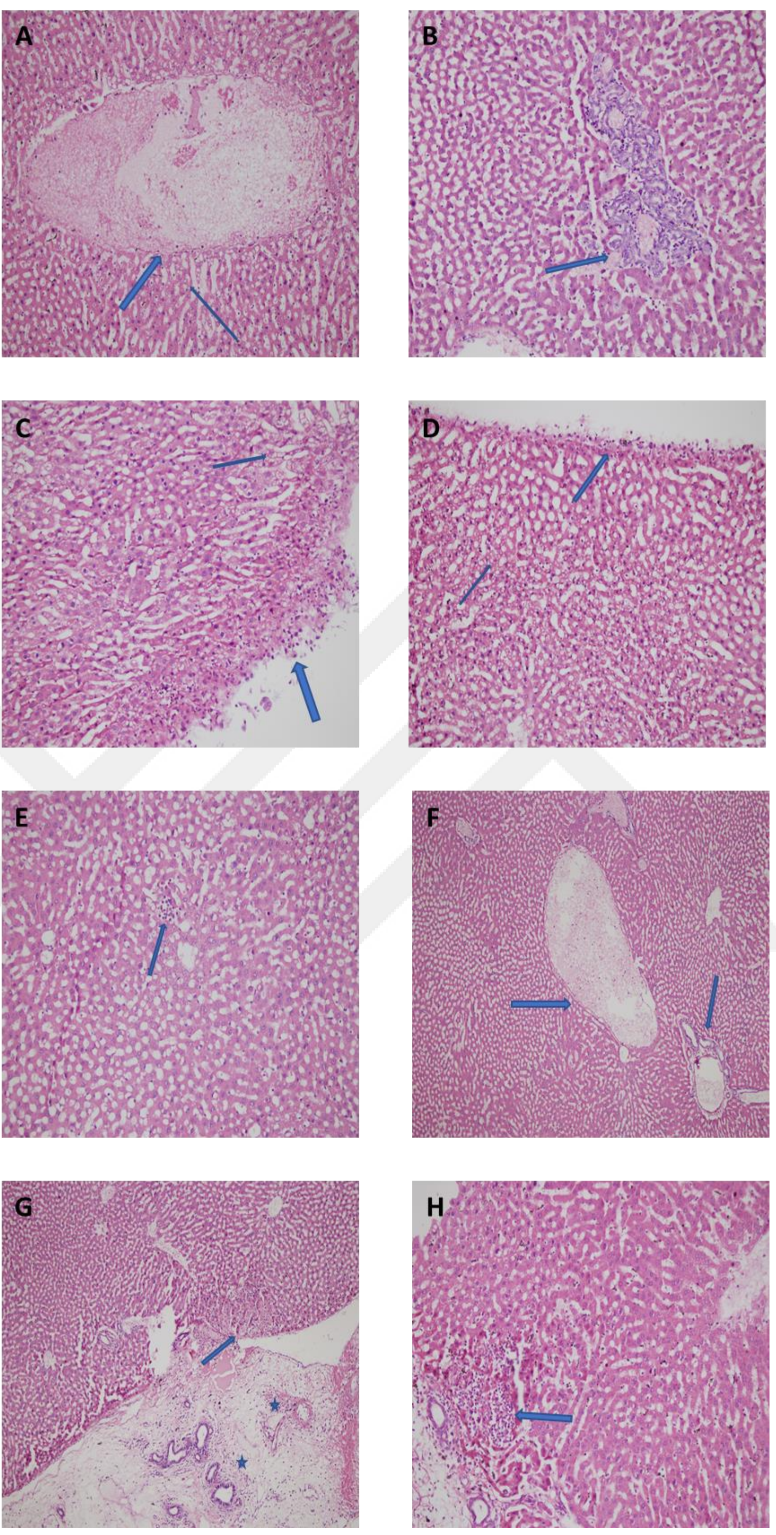


Figure 8. Liver histopathology findings of Group A (A and B), Group B (C and D), Group C (E and F) and Group D (G and H). (A) Central vein dilatation and congestion (thick arrow) and perivenular dilatation (thin arrow), **(B)** Bile duct proliferation (arrow), **(C and D)** Infiltrated inflammatory cells into the capsule (thick arrow) and vacuolar degeneration in the parenchyma cells (thin arrow), **(E)** Focal necrosis in parenchyma, **(F)** Perivenular (thick arrow) and

periportal (thin arrow) sinusoidal dilatation, **(G)** Inflammatory cells in the hilum region (star) and Parenchymal damage in a focal region around the surrounding parenchyma, **(H)** Inflammatory cells replacing the parenchymal cells around the focal region surrounding the hilum.

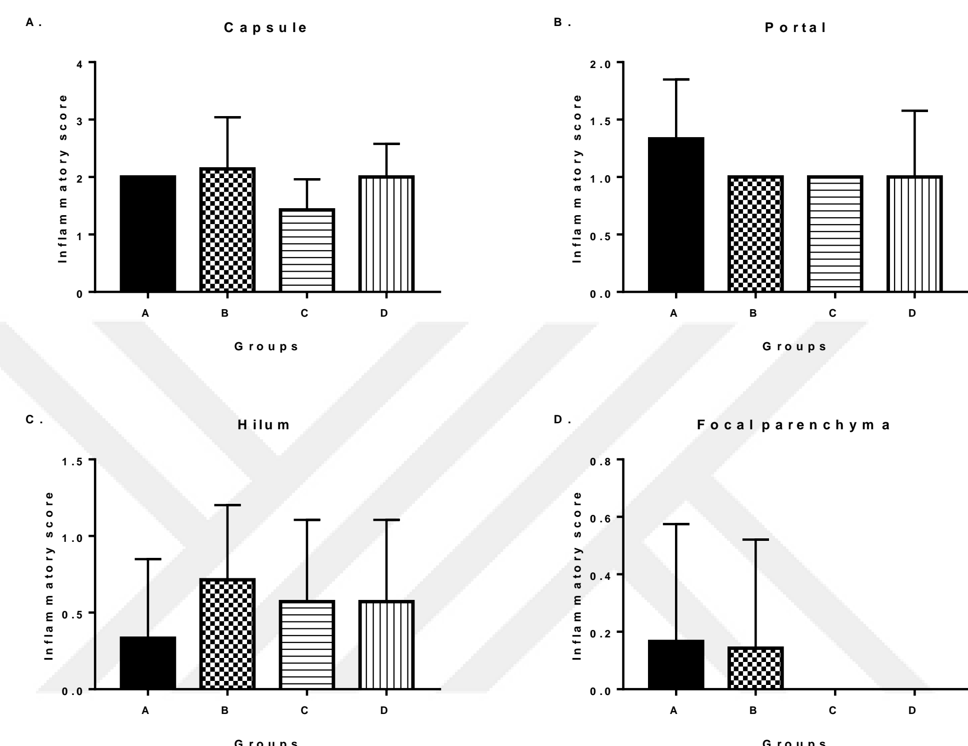


Figure 9. Inflammatory scores in (A) capsular, (B) portal, (C) hilum and (D) focal parenchyma regions. Data are mean ± SD. (n=7 animals in each group, except for the group A, n=6 animals)

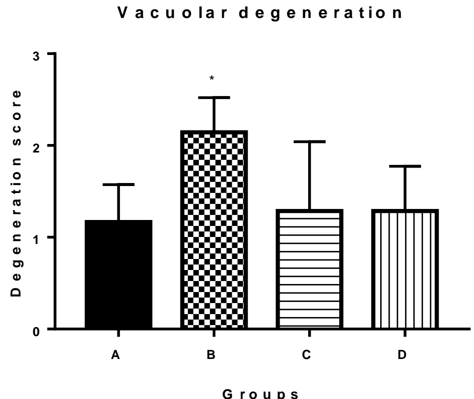


Figure 10. Vacuolar degeneration scores. Data are mean ± SD. (n=7 animals in each group, except for the group A, n=6 animals, *p<0.05)

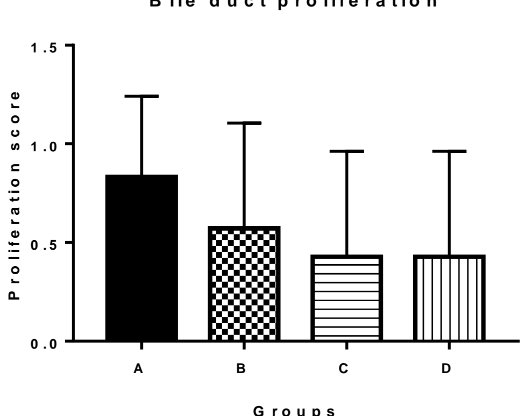


Figure 11. Bile duct proliferation scores. Data are mean ± SD. (n=7 animals in each group, except for the group A, n=6 animals)

5. DISCUSSION & CONCLUSION

Drug-induced hepatotoxicity (235) and nephrotoxicity (236) are two of the leading cause of drug withdrawal from the market. Chemotherapy-induced toxicity is one of the major problems encountered with during the treatment of cancer patients (237, 238). In this study, the effect of lycopene containing tomato extract on cisplatin-induced toxicities was investigated in a setup where lycopene was either administered prophylactically or therapeutically.

5.1. Body and Tissue Weights

Cisplatin administration have been previously indicated to lead to lower body weight in rodents (239-242). However, in our study body weight measurements taken on day of the sacrification showed that the weight of the animals after three days of cisplatin administration but not administered with lycopene (group D) had highest body weight compared to the other animals in the other groups (Table 4). The reason might be that the initial body weights were not measured, and it is possible that the higher body weight animals were placed in the group D by chance during the randomization. On the other hand, weight of the kidneys, liver, testes and heart did not differ between the groups. Relative weight of the tissues did not differ between the groups except that the group C in which the animals were administered with cisplatin on day 0 and did not receive lycopene. Previously, decrease in the heart weight reduction upon chemotherapy administration was reported in the literature. A study shown that doxorubicin administration contributed in a reduction in heart weight but not heart / body weight ratio (243), on the other hand, another study indicated increased apoptosis and decreased heart / body weight ratio upon doxorubicin treatment in rats (244). Doxorubicin also decreased both heart weight and heart / body weight ratio in juvenile mice (245). Additionally, a reduction in heart weight upon cisplatin treatment was shown in mice (240). It may be possible to suggest that the cisplatin induces apoptosis (154), as doxorubicin does, leading to cell death in the heart and resulting lower heart / body weight ratio. Additionally, group C had the lowest heart weight compared to other groups, but this difference was not significant. It may also be suggested that the lower heart weight and heart / body weight ratio can be related with timing of the cisplatin administration as group D had non-

significantly higher heart weight compared group C. However, one should also note that the body weight and the anatomy of the animal that contributes the weight of the heart.

5.2. Complete Blood Count Tests

Cytopenias (leukopenia, neutropenia, thrombocytopenia and anaemia) are some of the common side effects of cisplatin treatment (246). In an earlier study, 5 mg/kg cisplatin was found to reduce the leukocyte number 12-h after the administration in rats (247). Another study also reported decreased WBC counts upon a single dose of 10.5 mg/kg cisplatin administration in rats (248). In a study on mice, 16 mg/kg cisplatin led to reduction on WBC counts, however, it was normalized on day 8 (249). On the other hand, 5 mg/kg cisplatin significant increase in the WBC 5 days after the administration in rats (250). In our study, all groups exhibited significantly higher WBC counts on day 7, while only the group received cisplatin on day 7 and did not receive lycopene (Group D) exhibited significantly lower WBC counts compared to the baseline on day 10. However, there was no significant differences between the groups on any day of the measurements. LYM # was shown to be decreased upon cisplatin administration (251, 252), as well as the LYM (%) (250). In our study, we observed a significant decrease in LYM (%) on day 10 in all groups. Additionally, LYM # was unchanged in the animals that received a single dose of cisplatin and 10 days of lycopene (Group A) and in other groups, LYM # significantly increased on day 7 compared to baseline and decreased to the basal levels, except for the group D that exhibited significantly lower LYM # compared to baseline. On the other hand, there was no significant differences between the groups. Previously, neutrophil number and percentage of neutrophils was found significantly increased in the cisplatin-administered animals (250, 251), as well as increased basophil and eosinophil numbers (252).

In our study, we observed a significant increase in the GRAN (%) in all groups on day 10. On the other hand, GRAN # was not significantly changed in the Group A, while it was decreased in the group D and increased in group B on day 10 compared to day 0. Previously, platelet counts were significantly reduced in the cisplatin treated animals compared to the control animals (251), however, another study indicated no change in platelet counts but a decrease in MPV (250). In our study, there was significant differences among the groups on different time points, however, only the group who

received cisplatin on day 0 and did not receive lycopene (group C) was observed to be significantly increased PLT, while it increased non-significantly in group A and B and did not change in group D over time. On the other hand, MPV did not change over time and was not significantly different among the groups. PDW only increased significantly in group D on day 7 and reduced to its basal levels. PCT was not changed in all groups as indicated previously upon cisplatin treatment (250). A pharmacokinetic study stated only one subject out of 25 subjects showed decreased PLT upon lycopene administration (253). On the other hand, zearalenone-induced decrease in PLT was reversed by 20 mg/kg lycopene in mice (254). PDW was also unaffected at the end of experiment by cisplatin treatment, in parallel with a previous study (250). Additionally, in our study, we did not observe no difference among the groups with regards to the P-LCR (%).

