



## Morin and Hesperidin Ameliorate Cisplatin-Induced Hepatotoxicity and Nephrotoxicity in Rats: A Histopathological Study

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### Article Info

Received: 08/09/2017  
Accepted: 14/03/2018

### Keywords

Cisplatin  
Hepatotoxicity  
Hesperidin  
Histopathology  
Morin  
Nephrotoxicity

### Abstract

In this study, we investigated that the histopathological changes of rat kidney and liver tissues after cisplatin administration and potential beneficial effects of morin and hesperidin administration. Wistar rats were randomly divided in 7 groups: control, morin (M), hesperidin (H), cisplatin (CP), cisplatin+morin (CP+M), cisplatin+hesperidin (CP+H), cisplatin+morin+hesperidin (CP+M+H). Kidney and liver tissues were collected at the end of the experiment and were evaluated histopathological changes. Various histopathological changes in kidney and liver tissues of cisplatin-induced group were revealed. However, pre- and co-treatment of morin and/or hesperidin partially prevented these hepatotoxic and nephrotoxic changes in cisplatin-induced groups.

## 1. INTRODUCTION

Cisplatin (CP, cis-diamminedichloroplatinum II) is using for antineoplastic drug in the treatment of cancer however in the previous studies researchers reported that cisplatin caused many toxic effects [1,2]. It is known that CP is a cytotoxic agent, and their cytotoxic effect is probably via its interaction with DNA that the formation of covalent adducts between certain DNA bases [3,4]. Nasr (2014) was showed that CP caused hepatotoxic effects on male rats when it was given 7,5 mg/kg i.p to rats. And it also through the alterations of liver biomarkers, lipid peroxidation biomarker (Malondialdehyde, MDA), antioxidant enzymes including catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx) and Glutathione-S-transferase (GSH) and histological parameters [3].

Some authors showed that CP has accumulative effect on some organs like kidney and liver [5]. CP elicits number of toxic effects including vestibular toxicity [6], reproductive toxicity [7,8], cardiotoxicity [9], hepatic dysfunction [3], teratogenic effect [10]. In previous studies, it is reported that CP causes oxidative stress [11]. The cells have different mechanisms to alleviate oxidative stress which is offered by enzymatic and non-enzymatic antioxidants. Antioxidants have been shown to scavenge reactive oxygen species (ROS) [12-14]. Studies have demonstrated that exposure to CP caused production of ROS, and it changes the levels of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), Glutathione-S-transferase (GST) and malondialdehyde (MDA) in different tissues of experimental animals [13].

It is known that many antioxidant compounds and also antioxidant enzymes in biological systems prevent cell and tissue damages against oxidative stress [14]. It is well known that superoxide dismutase (SOD) catalase and glutathione peroxidase are the major endogenous antioxidant enzymes which play role in

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prevention of oxidative injury however the other exogenous radical scavenging agents include vitamin C, vitamin E, quercetin, catechin, hesperidin, morin etc. [13-15].

Hesperidin (H) and Morin (M) are recognized as antioxidants which prevent cellular molecules from reactive oxygen species inducing damages on different biological processes for example anti-inflammatory activity, anti-cancer activity [16-18]. Hesperidin is one of the widely found flavonoid in citrus fruits and called sometimes "Vitamin P". Similarly, to all other flavonoids Hesperidin shows antioxidant activity via its chemical structure as this flavonoid derives its antioxidant property from a hydroxyl group. It acts as an antioxidant and proved by other investigator to have anti-cancer and anti-inflammatory because of its anti-oxidative activities [19, 20]. Morin is also known as an antioxidant and it also found in the several therapeutic herbs such as fruits, vegetables, tea [18].

In this study, we prefer liver and kidney because liver is the organ where activation and detoxification of xenobiotic takes place [15]. Kidneys are vital organs which play important roles in excreting waste products and in maintaining electrolyte and water balance in the [21]. Therefore, the aim of this study was to determine for histopathological changes in the kidney and liver of rats after exposure to cisplatin and to assess the protective potential of morin and hesperidin.

## 2. MATERIAL AND METHODS

### 2.1. Animals, Drugs and Chemicals

Male Wistar albino rats (n=42, 200–250 g) were prepared for the current study. All experimental procedures were approved by the Local Ethics Committee for Animal Experiments, Gazi University (G.Ü.ET-12.070). Morin, hesperidin and cisplatin were bought from Sigma (St. Louis, MO). Other chemicals (analytical grade or higher) used in experiment were bought from Sigma (St. Louis, MO) or Merck (Darmstadt, Germany).

### 2.2. Experimental Design

Rats were singly housed per cage at room temperature with free access to food and water (and in a 12/12 h cycle). Rats were divided into the seven groups, each containing six rats. Control group was given distilled water only for 10 consecutive days by oral gavage. In flavonoid administration groups, throughout 10 consecutive days, morin (50 mg/kg) and/or hesperidin (200 mg/kg) which prepared in distilled water were given to rats as a single daily dose by oral gavage (Group H, M, CP+H, CP+M, CP+H+M). Cisplatin which prepared in distilled water was injected intraperitoneally as a single dose (7 mg/kg) on the 4th day in cisplatin-induced groups (Group CP, CP+H, CP+M, CP+H+M). On the 11th day, all rats were sacrificed with intracardiac blood aspiration under ketamine/xylazine anesthesia (intramuscularly, 50 and 5 mg/kg, respectively).

### 2.3. Histopathological Evaluation