Cisplatin has previously been indicated to cause anaemia (255), however, the impact of it on red blood cell parameters were variable in the previously published literature. In our study, RBC counts were found to be decreased on day 7 in all groups and it was significantly different from the baseline values in group B, C and D. This decrease was reversed on day 10 in the groups received lycopene and significantly higher RBC were found in the groups did not receive lycopene on day 10 compared to both day 0 and 7. HGB was not changed in all groups, however, it was significantly increased on day 10 compared to both baseline and day 7. On the other hand, HCT was increased significantly during the experimental period in all animals. Previous studies reported that cisplatin led to higher RBC counts three days after administration and no difference compared to control three days after second administration (251) and lower RBC counts after 15 days consecutive cisplatin administration (256). HGB levels were found to be increased (250) and decreased (251, 256). On the other hand, a recent study reported a dose-dependent increase in the RBC count, HGB levels and HCT (257). The significantly higher RBC count in only cisplatin-treated animals might be due to higher erythropoietin production upon cisplatin treatment as previously shown (258). MCV and MCH was found unchanged in group A, while they increased significantly in other groups on day 7 and were not statistically different on day 10 compared to basal levels. MCH was significantly decreased and MCV was significantly increased upon single dose of 7.5 mg/kg (259) and 7 mg/kg (260) cisplatin administration. On the other hand, another study reported that MCV and MCH increased significantly on different days at two different

doses (261). However, they were also reported unchanged in another study (250). MCHC, on the other hand, increased significantly on day 7 compared to day 0 and decreased to its basal levels on day 10 in all groups except the group D, which exhibited significantly lower MCHC levels on day 10 compared to day 0 and 7. In the literature MCHC was also found either unchanged (250, 251) or increased (261) or decreased (256) upon cisplatin treatment in different experimental setups. Lycopene, on the other hand, did not cause any difference in the red blood parameters in fluoride-exposed mice (262). Another study indicated that RBC and HGB decreased upon carboplatin treatment, reached a nadir and increased again and that was in parallel with an increase in EPO and reducing to the levels of the control rats (263). RDW-CV (%) was found increased on day 10 in group B and D significantly, while was unaffected on group A and C. Cisplatin did not change the RDW (%) (250). All in all, no major effects of either cisplatin or lycopene on the haematological parameters were observed.

5.3. Blood Biochemistry Parameters

The most common adverse effects of cisplatin are its nephrotoxicity and hepatotoxicity (191, 197-203). Increased AST (264, 265) and ALT (265) are two of determinants of the hepatotoxicity. In our study, we observed a significant decrease in the AST levels in group A on day 10 compared to day 0 and 7, while a significant decrease in group C and a significant increase in group D were observed on day 10 compared to day 7, both of which were not significantly different compared to day 0. On the other hand, ALT levels were significantly low in all groups at the end of experimental period compared to both day 0 and 7. Total bilirubin was only significantly higher in group B on day 10 compared to day 7 but not to day 0. Direct bilirubin levels did not significantly change in any groups. AST, ALT, total bilirubin and direct bilirubin did not differ between the groups. Cisplatin treatment significantly increases AST and ALT levels (251, 266, 267). However, a study showed a significant decrease in the ALT levels, while the AST levels were elevated over a 100-h period (268). ALT and AST was shown to reach a peak and decrease over time in a study where the rats were administered with a single dose of cisplatin at different doses (10, 25 and 50 mg/kg) intraperitoneally and followed up to 120 h (265). Lycopene, on the other hand, improved the liver enzyme levels in different models including high fat-induced fatty liver disease (269), methotrexate-

induced liver injury (231) and flutamide-induced liver toxicity (270) in rats. Additionally, lycopene also improved total and direct bilirubin levels in the d-galactosamine/lipopolysaccharide-induced hepatitis model of rats (271). Our study showed that lycopene treatment starting on the day 0 led to a significant reduction on the AST levels compared to day 0 and 7, while there were no significant differences between the groups, suggesting that lycopene administration, but not the prophylactic treatment, may contribute to improvement in the cisplatin-induced hepatotoxicity on the long term.

Amylase levels are tested to diagnose the acute pancreatitis in the clinics (272). There are reports stating the cisplatin-induced pancreatitis in the literature (273). Previously, rats received 7 mg/kg single dose of cisplatin showed pancreatitis with increased amylase levels compared to control in mice (274). On the other hand, lycopene pre-treatment was shown to be protective against the acute pancreatitis induced by sodium pentobarbital by attenuating the oxidative stress and reducing the apoptosis of pancreatic acinar cells, as well as by reducing the serum amylase levels in rats and improving the histological scores of the pancreas (275). Another study where the acute pancreatitis was induced by L-arginine administration in rats showed that lycopene pre-treatment for 10 days suppressed the inducible nitric oxide synthase and TNFα levels and decreased the serum α-amylase and lipase activities and also improved the pancreas histopathology (276). In our study, amylase activity was the lowest in the group D on the day 0. In group A, a decrease in the amylase activity was observed and this group exhibited the lowest amylase levels, which were significantly different from the groups B and D on day 10. When the two groups received lycopene and cisplatin on day 0 (groups A and C) were compared, it was observed that amylase levels on day 7 were significantly lower in the group received lycopene (group A), suggesting the positive impact of lycopene when co-administered with cisplatin. However, amylase activity was observed significantly higher in the group B compared to other groups, and lycopene administration did not lead to any difference (vs. Group D) on day 7 and 10, and this might be due to low basal amylase levels in group D. One should note that the amylase levels increased by 11 and 14 % of the baseline in group B and D, respectively, on day 10.

BUN and creatinine levels are analysed to investigate the renal functions in the clinics (277). Previously, BUN levels, as well as the creatinine levels, was shown to

increase within first 72 h after cisplatin administration and start to decrease afterwards, even down to its basal levels, depending on the administered concentration in rats (265). In cisplatin-induced AKI model of mice, 20 mg/kg (278) and 25 mg/kg (279) cisplatin administration led to significantly increased serum BUN and creatinine levels, as well as the tubular injury. In rats, cisplatin (7 mg/kg) administration also led to increased urea and creatinine levels (280). On the other hand, lycopene pre-treatment was found to improve the renal damage by non-significantly altering the BUN, creatinine and oxidative stress markers in renal ischemia-reperfusion model (281). On the other hand, 6 mg/kg tomato lycopene complex (6 % lycopene) administration for 10 days significantly reduced the BUN and creatinine, as well as the apoptotic activity, in cisplatin-injected (7 mg/kg, intraperitoneally) rats (26). Another study suggested lycopene as a potential adjuvant therapy in cancer patients to reduce the nephrotic complications caused by cisplatin administration (282). Another study in mice model of nephrotoxicity induced by colistin, an antibiotic used against Gram-negative bacteria, lycopene was found to reduce the nephrotic injury by reducing oxidative stress via upregulating NRF-2/HO-1 pathway and decreased the BUN and creatinine levels (283).

Creatinine kinase (CK) levels is a crucial laboratory test in order to evaluate the muscular damage, as well as the probable myopathies (284). CK level elevation was previously shown in breast cancer patients upon receiving the combination of 5-fluorouracil, adriamycin, and cyclophosphamide (285). On the other hand, chemotherapy-induced CK elevation was stated to not occur often upon cisplatin treatment (286). However, in rats, 5 mg/kg cisplatin administration four times every second day significantly increased the CK levels and oxidative stress that were significantly improved by Rutin (vitamin P1) (287). Additionally, single dose of 7 mg/kg cisplatin significantly increased CK levels and oxidative stress that were reduced by resveratrol treatment in rat model of cisplatin-induced cardiotoxicity (288). Previously, improvement in oxidative stress in rat model of isoproterenol-induced cardiotoxicity have been shown (289). Additionally, protective effects of lycopene (290) and carotenoids (291) were shown in adriamycin-induced cardiotoxicity models. In our study, therapeutic application of lycopene. In our study we observed the baseline CK levels were the highest in the Group A and it did not change significantly over the course of experiment. On the other hand, in other three groups we observed decreases in the CK levels at the end of the

experiment, but this was not significant. Interestingly, in the group C animals that received cisplatin on day 0 and did not receive lycopene throughout the experiment, CK levels on day 10 were found to be lower compared to day 7 measurements.

Na^+, K^+, Ca^{2+} and Cl^- levels were previously indicated to be altered by the cisplatin treatment in humans (292) and animals (293-296). In our study, Na^+, K^+, Ca^{2+} levels were found to be significantly increased in the Groups B, C and D but not in Group A on day 10 compared to day 0. This might be due to the alterations in the small intestinal structure of the animals leading to the altered fluid, therefore altered electrolyte absorption (294). On the other hand, a previous case report indicated hyperosmolarity and hyperglycaemia (297). Another case study observed diabetes mellitus during the cisplatin treatment period in 5% of the cancer patients (298). However, a study conducted on male albino rats revealed a dose-dependent decrease in the blood glucose levels (299). Another study indicated a significant increase in the urine glucose/creatinine ratio in the cisplatin treated in mice (300). A more recent study in rats showed that 5 mg/kg cisplatin injection led to significant increase in the blood glucose compared to control on day 2 but this was not observed on day 8 (301). Lycopene was found to decrease the increased blood Na^+, K^+ and Ca^{2+} levels in myocardial injury induced by isoproterenol in rats (99). Additionally, four (302) and eight (303) weeks of lycopene treatment reduced the blood glucose levels in the streptozotocin-induced diabetic rats. In our experiments we did not observe any change in blood glucose levels in any groups except the Group B that received cisplatin on day 7 and received lycopene for 10 days exhibited significantly higher blood glucose than both baseline and other groups on day 10.

5.4. Histopathological Examinations

While cisplatin is widely used for treating solid organ tumours, its major side effect is nephrotoxicity which increases with the dose and administration frequency (304) as high concentration of cisplatin promotes necrosis of proximal tubule cells, while lower concentrations lead to apoptosis (305). Cisplatin is removed from the kidney by tubular secretion and glomerular filtration (306), and studies reveal that it accumulates by renal parenchymal cells (307). Furthermore, it is metabolized to highly reactive thiols by cellular enzymatic activities (196). As a result, these molecules bind to cellular components, leading to increased intracellular calcium rates and cell death as a

consequence (308). In humans, most serious manifestation of cisplatin is kidney injury (AKI) which present 20–30% of patients (309). Via activating Nrf2/HO-1 pathway, lycopene can protect against nephrotoxicity induced by many drugs including cisplatin (26, 27). There are several in vivo studies which focus on cisplatin, investigating alleviating effects of various natural compounds on cisplatin mediated nephrotoxicity. Ilić et. al. (310) injected cisplatin intraperitoneally at a dose of 8 mg/kg and revealed that cisplatin leads to degenerated proximal tubular structure whereas distal tubules were not affected. On the contrary, degeneration was less prominent in cisplatin-quercetin group (310). In another study published on 2017 (311), authors investigated effect of rutin, which are flavonoid glycosides that attenuate lipid peroxidation and oxidative stress mediated decay. Rats were injected 5 mg/kg cisplatin intraperitoneally and received 30 mg/kg rutin for 14 days. Histopathological analysis revealed that cisplatin group showed acute tubular injury accompanied with apoptotic epithelial cells. However, group received rutin treatment combined with cisplatin showed only minimal findings related to cisplatin-mediated nephrotoxicity (311). Ma et. al. (312) induced cisplatin mediated toxicity via injecting 7mg/kg cisplatin intraperitoneally, and co-administered puerarin, which has antioxidant and anti-inflammatory effects and derived from radix *Puerariae* at the doses of 10, 30 or 50mg/kg intravenously for eight days. Severe necrosis and degeneration as well as leukocyte infiltrates were observed in cisplatin group. Puerarin ameliorated all these histological alterations in a dose dependent manner (312). Akca et. al. (313) reported that single dose of 16 mg/kg cisplatin resulted in tubular cell loss, tubular dilatation and peritubular capillary congestions along with oxidative damage, and carotenoid pigment astaxanthin administration showed protective effect (313). Lycopene has protective effects on impaired renal function (96), diabetes related oxidative stress and nephrotoxicity (314), as well as nephrotoxicity caused by either drugs such as colistin (283, 315), adriamycin (96) and even mercuric chloride ($HgCl_2$), which is a hazardous environment pollutant (316). However, it should be noted that even though lycopene treatment promotes antioxidant enzyme production, lycopene alone did not alter histological changes caused by HgCl2 such as tubular necrosis. Mahmoodnia et. al. suggested lycopene supplement is an effective adjuvant therapy to decrease cisplatin mediated nephrotoxicity in cancer patients (282). Moreover, lycopene has been shown to

be effective on nephrotoxicity caused by cisplatin in rat models before, but it should be noted that 7 mg/kg cisplatin was injected in both studies (26, 317).

Previous studies indicated the testicular toxicity of cisplatin administration in rats (219, 257, 318, 319). On the other hand, lycopene was found to improve the testicular damage and histopathology against the toxicity induced by adriamycin (25), cyclosporine A (320) and zearalenone (254). On the other hand, when the rats were administered with lycopene alone or in combination with selenium, cisplatin-induced testicular toxicity was improved (23). In our study, Johnsen scores showing the testicular histopathology were not significantly different between the groups. In contrast with the previous study in which the animals were administered with lycopene at a dose of 4 mg/kg/day for 10 days (23), our study showed that 10 mg/kg/day lycopene treatment in either prophylactic or therapeutic way did not change the testicular damage score.

Cisplatin is very well-known with its hepatotoxic properties as it induces oxidative stress (214, 264, 321, 322), increases inflammation (321, 322) and necrosis (321), causes central vein dilatation (214, 322) and bile duct proliferation (322). Lycopene, as an antioxidant and anti-inflammatory molecule, was suggested to be protective against chemotherapy-induced toxicities (28). Previous studies showed the protective effects of different natural compounds such as daidzein (321), silymarin (264), apocynin (322) and hesperidin (214) against the cisplatin-induced hepatotoxicity. Previously, hepatoprotective effects of lycopene was shown in the models of hepatotoxicity (96, 323, 324). In our study, we observed no difference between the groups with regards to inflammation score in the capsular, portal, hilum and focal parenchyma regions. Vacuolar degeneration score was significantly higher in the Group B that received 10 days of lycopene and administered with cisplatin on day 7 of the experiment. Bile duct proliferation was also present in all groups. In a model of alcohol-induced oxidative stress and inflammation in rats demonstrated that high dose of lycopene supplementation (3.3 mg/kg BW/day) for a two-week period increased the hepatic cytochrome P4502E1 and inflammation (325). In our study, the rats were administered with lycopene at a dose of 10 mg/day, which is even higher than the abovementioned study. Besides, lycopene is mainly accumulated and metabolized in the liver (47). Although lycopene was shown to be protective against the hepatic injury in different

models of hepatotoxicity, cisplatin and lycopene administration together may be additive action on the hepatic damage when the lycopene is administered as prophylactic.

This study investigated the therapeutic and prophylactic effects of lycopene on cisplatin-induced toxicities. One of the major weaknesses of the study was that the missing of a negative control group (untreated) and positive control (treated with a known compound for example silymarin). On the other hand, as a conclusion, our study is suggesting that prophylactic administration of the lycopene on cisplatin administration does not have a protective effect but may be dangerous.

6. REFERENCES

1. Lee, W.M., Drug-Induced Hepatotoxicity. *New England Journal of Medicine*. 2003; 349(5): 474-485.
2. Kim, S.Y. and A. Moon, Drug-induced nephrotoxicity and its biomarkers. *Biomol Ther (Seoul)*. 2012; 20(3): 268-72.
3. Papaioannou, E.H., M. Liakopoulou-Kyriakides, and A.J. Karabelas, Natural Origin Lycopene and Its "Green" Downstream Processing. *Crit Rev Food Sci Nutr*. 2016; 56(4): 686-709.
4. Hashimoto, H., C. Uragami, and R.J. Cogdell, Carotenoids and Photosynthesis. *Subcell Biochem*. 2016; 79: 111-39.
5. Pospisil, P., Production of Reactive Oxygen Species by Photosystem II as a Response to Light and Temperature Stress. *Front Plant Sci*. 2016; 7: 1950.
6. Namitha, K.K. and P.S. Negi, Chemistry and biotechnology of carotenoids. *Crit Rev Food Sci Nutr*. 2010; 50(8): 728-60.
7. Cho, K.S., M. Shin, S. Kim, et al., Recent Advances in Studies on the Therapeutic Potential of Dietary Carotenoids in Neurodegenerative Diseases. *Oxid Med Cell Longev*. 2018; 2018: 4120458.
8. Maiani, G., M.J. Caston, G. Catasta, et al., Carotenoids: actual knowledge on food sources, intakes, stability and bioavailability and their protective role in humans. *Mol Nutr Food Res*. 2009; 53 Suppl 2: S194-218.
9. Choksi, P.M. and V.Y. Joshi, A Review on Lycopene—Extraction, Purification, Stability and Applications. *International Journal of Food Properties*. 2007; 10(2): 289-298.
10. Story, E.N., R.E. Kopec, S.J. Schwartz, et al., An update on the health effects of tomato lycopene. *Annu Rev Food Sci Technol*. 2010; 1(1): 189-210.
11. Muhammad, N. and Z. Guo, Metal-based anticancer chemotherapeutic agents. *Curr Opin Chem Biol*. 2014; 19: 144-53.
12. Dasari, S. and P.B. Tchounwou, Cisplatin in cancer therapy: molecular mechanisms of action. *Eur J Pharmacol*. 2014; 740: 364-78.
13. de Jongh, F.E., R.N. van Veen, S.J. Veltman, et al., Weekly high-dose cisplatin is a feasible treatment option: analysis on prognostic factors for toxicity in 400 patients. *Br J Cancer*. 2003; 88(8): 1199-206.

14. Al-Majed, A.A., Carnitine deficiency provokes cisplatin-induced hepatotoxicity in rats. *Basic Clin Pharmacol Toxicol*. 2007; 100(3): 145-50.
15. Pezeshki, Z., A. Khosravi, M. Nekuei, et al., Time course of cisplatin-induced nephrotoxicity and hepatotoxicity. *J Nephropathol*. 2017; 6(3): 163-167.
16. Al-Majed, A.A., M.M. Sayed-Ahmed, A.A. Al-Yahya, et al., Propionyl-L-carnitine prevents the progression of cisplatin-induced cardiomyopathy in a carnitine-depleted rat model. *Pharmacol Res*. 2006; 53(3): 278-86.
17. Demkow, U. and A. Stelmaszczyk-Emmel, Cardiotoxicity of cisplatin-based chemotherapy in advanced non-small cell lung cancer patients. *Respiratory Physiology & Neurobiology*. 2013; 187(1): 64-67.
18. El-Awady el, S.E., Y.M. Moustafa, D.M. Abo-Elmatty, et al., Cisplatin-induced cardiotoxicity: Mechanisms and cardioprotective strategies. *Eur J Pharmacol*. 2011; 650(1): 335-41.
19. Lirdi, L.C., T. Stumpp, E. Sasso-Cerri, et al., Amifostine protective effect on cisplatin-treated rat testis. *Anat Rec (Hoboken)*. 2008; 291(7): 797-808.
20. Amin, A., A.A. Hamza, A. Kambal, et al., Herbal extracts counteract cisplatin-mediated cell death in rat testis. *Asian J Androl*. 2008; 10(2): 291-7.
21. Turk, G., A. Atessahin, M. Sonmez, et al., Improvement of cisplatin-induced injuries to sperm quality, the oxidant-antioxidant system, and the histologic structure of the rat testis by ellagic acid. *Fertil Steril*. 2008; 89(5 Suppl): 1474-81.
22. Leggas, M., K.L. Kuo, F. Robert, et al., Intensive anti-inflammatory therapy with dexamethasone in patients with non-small cell lung cancer: effect on chemotherapy toxicity and efficacy. *Cancer Chemother Pharmacol*. 2009; 63(4): 731-43.
23. Salem, E.A., N.A. Salem, A.M. Maarouf, et al., Selenium and lycopene attenuate cisplatin-induced testicular toxicity associated with oxidative stress in Wistar rats. *Urology*. 2012; 79(5): 1184.e1-6.
24. Atessahin, A., I. Karahan, G. Turk, et al., Protective role of lycopene on cisplatin-induced changes in sperm characteristics, testicular damage and oxidative stress in rats. *Reprod Toxicol*. 2006; 21(1): 42-7.
25. Atessahin, A., G. Turk, I. Karahan, et al., Lycopene prevents adriamycin-induced testicular toxicity in rats. *Fertil Steril*. 2006; 85 Suppl 1: 1216-22.

26. Dogukan, A., M. Tuzcu, C.A. Agca, et al., A tomato lycopene complex protects the kidney from cisplatin-induced injury via affecting oxidative stress as well as Bax, Bcl-2, and HSPs expression. *Nutr Cancer*. 2011; 63(3): 427-34.
27. Sahin, K., M. Tuzcu, N. Sahin, et al., Nrf2/HO-1 signaling pathway may be the prime target for chemoprevention of cisplatin-induced nephrotoxicity by lycopene. *Food Chem Toxicol*. 2010; 48(10): 2670-4.
28. Sahin, K., N. Sahin, and O. Kucuk, Lycopene and chemotherapy toxicity. *Nutr Cancer*. 2010; 62(7): 988-95.
29. Wang, Y., C. Zhang, S. Zhang, et al., Kanglaite sensitizes colorectal cancer cells to Taxol via NF-κB inhibition and connexin 43 upregulation. *Scientific Reports*. 2017; 7(1): 1280.
30. Mezzomo, N. and S.R.S. Ferreira, Carotenoids Functionality, Sources, and Processing by Supercritical Technology: A Review. *Journal of Chemistry*. 2016; 2016: 1-16.
31. Telfer, A., Singlet oxygen production by PSII under light stress: mechanism, detection and the protective role of beta-carotene. *Plant Cell Physiol*. 2014; 55(7): 1216-23.
32. Triantaphylides, C. and M. Havaux, Singlet oxygen in plants: production, detoxification and signaling. *Trends Plant Sci*. 2009; 14(4): 219-28.
33. Ramel, F., S. Birtic, S. Cuine, et al., Chemical quenching of singlet oxygen by carotenoids in plants. *Plant Physiol*. 2012; 158(3): 1267-78.
34. Zakar, T., H. Laczko-Dobos, T.N. Toth, et al., Carotenoids Assist in Cyanobacterial Photosystem II Assembly and Function. *Front Plant Sci*. 2016; 7: 295.
35. Khachik, F., Distribution and metabolism of dietary carotenoids in humans as a criterion for development of nutritional supplements. *Pure and Appl. Chem.* 2006; 78(8): 1551-1557.
36. Parker, R.S., Carotenoids in human blood and tissues. *J Nutr*. 1989; 119(1): 101-4.
37. Beecher, G.R. and F. Khachik, Qualitative relationship of dietary and plasma carotenoids in human beings. *Ann N Y Acad Sci*. 1992; 669: 320-1.

38. Fiedor, J. and K. Burda, Potential role of carotenoids as antioxidants in human health and disease. *Nutrients*. 2014; 6(2): 466-88.
39. van Het Hof, K.H., C.E. West, J.A. Weststrate, et al., Dietary factors that affect the bioavailability of carotenoids. *J Nutr*. 2000; 130(3): 503-6.
40. Borel, P., C. Desmarchelier, M. Nowicki, et al., A Combination of Single-Nucleotide Polymorphisms Is Associated with Interindividual Variability in Dietary beta-Carotene Bioavailability in Healthy Men. *J Nutr*. 2015; 145(8): 1740-7.
41. Borel, P., C. Desmarchelier, M. Nowicki, et al., Lycopene bioavailability is associated with a combination of genetic variants. *Free Radic Biol Med*. 2015; 83: 238-44.
42. Cardinault, N., V. Tyssandier, P. Grolier, et al., Comparison of the postprandial chylomicron carotenoid responses in young and older subjects. *Eur J Nutr*. 2003; 42(6): 315-23.
43. Homnick, D.N., J.H. Cox, M.J. DeLoof, et al., Carotenoid levels in normal children and in children with cystic fibrosis. *J Pediatr*. 1993; 122(5 Pt 1): 703-7.
44. Drai, J., P. Borel, H. Faure, et al., Fasting plasma carotenoids concentrations in Crohn's and pancreatic cancer patients compared to control subjects. *Int J Vitam Nutr Res*. 2009; 79(2): 87-94.
45. Granado-Lorencio, F., A. Simal-Anton, I. Blanco-Navarro, et al., Depletion of serum carotenoid and other fat-soluble vitamin concentrations following obesity surgery. *Obes Surg*. 2011; 21(10): 1605-11.
46. Johnson, E.J., R. Vishwanathan, T.M. Scott, et al., Serum carotenoids as a biomarker for carotenoid concentrations in the brain. *The FASEB Journal*. 2011; 25(1_supplement): 344.2-344.2.
47. Wang, X.D., Lycopene metabolism and its biological significance. *Am J Clin Nutr*. 2012; 96(5): 1214s-22s.
48. Kun, Y., U.S. Lule, and D. Xiao-Lin, Lycopene: Its properties and relationship to human health. *Food Rev Int*. 2006; 22(4): 309-333.
49. Agarwal, S. and A.V. Rao, Tomato lycopene and its role in human health and chronic diseases. *Cmaj*. 2000; 163(6): 739-44.

50. Fang, L., N. Pajkovic, Y. Wang, et al., Quantitative analysis of lycopene isomers in human plasma using high-performance liquid chromatography-tandem mass spectrometry. *Anal Chem*. 2003; 75(4): 812-7.
51. Di Mascio, P., S. Kaiser, and H. Sies, Lycopene as the most efficient biological carotenoid singlet oxygen quencher. *Arch Biochem Biophys*. 1989; 274(2): 532-8.
52. Miller, N.J., J. Sampson, L.P. Candeias, et al., Antioxidant activities of carotenes and xanthophylls. *FEBS Letters*. 1996; 384(3): 240-242.
53. Bandeira, A.C.B., T.P. da Silva, G.R. de Araujo, et al., Lycopene inhibits reactive oxygen species production in SK-Hep-1 cells and attenuates acetaminophen-induced liver injury in C57BL/6 mice. *Chem Biol Interact*. 2017; 263: 7-17.
54. van Breemen, R.B. and N. Pajkovic, Multitargeted therapy of cancer by lycopene. *Cancer Lett*. 2008; 269(2): 339-51.
55. Narisawa, T., Y. Fukaura, M. Hasebe, et al., Inhibitory effects of natural carotenoids, alpha-carotene, beta-carotene, lycopene and lutein, on colonic aberrant crypt foci formation in rats. *Cancer Lett*. 1996; 107(1): 137-42.
56. Narisawa, T., Y. Fukaura, M. Hasebe, et al., Prevention of N-methylnitrosourea-induced colon carcinogenesis in F344 rats by lycopene and tomato juice rich in lycopene. *Jpn J Cancer Res*. 1998; 89(10): 1003-8.
57. Sengupta, A., S. Ghosh, R.K. Das, et al., Chemopreventive potential of diallylsulfide, lycopene and theaflavin during chemically induced colon carcinogenesis in rat colon through modulation of cyclooxygenase-2 and inducible nitric oxide synthase pathways. *Eur J Cancer Prev*. 2006; 15(4): 301-5.
58. Wang, X., H.H. Yang, Y. Liu, et al., Lycopene Consumption and Risk of Colorectal Cancer: A Meta-Analysis of Observational Studies. *Nutr Cancer*. 2016; 68(7): 1083-96.
59. Cohen, L.A., Z. Zhao, B. Pittman, et al., Effect of dietary lycopene on N-methylnitrosourea-induced mammary tumorigenesis. *Nutr Cancer*. 1999; 34(2): 153-9.
60. Moselhy, S.S. and M.A. Al mslmani, Chemopreventive effect of lycopene alone or with melatonin against the genesis of oxidative stress and mammary tumors

induced by 7,12 dimethyl(a)benzanthracene in sprague dawely female rats. *Mol Cell Biochem*. 2008; 319(1-2): 175-80.

61. Sahin, K., M. Tuzcu, N. Sahin, et al., Inhibitory effects of combination of lycopene and genistein on 7,12- dimethyl benz(a)anthracene-induced breast cancer in rats. *Nutr Cancer*. 2011; 63(8): 1279-86.

62. Al-Malki, A.L., S.S. Moselhy, and M.Y. Refai, Synergistic effect of lycopene and tocopherol against oxidative stress and mammary tumorigenesis induced by 7,12-dimethyl[a]benzanthracene in female rats. *Toxicol Ind Health*. 2012; 28(6): 542-8.

63. Nagasawa, H., T. Mitamura, S. Sakamoto, et al., Effects of lycopene on spontaneous mammary tumour development in SHN virgin mice. *Anticancer Res*. 1995; 15(4): 1173-8.

64. Thomson, C.A., N.R. Stendell-Hollis, C.L. Rock, et al., Plasma and dietary carotenoids are associated with reduced oxidative stress in women previously treated for breast cancer. *Cancer Epidemiol Biomarkers Prev*. 2007; 16(10): 2008-15.

65. Voskuil, D.W., A. Vrieling, C.M. Korse, et al., Effects of lycopene on the insulin-like growth factor (IGF) system in premenopausal breast cancer survivors and women at high familial breast cancer risk. *Nutr Cancer*. 2008; 60(3): 342-53.

66. Kabat, G.C., M. Kim, L.L. Adams-Campbell, et al., Longitudinal study of serum carotenoid, retinol, and tocopherol concentrations in relation to breast cancer risk among postmenopausal women. *Am J Clin Nutr*. 2009; 90(1): 162-9.

67. Eliassen, A.H., X. Liao, B. Rosner, et al., Plasma carotenoids and risk of breast cancer over 20 y of follow-up. *Am J Clin Nutr*. 2015; 101(6): 1197-205.

68. Eliassen, A.H., S.J. Hendrickson, L.A. Brinton, et al., Circulating carotenoids and risk of breast cancer: pooled analysis of eight prospective studies. *J Natl Cancer Inst*. 2012; 104(24): 1905-16.

69. Kim, D.J., N. Takasuka, J.M. Kim, et al., Chemoprevention by lycopene of mouse lung neoplasia after combined initiation treatment with DEN, MNU and DMH. *Cancer Lett*. 1997; 120(1): 15-22.

70. Lian, F., D.E. Smith, H. Ernst, et al., Apo-10'-lycopenoic acid inhibits lung cancer cell growth in vitro, and suppresses lung tumorigenesis in the A/J mouse model in vivo. *Carcinogenesis*. 2007; 28(7): 1567-74.
71. Liu, C., F. Lian, D.E. Smith, et al., Lycopene supplementation inhibits lung squamous metaplasia and induces apoptosis via up-regulating insulin-like growth factor-binding protein 3 in cigarette smoke-exposed ferrets. *Cancer Res*. 2003; 63(12): 3138-44.
72. Boileau, T.W., Z. Liao, S. Kim, et al., Prostate carcinogenesis in N-methyl-N-nitrosourea (NMU)-testosterone-treated rats fed tomato powder, lycopene, or energy-restricted diets. *J Natl Cancer Inst*. 2003; 95(21): 1578-86.
73. Imaida, K., S. Tamano, K. Kato, et al., Lack of chemopreventive effects of lycopene and curcumin on experimental rat prostate carcinogenesis. *Carcinogenesis*. 2001; 22(3): 467-72.
74. Tang, L., T. Jin, X. Zeng, et al., Lycopene inhibits the growth of human androgen-independent prostate cancer cells in vitro and in BALB/c nude mice. *J Nutr*. 2005; 135(2): 287-90.
75. Yang, C.M., Y.T. Yen, C.S. Huang, et al., Growth inhibitory efficacy of lycopene and beta-carotene against androgen-independent prostate tumor cells xenografted in nude mice. *Mol Nutr Food Res*. 2011; 55(4): 606-12.
76. Jiang, L.N., Y.B. Liu, and B.H. Li, Lycopene exerts anti-inflammatory effect to inhibit prostate cancer progression. *Asian J Androl*. 2018.
77. Limpens, J., F.H. Schroder, C.M. de Ridder, et al., Combined lycopene and vitamin E treatment suppresses the growth of PC-346C human prostate cancer cells in nude mice. *J Nutr*. 2006; 136(5): 1287-93.
78. Mariani, S., L. Lionetto, M. Cavallari, et al., Low prostate concentration of lycopene is associated with development of prostate cancer in patients with high-grade prostatic intraepithelial neoplasia. *Int J Mol Sci*. 2014; 15(1): 1433-40.
79. Kucuk, O., F.H. Sarkar, Z. Djuric, et al., Effects of lycopene supplementation in patients with localized prostate cancer. *Exp Biol Med (Maywood)*. 2002; 227(10): 881-5.
80. Paur, I., W. Lilleby, S.K. Bohn, et al., Tomato-based randomized controlled trial in prostate cancer patients: Effect on PSA. *Clin Nutr*. 2017; 36(3): 672-679.

81. Antwi, S.O., S.E. Steck, H. Zhang, et al., Plasma carotenoids and tocopherols in relation to prostate-specific antigen (PSA) levels among men with biochemical recurrence of prostate cancer. *Cancer Epidemiol*. 2015; 39(5): 752-62.
82. Beilby, J., G.L. Ambrosini, E. Rossi, et al., Serum levels of folate, lycopene, beta-carotene, retinol and vitamin E and prostate cancer risk. *Eur J Clin Nutr*. 2010; 64(10): 1235-8.
83. Jatoi, A., P. Burch, D. Hillman, et al., A tomato-based, lycopene-containing intervention for androgen-independent prostate cancer: results of a Phase II study from the North Central Cancer Treatment Group. *Urology*. 2007; 69(2): 289-94.
84. Clark, P.E., M.C. Hall, L.S. Borden, Jr., et al., Phase I-II prospective dose-escalating trial of lycopene in patients with biochemical relapse of prostate cancer after definitive local therapy. *Urology*. 2006; 67(6): 1257-61.
85. Gann, P.H., R.J. Deaton, E.E. Rueter, et al., A Phase II Randomized Trial of Lycopene-Rich Tomato Extract Among Men with High-Grade Prostatic Intraepithelial Neoplasia. *Nutr Cancer*. 2015; 67(7): 1104-12.
86. Wang, Y., L.M. Ausman, A.S. Greenberg, et al., Dietary lycopene and tomato extract supplementations inhibit nonalcoholic steatohepatitis-promoted hepatocarcinogenesis in rats. *Int J Cancer*. 2010; 126(8): 1788-1796.
87. Sahin, K., C. Orhan, M. Tuzcu, et al., Orally administered lycopene attenuates diethylnitrosamine-induced hepatocarcinogenesis in rats by modulating Nrf-2/HO-1 and Akt/mTOR pathways. *Nutr Cancer*. 2014; 66(4): 590-8.
88. Ip, B.C., C. Liu, L.M. Ausman, et al., Lycopene attenuated hepatic tumorigenesis via differential mechanisms depending on carotenoid cleavage enzyme in mice. *Cancer Prev Res (Phila)*. 2014; 7(12): 1219-27.
89. Koul, A., N. Arora, and L. Tanwar, Lycopene mediated modulation of 7,12 Dimethlybenz (a) anthracene induced hepatic clastogenicity in male Balb/c mice. *Nutr Hosp*. 2010; 25(2): 304-10.
90. Watanabe, S., Y. Kitade, T. Masaki, et al., Effects of lycopene and Sho-saiko-to on hepatocarcinogenesis in a rat model of spontaneous liver cancer. *Nutr Cancer*. 2001; 39(1): 96-101.

91. Bock, C.H., J.J. Ruterbusch, A.N. Holowatyj, et al., Renal cell carcinoma risk associated with lower intake of micronutrients. *Cancer Med*. 2018; 7(8): 4087-4097.
92. Sahin, K., B. Cross, N. Sahin, et al., Lycopene in the prevention of renal cell cancer in the TSC2 mutant Eker rat model. *Arch Biochem Biophys*. 2015; 572: 36-39.
93. Ollberding, N.J., G. Maskarinec, S.M. Conroy, et al., Prediagnostic circulating carotenoid levels and the risk of non-Hodgkin lymphoma: the Multiethnic Cohort. *Blood*. 2012; 119(24): 5817-23.
94. Chen, F., J. Hu, P. Liu, et al., Carotenoid intake and risk of non-Hodgkin lymphoma: a systematic review and dose-response meta-analysis of observational studies. *Ann Hematol*. 2017; 96(6): 957-965.
95. Psaltopoulou, T., I. Ntanasis-Stathopoulos, D.I. Tsilimigras, et al., Micronutrient Intake and Risk of Hematological Malignancies in Adults: A Systematic Review and Meta-analysis of Cohort Studies. *Nutr Cancer*. 2018: 1-19.
96. Yilmaz, S., A. Atessahin, E. Sahna, et al., Protective effect of lycopene on adriamycin-induced cardiotoxicity and nephrotoxicity. *Toxicology*. 2006; 218(2-3): 164-71.
97. Bansal, P., S.K. Gupta, S.K. Ojha, et al., Cardioprotective effect of lycopene in the experimental model of myocardial ischemia-reperfusion injury. *Mol Cell Biochem*. 2006; 289(1-2): 1-9.
98. Ojha, S., S. Goyal, C. Sharma, et al., Cardioprotective effect of lycopene against isoproterenol-induced myocardial infarction in rats. *Hum Exp Toxicol*. 2013; 32(5): 492-503.
99. Aman, U., P. Vaibhav, and R. Balaraman, Tomato lycopene attenuates myocardial infarction induced by isoproterenol: electrocardiographic, biochemical and anti-apoptotic study. *Asian Pac J Trop Biomed*. 2012; 2(5): 345-51.
100. Wang, X., H. Lv, Y. Gu, et al., Protective effect of lycopene on cardiac function and myocardial fibrosis after acute myocardial infarction in rats via the modulation of p38 and MMP-9. *J Mol Histol*. 2014; 45(1): 113-20.

101. He, Q., W. Zhou, C. Xiong, et al., Lycopene attenuates inflammation and apoptosis in post-myocardial infarction remodeling by inhibiting the nuclear factor-kappaB signaling pathway. *Mol Med Rep*. 2015; 11(1): 374-8.

102. Tong, C., C. Peng, L. Wang, et al., Intravenous Administration of Lycopene, a Tomato Extract, Protects against Myocardial Ischemia-Reperfusion Injury. *Nutrients*. 2016; 8(3): 138.

103. Hsiao, G., Y. Wang, N.H. Tzu, et al., Inhibitory effects of lycopene on in vitro platelet activation and in vivo prevention of thrombus formation. *J Lab Clin Med*. 2005; 146(4): 216-26.

104. Lin, J., H.X. Li, J. Xia, et al., The chemopreventive potential of lycopene against atrazine-induced cardiotoxicity: modulation of ionic homeostasis. *Sci Rep*. 2016; 6: 24855.

105. Zhu, J., C.G. Wang, and Y.G. Xu, Lycopene attenuates endothelial dysfunction in streptozotocin-induced diabetic rats by reducing oxidative stress. *Pharm Biol*. 2011; 49(11): 1144-9.

106. Chopra, M., M.E. O'Neill, N. Keogh, et al., Influence of increased fruit and vegetable intake on plasma and lipoprotein carotenoids and LDL oxidation in smokers and nonsmokers. *Clin Chem*. 2000; 46(11): 1818-29.

107. Lim, P.S., N.P. Wang, T.C. Lu, et al., Evidence for alterations in circulating low-molecular-weight antioxidants and increased lipid peroxidation in smokers on hemodialysis. *Nephron*. 2001; 88(2): 127-33.

108. Maruyama, C., K. Imamura, S. Oshima, et al., Effects of tomato juice consumption on plasma and lipoprotein carotenoid concentrations and the susceptibility of low density lipoprotein to oxidative modification. *J Nutr Sci Vitaminol (Tokyo)*. 2001; 47(3): 213-21.

109. Visioli, F., P. Riso, S. Grande, et al., Protective activity of tomato products on in vivo markers of lipid oxidation. *Eur J Nutr*. 2003; 42(4): 201-6.

110. Engelhard, Y.N., B. Gazer, and E. Paran, Natural antioxidants from tomato extract reduce blood pressure in patients with grade-1 hypertension: a double-blind, placebo-controlled pilot study. *Am Heart J*. 2006; 151(1): 100.

111. Paran, E., V. Novack, Y.N. Engelhard, et al., The effects of natural antioxidants from tomato extract in treated but uncontrolled hypertensive patients. *Cardiovasc Drugs Ther*. 2009; 23(2): 145-51.

112. Stangl, V., C. Kuhn, S. Hentschel, et al., Lack of effects of tomato products on endothelial function in human subjects: results of a randomised, placebo-controlled cross-over study. *Br J Nutr*. 2011; 105(2): 263-7.

113. Shidfar, F., N. Froghifar, M. Vafa, et al., The effects of tomato consumption on serum glucose, apolipoprotein B, apolipoprotein A-I, homocysteine and blood pressure in type 2 diabetic patients. *Int J Food Sci Nutr*. 2011; 62(3): 289-94.

114. Kim, J.Y., J.K. Paik, O.Y. Kim, et al., Effects of lycopene supplementation on oxidative stress and markers of endothelial function in healthy men. *Atherosclerosis*. 2011; 215(1): 189-95.

115. Xaplanteris, P., C. Vlachopoulos, P. Pietri, et al., Tomato paste supplementation improves endothelial dynamics and reduces plasma total oxidative status in healthy subjects. *Nutr Res*. 2012; 32(5): 390-4.

116. Gajendragadkar, P.R., A. Hubsch, K.M. Maki-Petaja, et al., Effects of oral lycopene supplementation on vascular function in patients with cardiovascular disease and healthy volunteers: a randomised controlled trial. *PLoS One*. 2014; 9(6): e99070.

117. Zou, Z.Y., X.R. Xu, X.M. Lin, et al., Effects of lutein and lycopene on carotid intima-media thickness in Chinese subjects with subclinical atherosclerosis: a randomised, double-blind, placebo-controlled trial. *Br J Nutr*. 2014; 111(3): 474-80.

118. Burton-Freeman, B., J. Talbot, E. Park, et al., Protective activity of processed tomato products on postprandial oxidation and inflammation: a clinical trial in healthy weight men and women. *Mol Nutr Food Res*. 2012; 56(4): 622-31.

119. Thies, F., L.F. Masson, A. Rudd, et al., Effect of a tomato-rich diet on markers of cardiovascular disease risk in moderately overweight, disease-free, middle-aged adults: a randomized controlled trial. *Am J Clin Nutr*. 2012; 95(5): 1013-22.

120. Misra, R., S. Mangi, S. Joshi, et al., LycoRed as an alternative to hormone replacement therapy in lowering serum lipids and oxidative stress markers: a randomized controlled clinical trial. *J Obstet Gynaecol Res*. 2006; 32(3): 299-304.

121. Riccioni, G., L. Scotti, E. Di Ilio, et al., Lycopene and preclinical carotid atherosclerosis. *J Biol Regul Homeost Agents*. 2011; 25(3): 435-41.

122. Biddle, M.J., T.A. Lennie, G.V. Bricker, et al., Lycopene dietary intervention: a pilot study in patients with heart failure. *J Cardiovasc Nurs*. 2015; 30(3): 205-12.

123. McEneny, J., L. Wade, I.S. Young, et al., Lycopene intervention reduces inflammation and improves HDL functionality in moderately overweight middle-aged individuals. *J Nutr Biochem*. 2013; 24(1): 163-8.

124. Colman-Martinez, M., M. Martinez-Huelamo, P. Valderas-Martinez, et al., trans-Lycopene from tomato juice attenuates inflammatory biomarkers in human plasma samples: An intervention trial. *Mol Nutr Food Res*. 2017; 61(11).

125. Blum, A., M. Monir, K. Khazim, et al., Tomato-rich (Mediterranean) diet does not modify inflammatory markers. *Clin Invest Med*. 2007; 30(2): E70-4.

126. Ali, M.M. and F.G. Agha, Amelioration of streptozotocin-induced diabetes mellitus, oxidative stress and dyslipidemia in rats by tomato extract lycopene. *Scand J Clin Lab Invest*. 2009; 69(3): 371-9.

127. Kuhad, A., R. Sethi, and K. Chopra, Lycopene attenuates diabetes-associated cognitive decline in rats. *Life Sci*. 2008; 83(3-4): 128-34.

128. Kuhad, A. and K. Chopra, Lycopene ameliorates thermal hyperalgesia and cold allodynia in STZ-induced diabetic rat. *Indian J Exp Biol*. 2008; 46(2): 108-11.

129. Wu, A., R. Liu, W. Dai, et al., Lycopene attenuates early brain injury and inflammation following subarachnoid hemorrhage in rats. *Int J Clin Exp Med*. 2015; 8(8): 14316-22.

130. Liu, T.Y. and S.B. Chen, Sarcandra glabra combined with lycopene protect rats from lipopolysaccharide induced acute lung injury via reducing inflammatory response. *Biomed Pharmacother*. 2016; 84: 34-41.

131. Lin, H.Y., B.R. Huang, W.L. Yeh, et al., Antineuroinflammatory effects of lycopene via activation of adenosine monophosphate-activated protein kinase-alpha1/heme oxygenase-1 pathways. *Neurobiol Aging*. 2014; 35(1): 191-202.

132. Boeira, S.P., V.R. Funck, C. Borges Filho, et al., Lycopene protects against acute zearalenone-induced oxidative, endocrine, inflammatory and reproductive damages in male mice. *Chem Biol Interact*. 2015; 230: 50-7.

133. Renju, G.L., G.M. Kurup, and C.H. Saritha Kumari, Effect of lycopene from Chlorella marina on high cholesterol-induced oxidative damage and inflammation in rats. *Inflammopharmacology*. 2014; 22(1): 45-54.
134. Oliveira, G.R., P.K. Vargas-Sanchez, R.R. Fernandes, et al., Lycopene influences osteoblast functional activity and prevents femur bone loss in female rats submitted to an experimental model of osteoporosis. *J Bone Miner Metab*. 2018.
135. Ardawi, M.M., M.H. Badawoud, S.M. Hassan, et al., Lycopene treatment against loss of bone mass, microarchitecture and strength in relation to regulatory mechanisms in a postmenopausal osteoporosis model. *Bone*. 2016; 83: 127-140.
136. Kaur, H., S. Chauhan, and R. Sandhir, Protective effect of lycopene on oxidative stress and cognitive decline in rotenone induced model of Parkinson's disease. *Neurochem Res*. 2011; 36(8): 1435-43.
137. Wang, J., L. Li, Z. Wang, et al., Supplementation of lycopene attenuates lipopolysaccharide-induced amyloidogenesis and cognitive impairments via mediating neuroinflammation and oxidative stress. *J Nutr Biochem*. 2018; 56: 16-25.
138. Prema, A., U. Janakiraman, T. Manivasagam, et al., Neuroprotective effect of lycopene against MPTP induced experimental Parkinson's disease in mice. *Neurosci Lett*. 2015; 599: 12-9.
139. Zhao, X., G. Aldini, E.J. Johnson, et al., Modification of lymphocyte DNA damage by carotenoid supplementation in postmenopausal women. *Am J Clin Nutr*. 2006; 83(1): 163-9.
140. Neyestani, T.R., N. Shariat-Zadeh, A. Gharavi, et al., The opposite associations of lycopene and body fat mass with humoral immunity in type 2 diabetes mellitus: a possible role in atherogenesis. *Iran J Allergy Asthma Immunol*. 2007; 6(2): 79-87.
141. Mackinnon, E.S., A.V. Rao, R.G. Josse, et al., Supplementation with the antioxidant lycopene significantly decreases oxidative stress parameters and the bone resorption marker N-telopeptide of type I collagen in postmenopausal women. *Osteoporos Int*. 2011; 22(4): 1091-101.
142. Falk, B., R. Gorev, L. Zigel, et al., Effect of lycopene supplementation on lung function after exercise in young athletes who complain of exercise-induced

bronchoconstriction symptoms. *Ann Allergy Asthma Immunol*. 2005; 94(4): 480-5.

143. Jacob, K., M.J. Periago, V. Bohm, et al., Influence of lycopene and vitamin C from tomato juice on biomarkers of oxidative stress and inflammation. *Br J Nutr*. 2008; 99(1): 137-46.
144. Ghavipour, M., A. Saedisomeolia, M. Djalali, et al., Tomato juice consumption reduces systemic inflammation in overweight and obese females. *Br J Nutr*. 2013; 109(11): 2031-5.
145. Lampe, J.W., Dairy products and cancer. *J Am Coll Nutr*. 2011; 30(5 Suppl 1): 464s-70s.
146. Arruebo, M., N. Vilaboa, B. Saez-Gutierrez, et al., Assessment of the evolution of cancer treatment therapies. *Cancers (Basel)*. 2011; 3(3): 3279-330.
147. Dawood, S. and B. Leyland-Jones, Pharmacology and pharmacogenetics of chemotherapeutic agents. *Cancer Invest*. 2009; 27(5): 482-8.
148. Magalhaes, L.G., L.L.G. Ferreira, and A.D. Andricopulo, Recent Advances and Perspectives in Cancer Drug Design. *An Acad Bras Cienc*. 2018; 90(1 Suppl 2): 1233-1250.
149. Dilruba, S. and G.V. Kalayda, Platinum-based drugs: past, present and future. *Cancer Chemother Pharmacol*. 2016; 77(6): 1103-24.
150. Kang, B.W., J.G. Kim, O.K. Kwon, et al., Non-platinum-based chemotherapy for treatment of advanced gastric cancer: 5-fluorouracil, taxanes, and irinotecan. *World J Gastroenterol*. 2014; 20(18): 5396-402.
151. Rosenberg, B., L. Vancamp, and T. Krigas, INHIBITION OF CELL DIVISION IN ESCHERICHIA COLI BY ELECTROLYSIS PRODUCTS FROM A PLATINUM ELECTRODE. *Nature*. 1965; 205: 698-9.
152. Rosenberg, B., L. VanCamp, J.E. Trosko, et al., Platinum compounds: a new class of potent antitumour agents. *Nature*. 1969; 222(5191): 385-6.
153. Kelland, L., The resurgence of platinum-based cancer chemotherapy. *Nat Rev Cancer*. 2007; 7(8): 573-84.
154. Wang, D. and S.J. Lippard, Cellular processing of platinum anticancer drugs. *Nat Rev Drug Discov*. 2005; 4(4): 307-20.

155. Rabik, C.A. and M.E. Dolan, Molecular mechanisms of resistance and toxicity associated with platinating agents. *Cancer Treat Rev*. 2007; 33(1): 9-23.
156. Fuertes, M.A., C. Alonso, and J.M. Perez, Biochemical modulation of Cisplatin mechanisms of action: enhancement of antitumor activity and circumvention of drug resistance. *Chem Rev*. 2003; 103(3): 645-62.
157. Di Francesco, A.M., A. Ruggiero, and R. Riccardi, Cellular and molecular aspects of drugs of the future: oxaliplatin. *Cell Mol Life Sci*. 2002; 59(11): 1914-27.
158. Misset, J.L., H. Bleiberg, W. Sutherland, et al., Oxaliplatin clinical activity: a review. *Crit Rev Oncol Hematol*. 2000; 35(2): 75-93.
159. Cohen, S.M. and S.J. Lippard, Cisplatin: from DNA damage to cancer chemotherapy. *Prog Nucleic Acid Res Mol Biol*. 2001; 67: 93-130.
160. Chen, X., Y. Wu, H. Dong, et al., Platinum-based agents for individualized cancer treatment. *Curr Mol Med*. 2013; 13(10): 1603-12.
161. Aisner, J. and J. Abrams, Cisplatin for small-cell lung cancer. *Semin Oncol*. 1989; 16(4 Suppl 6): 2-9.
162. Forastiere, A.A., Overview of platinum chemotherapy in head and neck cancer. *Semin Oncol*. 1994; 21(5 Suppl 12): 20-7.
163. Vassilomanolakis, M., G. Koumakis, V. Barbounis, et al., First-line chemotherapy with docetaxel and cisplatin in metastatic breast cancer. *Breast*. 2005; 14(2): 136-41.
164. Chen, Y.J. and G. Del Priore, The role of cisplatin alternative regimens with radiotherapy in cervical cancer. *Gynecol Oncol Rep*. 2015; 11: 38-40.
165. Bergamini, A., C. Pisano, M. Di Napoli, et al., Cisplatin can be safely administered to ovarian cancer patients with hypersensitivity to carboplatin. *Gynecol Oncol*. 2017; 144(1): 72-76.
166. Holzer, A.K., G.H. Manorek, and S.B. Howell, Contribution of the major copper influx transporter CTR1 to the cellular accumulation of cisplatin, carboplatin, and oxaliplatin. *Mol Pharmacol*. 2006; 70(4): 1390-94.
167. Siddik, Z.H., Cisplatin: mode of cytotoxic action and molecular basis of resistance. *Oncogene*. 2003; 22(47): 7265-79.
168. Dutta, A., J.M. Ruppert, J.C. Aster, et al., Inhibition of DNA replication factor RPA by p53. *Nature*. 1993; 365(6441): 79-82.

169. Gumulec, J., J. Balvan, M. Sztalmachova, et al., Cisplatin-resistant prostate cancer model: Differences in antioxidant system, apoptosis and cell cycle. *Int J Oncol*. 2014; 44(3): 923-33.
170. Kischkel, F.C., S. Hellbardt, I. Behrmann, et al., Cytotoxicity-dependent APO-1 (Fas/CD95)-associated proteins form a death-inducing signaling complex (DISC) with the receptor. *Embo j*. 1995; 14(22): 5579-88.
171. Elmore, S., Apoptosis: a review of programmed cell death. *Toxicol Pathol*. 2007; 35(4): 495-516.
172. Kelland, L.R., P. Mistry, G. Abel, et al., Mechanism-related circumvention of acquired cis-diamminedichloroplatinum(II) resistance using two pairs of human ovarian carcinoma cell lines by ammine/amine platinum(IV) dicarboxylates. *Cancer Res*. 1992; 52(14): 3857-64.
173. Holzer, A.K. and S.B. Howell, The internalization and degradation of human copper transporter 1 following cisplatin exposure. *Cancer Res*. 2006; 66(22): 10944-52.
174. Safaei, R., A.K. Holzer, K. Katano, et al., The role of copper transporters in the development of resistance to Pt drugs. *J Inorg Biochem*. 2004; 98(10): 1607-13.
175. Ferry, K.V., T.C. Hamilton, and S.W. Johnson, Increased nucleotide excision repair in cisplatin-resistant ovarian cancer cells: role of ERCC1-XPF. *Biochem Pharmacol*. 2000; 60(9): 1305-13.
176. Guengerich, F.P., Mechanisms of drug toxicity and relevance to pharmaceutical development. *Drug Metab Pharmacokinet*. 2011; 26(1): 3-14.
177. Singh, D., W.C. Cho, and G. Upadhyay, Drug-Induced Liver Toxicity and Prevention by Herbal Antioxidants: An Overview. *Front Physiol*. 2015; 6: 363.
178. Larrey, D. and G.P. Pageaux, Drug-induced acute liver failure. *Eur J Gastroenterol Hepatol*. 2005; 17(2): 141-3.
179. Lee, W.M., Drug-induced acute liver failure. *Clin Liver Dis*. 2013; 17(4): 575-86, viii.
180. Fontana, R.J., P.H. Hayashi, J. Gu, et al., Idiosyncratic drug-induced liver injury is associated with substantial morbidity and mortality within 6 months from onset. *Gastroenterology*. 2014; 147(1): 96-108.e4.

181. Chen, M., A. Suzuki, J. Borlak, et al., Drug-induced liver injury: Interactions between drug properties and host factors. *J Hepatol*. 2015; 63(2): 503-14.
182. Pazhayattil, G.S. and A.C. Shirali, Drug-induced impairment of renal function. *Int J Nephrol Renovasc Dis*. 2014; 7: 457-68.
183. Mehta, R.L., M.T. Pascual, S. Soroko, et al., Spectrum of acute renal failure in the intensive care unit: the PICARD experience. *Kidney Int*. 2004; 66(4): 1613-21.
184. Uchino, S., J.A. Kellum, R. Bellomo, et al., Acute renal failure in critically ill patients: a multinational, multicenter study. *Jama*. 2005; 294(7): 813-8.
185. Hoste, E.A., S.M. Bagshaw, R. Bellomo, et al., Epidemiology of acute kidney injury in critically ill patients: the multinational AKI-EPI study. *Intensive Care Med*. 2015; 41(8): 1411-23.
186. Ferri, N., P. Siegl, A. Corsini, et al., Drug attrition during pre-clinical and clinical development: understanding and managing drug-induced cardiotoxicity. *Pharmacol Ther*. 2013; 138(3): 470-84.
187. Sweidan, A.J., N.K. Singh, N. Dang, et al., Amiodarone-Induced Pulmonary Toxicity - A Frequently Missed Complication. *Clin Med Insights Case Rep*. 2016; 9: 91-94.
188. Stone, J.B. and L.M. DeAngelis, Cancer-treatment-induced neurotoxicity--focus on newer treatments. *Nat Rev Clin Oncol*. 2016; 13(2): 92-105.
189. Payne, L.E., D.J. Gagnon, R.R. Riker, et al., Cefepime-induced neurotoxicity: a systematic review. *Crit Care*. 2017; 21(1): 276.
190. Grigorian, A. and C.B. O'Brien, Hepatotoxicity Secondary to Chemotherapy. *J Clin Transl Hepatol*. 2014; 2(2): 95-102.
191. Miller, R.P., R.K. Tadagavadi, G. Ramesh, et al., Mechanisms of Cisplatin nephrotoxicity. *Toxins (Basel)*. 2010; 2(11): 2490-518.
192. Karasawa, T. and P.S. Steyger, An integrated view of cisplatin-induced nephrotoxicity and ototoxicity. *Toxicol Lett*. 2015; 237(3): 219-27.
193. Pabla, N., R.F. Murphy, K. Liu, et al., The copper transporter Ctr1 contributes to cisplatin uptake by renal tubular cells during cisplatin nephrotoxicity. *Am J Physiol Renal Physiol*. 2009; 296(3): F505-11.

194. Filipski, K.K., R.H. Mathijssen, T.S. Mikkelsen, et al., Contribution of organic cation transporter 2 (OCT2) to cisplatin-induced nephrotoxicity. *Clin Pharmacol Ther*. 2009; 86(4): 396-402.

195. Okuda, M., H. Saito, Y. Urakami, et al., cDNA cloning and functional expression of a novel rat kidney organic cation transporter, OCT2. *Biochem Biophys Res Commun*. 1996; 224(2): 500-7.

196. Townsend, D.M., M. Deng, L. Zhang, et al., Metabolism of Cisplatin to a nephrotoxin in proximal tubule cells. *J Am Soc Nephrol*. 2003; 14(1): 1-10.

197. Cersosimo, R.J., Hepatotoxicity associated with cisplatin chemotherapy. *Ann Pharmacother*. 1993; 27(4): 438-41.

198. Ettinger, L.J., M.D. Krailo, P.S. Gaynon, et al., A phase I study of carboplatin in children with acute leukemia in bone marrow relapse. A report from the Childrens Cancer Group. *Cancer*. 1993; 72(3): 917-22.

199. Lee, K.W., S.A. Im, T. Yun, et al., Phase II trial of low-dose paclitaxel and cisplatin in patients with advanced gastric cancer. *Jpn J Clin Oncol*. 2005; 35(12): 720-6.

200. Esteban, E., J. Fra, M. Sala, et al., Phase I/II study of gemcitabine and vinorelbine plus cisplatin in non-small cell lung cancer. *Invest New Drugs*. 2002; 20(3): 317-26.

201. Kusaba, H., T. Tamura, T. Shimoyama, et al., Phase I/II study of 3-week cycle cisplatin-gemcitabine in advanced non-small cell lung cancer. *Jpn J Clin Oncol*. 2002; 32(2): 43-7.

202. Mao, Y., X. Wan, W. Lv, et al., Relapsed or refractory gestational trophoblastic neoplasia treated with the etoposide and cisplatin/etoposide, methotrexate, and actinomycin D (EP-EMA) regimen. *Int J Gynaecol Obstet*. 2007; 98(1): 44-7.

203. Yuan, J.N., Y. Chao, W.P. Lee, et al., Chemotherapy with etoposide, doxorubicin, cisplatin, 5-fluorouracil, and leucovorin for patients with advanced hepatocellular carcinoma. *Med Oncol*. 2008; 25(2): 201-6.

204. King, P.D. and M.C. Perry, Hepatotoxicity of chemotherapy. *Oncologist*. 2001; 6(2): 162-76.

205. Stewart, D.J., R.S. Benjamin, M. Luna, et al., Human tissue distribution of platinum after cis-diamminedichloroplatinum. *Cancer Chemother Pharmacol*. 1982; 10(1): 51-4.
206. Yang, Y., H. Liu, F. Liu, et al., Mitochondrial dysregulation and protection in cisplatin nephrotoxicity. *Arch Toxicol*. 2014; 88(6): 1249-56.
207. Hosohata, K., Role of Oxidative Stress in Drug-Induced Kidney Injury. *Int J Mol Sci*. 2016; 17(11).
208. Liu, H. and R. Baliga, Cytochrome P450 2E1 null mice provide novel protection against cisplatin-induced nephrotoxicity and apoptosis. *Kidney Int*. 2003; 63(5): 1687-96.
209. Soni, H., D. Kaminski, R. Gangaraju, et al., Cisplatin-induced oxidative stress stimulates renal Fas ligand shedding. *Ren Fail*. 2018; 40(1): 314-322.
210. El-Shitany, N.A. and B. Eid, Proanthocyanidin protects against cisplatin-induced oxidative liver damage through inhibition of inflammation and NF-kappabeta/TLR-4 pathway. *Environ Toxicol*. 2017; 32(7): 1952-1963.
211. Omar, H.A., W.R. Mohamed, H.H. Arab, et al., Tangeretin Alleviates Cisplatin-Induced Acute Hepatic Injury in Rats: Targeting MAPKs and Apoptosis. *PLoS One*. 2016; 11(3): e0151649.
212. Waseem, M., M. Bhardwaj, H. Tabassum, et al., Cisplatin hepatotoxicity mediated by mitochondrial stress. *Drug Chem Toxicol*. 2015; 38(4): 452-9.
213. Cure, M.C., E. Cure, Y. Kalkan, et al., Infliximab Modulates Cisplatin-Induced Hepatotoxicity in Rats. *Balkan Med J*. 2016; 33(5): 504-511.
214. Omar, H.A., W.R. Mohamed, S.A. Arafa el, et al., Hesperidin alleviates cisplatin-induced hepatotoxicity in rats without inhibiting its antitumor activity. *Pharmacol Rep*. 2016; 68(2): 349-56.
215. Abramavicius, S., M. Zemaitis, V. Pilvinis, et al., Cisplatin-induced sudden cardiac death with hemodynamic collapse: a severe adverse drug reaction: Case report. *Medicine (Baltimore)*. 2017; 96(48): e8995.
216. Hanchate, L.P., S.R. Sharma, and S. Madyalkar, Cisplatin Induced Acute Myocardial Infarction and Dyslipidemia. *J Clin Diagn Res*. 2017; 11(6): Od05-od07.

217. Patane, S., Cardiotoxicity: cisplatin and long-term cancer survivors. *Int J Cardiol*. 2014; 175(1): 201-2.

218. Fallahzadeh, A.R., Z. Rezaei, H.R. Rahimi, et al., Evaluation of the Effect of Pentoxifylline on Cisplatin-Induced Testicular Toxicity in Rats. *Toxicol Res*. 2017; 33(3): 255-263.

219. Sherif, I.O., A. Abdel-Aziz, and O.M. Sarhan, Cisplatin-induced testicular toxicity in rats: the protective effect of arjunolic acid. *J Biochem Mol Toxicol*. 2014; 28(11): 515-21.

220. Torino, F., A. Barnabei, L. De Vecchis, et al., Chemotherapy-induced ovarian toxicity in patients affected by endocrine-responsive early breast cancer. *Crit Rev Oncol Hematol*. 2014; 89(1): 27-42.

221. Pandir, D., O. Kara, and M. Kara, Protective effect of bilberry (Vaccinium myrtillus L.) on cisplatin induced ovarian damage in rat. *Cytotechnology*. 2014; 66(4): 677-85.

222. Glei, M., U.M. Liegibel, M.N. Ebert, et al., beta-Carotene reduces bleomycin-induced genetic damage in human lymphocytes. *Toxicol Appl Pharmacol*. 2002; 179(2): 65-73.

223. Zhou, C., W. Han, P. Zhang, et al., Lycopene from tomatoes partially alleviates the bleomycin-induced experimental pulmonary fibrosis in rats. *Nutr Res*. 2008; 28(2): 122-30.

224. Koul, A., Shubrant, and P. Gupta, Phytomodulatory potential of lycopene from Lycopersicum esculentum against doxorubicin induced nephrotoxicity. *Indian J Exp Biol*. 2013; 51(8): 635-45.

225. Anjos Ferreira, A.L., R.M. Russell, N. Rocha, et al., Effect of lycopene on doxorubicin-induced cardiotoxicity: an echocardiographic, histological and morphometrical assessment. *Basic Clin Pharmacol Toxicol*. 2007; 101(1): 16-24.

226. Ferreira, A.L., D.M. Salvadori, M.C. Nascimento, et al., Tomato-oleoresin supplement prevents doxorubicin-induced cardiac myocyte oxidative DNA damage in rats. *Mutat Res*. 2007; 631(1): 26-35.

227. Ceribasi, A.O., G. Turk, M. Sonmez, et al., Toxic effect of cyclophosphamide on sperm morphology, testicular histology and blood oxidant-antioxidant balance,

and protective roles of lycopene and ellagic acid. *Basic Clin Pharmacol Toxicol*. 2010; 107(3): 730-6.

228. Turk, G., A.O. Ceribasi, F. Sakin, et al., Antiperoxidative and anti-apoptotic effects of lycopene and ellagic acid on cyclophosphamide-induced testicular lipid peroxidation and apoptosis. *Reprod Fertil Dev*. 2010; 22(4): 587-96.
229. Oguz, E., S. Kocarslan, S. Tabur, et al., Effects of Lycopene Alone or Combined with Melatonin on Methotrexate-Induced Nephrotoxicity in Rats. *Asian Pac J Cancer Prev*. 2015; 16(14): 6061-6.
230. Yucel, Y., S. Tabur, O. Gozeneli, et al., The effects of lycopene on intestinal injury due to methotrexate in rats. *Redox Rep*. 2016; 21(3): 113-8.
231. Yucel, Y., E. Oguz, S. Kocarslan, et al., The effects of lycopene on methotrexate-induced liver injury in rats. *Bratisl Lek Listy*. 2017; 118(4): 212-216.
232. Sendao, M.C., E.B. Behling, R.A. dos Santos, et al., Comparative effects of acute and subacute lycopene administration on chromosomal aberrations induced by cisplatin in male rats. *Food Chem Toxicol*. 2006; 44(8): 1334-9.
233. Ozkiris, M., Z. Kapusuz, S. Karacavus, et al., The effects of lycopene on cisplatin-induced ototoxicity. *Eur Arch Otorhinolaryngol*. 2013; 270(12): 3027-33.
234. Johnsen, S.G., Testicular biopsy score count--a method for registration of spermatogenesis in human testes: normal values and results in 335 hypogonadal males. *Hormones*. 1970; 1(1): 2-25.
235. Babai, S., L. Auclert, and H. Le-Louet, Safety data and withdrawal of hepatotoxic drugs. *Therapie*. 2018.
236. Benedetti, G., B. van de Water, and M. de Graauw, *Chapter 5.2 - An Overview of Toxicogenomics Approaches to Mechanistically Understand and Predict Kidney Toxicity*, in *Toxicogenomics-Based Cellular Models*, J. Kleinjans, Editor. 2014, Academic Press: San Diego. p. 213-234.
237. Weijl, N.I., T.J. Elsendoorn, E.G. Lentjes, et al., Supplementation with antioxidant micronutrients and chemotherapy-induced toxicity in cancer patients treated with cisplatin-based chemotherapy: a randomised, double-blind, placebo-controlled study. *Eur J Cancer*. 2004; 40(11): 1713-23.

238. Phaibulvatanapong, E., V. Srinonprasert, and S. Ithimakin, Risk Factors for Chemotherapy-Related Toxicity and Adverse Events in Elderly Thai Cancer Patients: A Prospective Study. *Oncology*. 2018; 94(3): 149-160.

239. Woo, S.M., Y.K. Choi, A.J. Kim, et al., Sip-jeon-dea-bo-tang, a traditional herbal medicine, ameliorates cisplatin-induced anorexia via the activation of JAK1/STAT3-mediated leptin and IL-6 production in the fat tissue of mice. *Mol Med Rep*. 2016; 13(4): 2967-72.

240. Hojman, P., J. Fjelbye, B. Zerahn, et al., Voluntary exercise prevents cisplatin-induced muscle wasting during chemotherapy in mice. *PLoS One*. 2014; 9(9): e109030.

241. Afsar, T., S. Razak, A. Almajwal, et al., Acacia hydaspica R. Parker ameliorates cisplatin induced oxidative stress, DNA damage and morphological alterations in rat pulmonary tissue. *BMC Complement Altern Med*. 2018; 18(1): 49.

242. Terada, Y., K. Inoue, T. Matsumoto, et al., 5-Aminolevulinic acid protects against cisplatin-induced nephrotoxicity without compromising the anticancer efficiency of cisplatin in rats in vitro and in vivo. *PLoS One*. 2013; 8(12): e80850.

243. Shaker, R.A., S.H. Abboud, H.C. Assad, et al., Enoxaparin attenuates doxorubicin induced cardiotoxicity in rats via interfering with oxidative stress, inflammation and apoptosis. *BMC Pharmacol Toxicol*. 2018; 19(1): 3.

244. Bennink, R.J., M.J. van den Hoff, F.J. van Hemert, et al., Annexin V imaging of acute doxorubicin cardiotoxicity (apoptosis) in rats. *J Nucl Med*. 2004; 45(5): 842-8.

245. Longo, M., P. Della Torre, C. Allievi, et al., Tolerability and toxicological profile of pixantrone (Pixuvri(R)) in juvenile mice. Comparative study with doxorubicin. *Reprod Toxicol*. 2014; 46: 20-30.

246. Oun, R., Y.E. Moussa, and N.J. Wheate, The side effects of platinum-based chemotherapy drugs: a review for chemists. *Dalton Trans*. 2018; 47(19): 6645-6653.

247. Degirmenci, I., A. Basaran, K. Erol, et al., Effects of S-CMC on the cisplatin toxicity in rats. *Urol Int*. 1996; 57(2): 99-103.

248. Chiarandini Fiore, J.P., S.L. Fanelli, E.C. de Ferreyra, et al., Diallyl disulfide prevention of cis-Diamminedichloroplatinum-induced nephrotoxicity and leukopenia in rats: potential adjuvant effects. *Nutr Cancer*. 2008; 60(6): 784-91.
249. Nojiri, T., H. Hosoda, M. Zenitani, et al., Atrial natriuretic peptide protects against cisplatin-induced granulocytopenia. *Cancer Chemother Pharmacol*. 2016; 78(1): 191-7.
250. Vardin, A.M., B. Abdollahi, M. Kosari-Nasab, et al., Effects of *Cornus mas* fruit hydro-methanolic extract on serum antioxidants, lipid profile, and hematologic parameters following cisplatin-induced changes in rats. *Res Pharm Sci*. 2017; 12(6): 510-516.
251. Salem, S.I.A.E.-B., A.A.; Mohammed, F. F., Cytoprotective Effect of Silymarin on Cisplatin Induced Hepatotoxicity and Bone Marrow Toxicity in Rats. *Asian Journal of Animal Sciences*. 2017; 11(3): 140-152.
252. Kim, H.S., H.G. Kim, H.J. Im, et al., Antiemetic and Myeloprotective Effects of *Rhus verniciflua Stoke* in a Cisplatin-Induced Rat Model. *Evid Based Complement Alternat Med*. 2017; 2017: 9830342.
253. Gustin, D.M., K.A. Rodvold, J.A. Sosman, et al., Single-dose pharmacokinetic study of lycopene delivered in a well-defined food-based lycopene delivery system (tomato paste-oil mixture) in healthy adult male subjects. *Cancer Epidemiol Biomarkers Prev*. 2004; 13(5): 850-60.
254. Boeira, S.P., C.B. Filho, L. Del'Fabbro, et al., Lycopene treatment prevents hematological, reproductive and histopathological damage induced by acute zearalenone administration in male Swiss mice. *Exp Toxicol Pathol*. 2014; 66(4): 179-85.
255. Wood, P.A. and W.J. Hrushesky, Cisplatin-associated anemia: an erythropoietin deficiency syndrome. *J Clin Invest*. 1995; 95(4): 1650-9.
256. Bhalchandra, W., Y.A. Alqadhi, and A.S. Ninawe, Ameliorative Role Of Bee Honey And Royal Jelly Against Cisplatin Induced Alteration In Hematological Parameters In Male Wister Albino Rat. *Int J Pharm Pharm Sci*. 2018; 10(4): 110-114.

257. Soni, K.K., H.K. Kim, B.R. Choi, 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: 3959-3968.
258. Unami, A., N. Nishina, T. Terai, et al., Effects of cisplatin on erythropoietin production in rats. *J Toxicol Sci*. 1996; 21(3): 157-65.
259. Nasr, A.Y., Protective effect of aged garlic extract against the oxidative stress induced by cisplatin on blood cells parameters and hepatic antioxidant enzymes in rats. *Toxicol Rep*. 2014; 1: 682-691.
260. Geyikoglu, F., S. Colak, H. Turkez, et al., Oleuropein Ameliorates Cisplatin-induced Hematological Damages Via Restraining Oxidative Stress and DNA Injury. *Indian J Hematol Blood Transfus*. 2017; 33(3): 348-354.
261. Molyneux, G., M. Andrews, W. Sones, et al., Haemotoxicity of busulphan, doxorubicin, cisplatin and cyclophosphamide in the female BALB/c mouse using a brief regimen of drug administration. *Cell Biol Toxicol*. 2011; 27(1): 13-40.
262. Sharma, S., P. Parashar, S. Sharma, et al., Ameliorating Role of Lycopene, Tomato Puree, and Spirulina + Tomato Puree on the Hematology of Fluoride-Exposed Swiss Albino Mice. *J Diet Suppl*. 2018; 15(6): 827-841.
263. Woo, S., W. Krzyzanski, and W.J. Jusko, Pharmacodynamic model for chemotherapy-induced anemia in rats. *Cancer Chemother Pharmacol*. 2008; 62(1): 123-33.
264. Mansour, H.H., H.F. Hafez, and N.M. Fahmy, Silymarin modulates Cisplatin-induced oxidative stress and hepatotoxicity in rats. *J Biochem Mol Biol*. 2006; 39(6): 656-61.
265. Palipoch, S. and C. Punsawad, Biochemical and histological study of rat liver and kidney injury induced by Cisplatin. *J Toxicol Pathol*. 2013; 26(3): 293-9.
266. Salem, M.M. and Z.M. Altayeb, Light and Electron Microscopic Study on the Possible Protective Effect of Nigella Sativa Oil on Cisplatin Hepatotoxicity in Albino Rats. *Egyptian Journal of Histology*. 2017; 40(1): 68-79.
267. Mir, M., M.R. Arab, M.R. Shahraki, et al., Toxic Effects of Cisplatin on Hepatocytes and Liver Enzymes of Rats. *Anatomical Sciences Journal*. 2015; 12(4): 171-176.

268. Pezeshki, Z., A. Khosravi, M. Nekuei, et al., Time course of cisplatin-induced nephrotoxicity and hepatotoxicity. *J Nephropathol*. 2017; 6(3): 163-167.

269. Jiang, W., M.H. Guo, and X. Hai, Hepatoprotective and antioxidant effects of lycopene on non-alcoholic fatty liver disease in rat. *World J Gastroenterol*. 2016; 22(46): 10180-10188.

270. Hemieda, F.A.-K.E.-S., H.A. Hassan, E.E.-b. Ibrahim, et al., Protective impact of lycopene on flutamide-induced hepatotoxicity in male rats. *The Egyptian Journal of Experimental Biology (Zoology)*. 2017; 13(1): 1-10.

271. Sheriff, S.A. and T. Devaki, Lycopene stabilizes lipoprotein levels during D-galactosamine/lipopolysaccharide induced hepatitis in experimental rats. *Asian Pac J Trop Biomed*. 2012; 2(12): 975-80.

272. Beauregard, J.M., J.A. Lyon, and C. Slovis, Using the literature to evaluate diagnostic tests: amylase or lipase for diagnosing acute pancreatitis? *J Med Libr Assoc*. 2007; 95(2): 121-6.

273. Nashwan, A.J., M.A. Yassin, and S.L. Nair, Acute pancreatitis-induced by platinum compounds in patients with cancer: a review of the literature. *2017*. 2017; 4(2): 4.

274. Bakir, M., F. Geyikoglu, K. Koc, et al., Therapeutic effects of oleuropein on cisplatin-induced pancreas injury in rats. *J Cancer Res Ther*. 2018; 14(3): 671-678.

275. Lv, J.C., G. Wang, S.H. Pan, et al., Lycopene protects pancreatic acinar cells against severe acute pancreatitis by abating the oxidative stress through JNK pathway. *Free Radic Res*. 2015; 49(2): 151-63.

276. El-Ashmawy, N.E., N.F. Khedr, H.A. El-Bahrawy, et al., Suppression of inducible nitric oxide synthase and tumor necrosis factor-alpha level by lycopene is comparable to methylprednisolone in acute pancreatitis. *Dig Liver Dis*. 2018; 50(6): 601-607.

277. Lyman, J.L., Blood urea nitrogen and creatinine. *Emerg Med Clin North Am*. 1986; 4(2): 223-33.

278. Xu, Y., H. Ma, J. Shao, et al., A Role for Tubular Necroptosis in Cisplatin-Induced AKI. *J Am Soc Nephrol*. 2015; 26(11): 2647-58.

279. Ozkok, A., K. Ravichandran, Q. Wang, et al., NF-kappaB transcriptional inhibition ameliorates cisplatin-induced acute kidney injury (AKI). *Toxicol Lett*. 2016; 240(1): 105-13.

280. Karafakioglu, Y.S., M.F. Bozkurt, O. Hazman, et al., Efficacy of safranal to cisplatin-induced nephrotoxicity. *Biochem J*. 2017; 474(7): 1195-1203.

281. Kaya, C., R. Karabulut, Z. Turkyilmaz, et al., Lycopene has reduced renal damage histopathologically and biochemically in experimental renal ischemia-reperfusion injury. *Ren Fail*. 2015; 37(8): 1390-5.

282. Mahmoodnia, L., K. Mohammadi, and R. Masumi, Ameliorative effect of lycopene effect on cisplatin-induced nephropathy in patient. *J Nephropathol*. 2017; 6(3): 144-149.

283. Dai, C., S. Tang, S. Deng, et al., Lycopene attenuates colistin-induced nephrotoxicity in mice via activation of the Nrf2/HO-1 pathway. *Antimicrob Agents Chemother*. 2015; 59(1): 579-85.

284. George, M.D., N.K. McGill, and J.F. Baker, Creatine kinase in the U.S. population: Impact of demographics, comorbidities, and body composition on the normal range. *Medicine (Baltimore)*. 2016; 95(33): e4344.

285. Amin, K.A., B.M. Mohamed, M.A. El-Wakil, et al., Impact of breast cancer and combination chemotherapy on oxidative stress, hepatic and cardiac markers. *J Breast Cancer*. 2012; 15(3): 306-12.

286. Soffietti, R., E. Trevisan, and R. Ruda, Neurologic complications of chemotherapy and other newer and experimental approaches. *Handb Clin Neurol*. 2014; 121: 1199-218.

287. Topal, I., A. Ozbek Bilgin, F. Keskin Cimen, et al., The effect of rutin on cisplatin-induced oxidative cardiac damage in rats. *Anatol J Cardiol*. 2018; 20(3): 136-142.

288. Wang, J., D. He, Q. Zhang, et al., Resveratrol protects against Cisplatin-induced cardiotoxicity by alleviating oxidative damage. *Cancer Biother Radiopharm*. 2009; 24(6): 675-80.

289. Mohamadin, A.M., A.A. Elberry, A.D. Mariee, et al., Lycopene attenuates oxidative stress and heart lysosomal damage in isoproterenol induced cardiotoxicity in rats: A biochemical study. *Pathophysiology*. 2012; 19(2): 121-30.

290. Karimi, G., M. Ramezani, and A. Abdi, Protective effects of lycopene and tomato extract against doxorubicin-induced cardiotoxicity. *Phytother Res*. 2005; 19(10): 912-4.

291. Indu, R., T.S. Azhar, A. Nair, et al., Amelioration of doxorubicin induced cardio- and hepato-toxicity by carotenoids. *J Cancer Res Ther*. 2014; 10(1): 62-7.

292. Arunkumar, P.A., G.L. Viswanatha, N. Radheshyam, et al., Science behind cisplatin-induced nephrotoxicity in humans: a clinical study. *Asian Pac J Trop Biomed*. 2012; 2(8): 640-4.

293. Noori, S. and T. Mahboob, Sodium selenite attenuated cisplatin-induced toxicity in rats: role of electrolytes homeostasis. *Indian J Exp Biol*. 2011; 49(10): 791-4.

294. Bearcroft, C.P., P. Domizio, F.H. Mourad, et al., Cisplatin impairs fluid and electrolyte absorption in rat small intestine: a role for 5-hydroxytryptamine. *Gut*. 1999; 44(2): 174-9.

295. Francescato, H.D.C., L.F. Almeida, N.G. Reis, et al., Previous Exercise Effects in Cisplatin-Induced Renal Lesions in Rats. *Kidney Blood Press Res*. 2018; 43(2): 582-593.

296. Adeneye, A.A. and A.S. Benebo, Chemopreventive Effect of Tadalafil in Cisplatin-Induced Nephrotoxicity in Rats. *Niger J Physiol Sci*. 2016; 31(1): 1-10.

297. Komdeur, R., J. Derksen, M.C. Legdeur, et al., Cisplatin-induced hyperglycemic hyperosmolar coma. *Neth J Med*. 2007; 65(1): 36-7.

298. Nan, D.N., M. Fernandez-Ayala, M.E. Vega Villegas, et al., Diabetes mellitus following cisplatin treatment. *Acta Oncol*. 2003; 42(1): 75-8.

299. Abdullah, M.A.A., A.A.; Baker, S.A., A Biochemical Study of the Effect of Quercetin on Cisplatin Induced Rat Tissues Toxicity. *American Journal of Biochemistry*. 2018; 8(5): 87-92.

300. Portilla, D., S. Li, K.K. Nagothu, et al., Metabolomic study of cisplatin-induced nephrotoxicity. *Kidney Int*. 2006; 69(12): 2194-204.

301. Ryu, S.H., J.D. Lee, J.W. Kim, et al., (1)H NMR toxicometabolomics following cisplatin-induced nephrotoxicity in male rats. *J Toxicol Sci*. 2019; 44(1): 57-71.

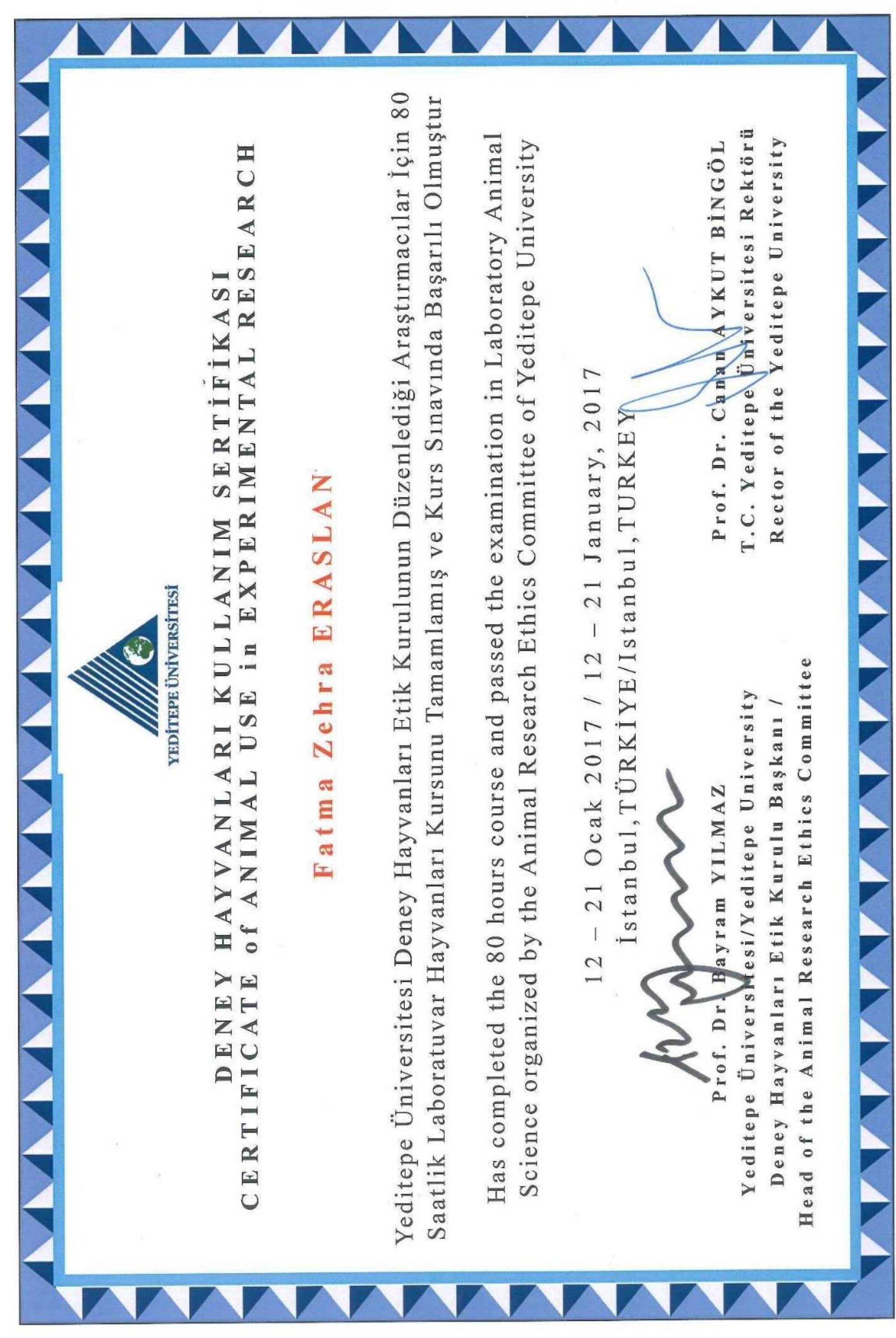
302. Daniel, E.E.M., A.; Tanko, Y.; Ahmed, A., Effect of Lycopene on Altered Kidney Antioxidant Enzymes Activity and Functions in Streptozotocin-Induced Diabetic Wistar Rats. *Cell Biology*. 2015; 3(1): 1-13.

303. Aydin, M. and S. ÇElİK, Effects of lycopene on plasma glucose, insulin levels, oxidative stress, and body weights of streptozotocin-induced diabetic rats. *Turkish Journal of Medical Sciences*. 2012; 42(Sup.2): 1406-1413.
304. Madias, N.E. and J.T. Harrington, Platinum nephrotoxicity. *Am J Med*. 1978; 65(2): 307-14.
305. Lieberthal, W., V. Triaca, and J. Levine, Mechanisms of death induced by cisplatin in proximal tubular epithelial cells: apoptosis vs. necrosis. *Am J Physiol*. 1996; 270(4 Pt 2): F700-8.
306. Yao, X., K. Panichpisal, N. Kurtzman, et al., Cisplatin nephrotoxicity: a review. *Am J Med Sci*. 2007; 334(2): 115-24.
307. Safirstein, R., P. Miller, and J.B. Guttenplan, Uptake and metabolism of cisplatin by rat kidney. *Kidney Int*. 1984; 25(5): 753-8.
308. Chen, Q., K. Yu, and J.L. Stevens, Regulation of the cellular stress response by reactive electrophiles. The role of covalent binding and cellular thiols in transcriptional activation of the 70-kilodalton heat shock protein gene by nephrotoxic cysteine conjugates. *J Biol Chem*. 1992; 267(34): 24322-7.
309. Goldstein, R.S. and G.H. Mayor, Minireview. The nephrotoxicity of cisplatin. *Life Sci*. 1983; 32(7): 685-90.
310. Ilic, S., N. Stojiljkovic, M. Veljkovic, et al., Protective effect of quercetin on cisplatin-induced nephrotoxicity in rats. *Medicine and Biology*. 2014; 16(2): 71-75.
311. Alhoshani, A.R., M.M. Hafez, S. Husain, et al., Protective effect of rutin supplementation against cisplatin-induced Nephrotoxicity in rats. *BMC Nephrol*. 2017; 18(1): 194.
312. Ma, X., L. Yan, Q. Zhu, et al., Puerarin attenuates cisplatin-induced rat nephrotoxicity: The involvement of TLR4/NF-kappaB signaling pathway. *PLoS One*. 2017; 12(2): e0171612.
313. Akca, G., H. Eren, L. Tumkaya, et al., The protective effect of astaxanthin against cisplatin-induced nephrotoxicity in rats. *Biomed Pharmacother*. 2018; 100: 575-582.

314. Pierine, D.T., M.E. Navarro, I.O. Minatel, et al., Lycopene supplementation reduces TNF-alpha via RAGE in the kidney of obese rats. *Nutr Diabetes*. 2014; 4: e142.
315. Loho, T. and A. Dharmayanti, Colistin: an antibiotic and its role in multiresistant Gram-negative infections. *Acta Med Indones*. 2015; 47(2): 157-68.
316. Augusti, P.R., G.M. Conterato, S. Somacal, et al., Effect of lycopene on nephrotoxicity induced by mercuric chloride in rats. *Basic Clin Pharmacol Toxicol*. 2007; 100(6): 398-402.
317. Atessahin, A., S. Yilmaz, I. Karahan, et al., Effects of lycopene against cisplatin-induced nephrotoxicity and oxidative stress in rats. *Toxicology*. 2005; 212(2-3): 116-23.
318. Soni, K.K., L.T. Zhang, J.H. You, et al., The effects of MOTILIPERM on cisplatin induced testicular toxicity in Sprague-Dawley rats. *Cancer Cell Int*. 2015; 15: 121.
319. Vawda, A.I., Effect of testosterone on cisplatin-induced testicular damage. *Arch Androl*. 1994; 32(1): 53-7.
320. Turk, G., A. Atessahin, M. Sonmez, et al., Lycopene protects against cyclosporine A-induced testicular toxicity in rats. *Theriogenology*. 2007; 67(4): 778-85.
321. Karale, S. and J.V. Kamath, Effect of daidzein on cisplatin-induced hematotoxicity and hepatotoxicity in experimental rats. *Indian J Pharmacol*. 2017; 49(1): 49-54.
322. Cagin, Y.F., M.A. Erdogan, N. Sahin, et al., Protective Effects of Apocynin on Cisplatin-induced Hepatotoxicity in Rats. *Arch Med Res*. 2015; 46(7): 517-26.
323. Joshi, S.S., F.M. Tadavi, M. T. A., et al., Study of evaluation of hepatoprotective potential of lycopene in rat models of paracetamol and antitubercular drugs (isoniazid + rifampicin) induced hepatotoxicity. *2017*. 2017; 6(7): 6.
324. Pinto, C., B. Rodríguez-Galdón, J.J. Cestero, et al., Hepatoprotective effects of lycopene against carbon tetrachloride-induced acute liver injury in rats. *Journal of Functional Foods*. 2013; 5(4): 1601-1610.
325. Veeramachaneni, S., L.M. Ausman, S.W. Choi, et al., High dose lycopene supplementation increases hepatic cytochrome P4502E1 protein and inflammation in alcohol-fed rats. *J Nutr*. 2008; 138(7): 1329-35.

7. Appendix

7.1. Certificate of Animal Use in Experimental Research



YEDİTEPE ÜNİVERSİTESİ

DENEY HAYVANLARI KULLANIM SERTİFİKASI

CERTIFICATE of ANIMAL USE in EXPERIMENTAL RESEARCH

Fatma Zehra ERASLAN

Yeditepe Üniversitesi Deney Hayvanları Etik Kurulunun Düzenlediği Araştırmacılar İçin 80 Saatlik Laboratuvar Hayvanları Kursunu Tamamlamış ve Kurs Sınavında Başarılı Olmuştur

Has completed the 80 hours course and passed the examination in Laboratory Animal Science organized by the Animal Research Ethics Committee of Yeditepe University

12 – 21 Ocak 2017 / 12 – 21 January, 2017

İstanbul, TÜRKİYE/Istanbul, TURKEY

Prof. Dr. Bayram YILMAZ
Yeditepe Üniversitesi/Yeditepe University
Deney Hayvanları Etik Kurulu Başkanı /
Head of the Animal Research Ethics Committee

Prof. Dr. Canan AYKUT BİNGÖL
T.C. Yeditepe Üniversitesi Rektörü
Rector of the Yeditepe University

7.2. Animal Research Ethics Committee Approval

T.C. YEDİTEPE ÜNİVERSİTESİ, DENEY HAYVANLARI ETİK KURULU
(YÜDHEK)
ETİK KURUL KARARI

Toplantı Tarihi	Karar No	İlgi	Proje Yürütücüsü
20.04.2018	663	10.04.2018	Prof. Dr. Erdem YEŞİLADA

"Sisplatin Toksisitesinde Likopen'in Etkilerinin Araştırılması" adlı bilimsel çalışma etik kurulumuzda görüşülmüş olup, çalışmanın etik kurallara uygun olduğuna oy birliğiyle karar verilmiştir.

Etik Onay Geçerlilik Süresi: 3 Yıl	**Hayvan Türü ve cinsiyeti:** Sıçan ♂	**Hayvan Sayısı:** 28

GÖREVİ	ADI SOYADI	
Başkan	Prof. Dr. Bayram YILMAZ	
Başkan Yardımcısı	Prof. Dr. Erdem YEŞİLADA	KATILMADI
Raportör	Vet. Hekim Engin SÜMER	
Üye	Prof. Dr. M. Ece GENÇ	
Üye	Prof. Dr. Rukset ATTAR	
Üye	Doç. Dr. Soner DOĞAN	
Üye	Doç. Dr. Ediz DENİZ	
Üye	Prof. Dr. Gamze TORUN KÖSE	KATILMADI
Üye	Doç. Dr. Aylin YABA UÇAR	
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Üye	Ahmet ŞENKARDEŞLER	

8. Curriculum Vıtae

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Medical Doctor	University of Health Science – Ümraniye Training and Research Hospital – İnternal Medicine Department	2017 - 2018
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Medical Doctor	Marmara University Pendik Training and Research Hospital – İnternal Medicine Department	2018 - 2019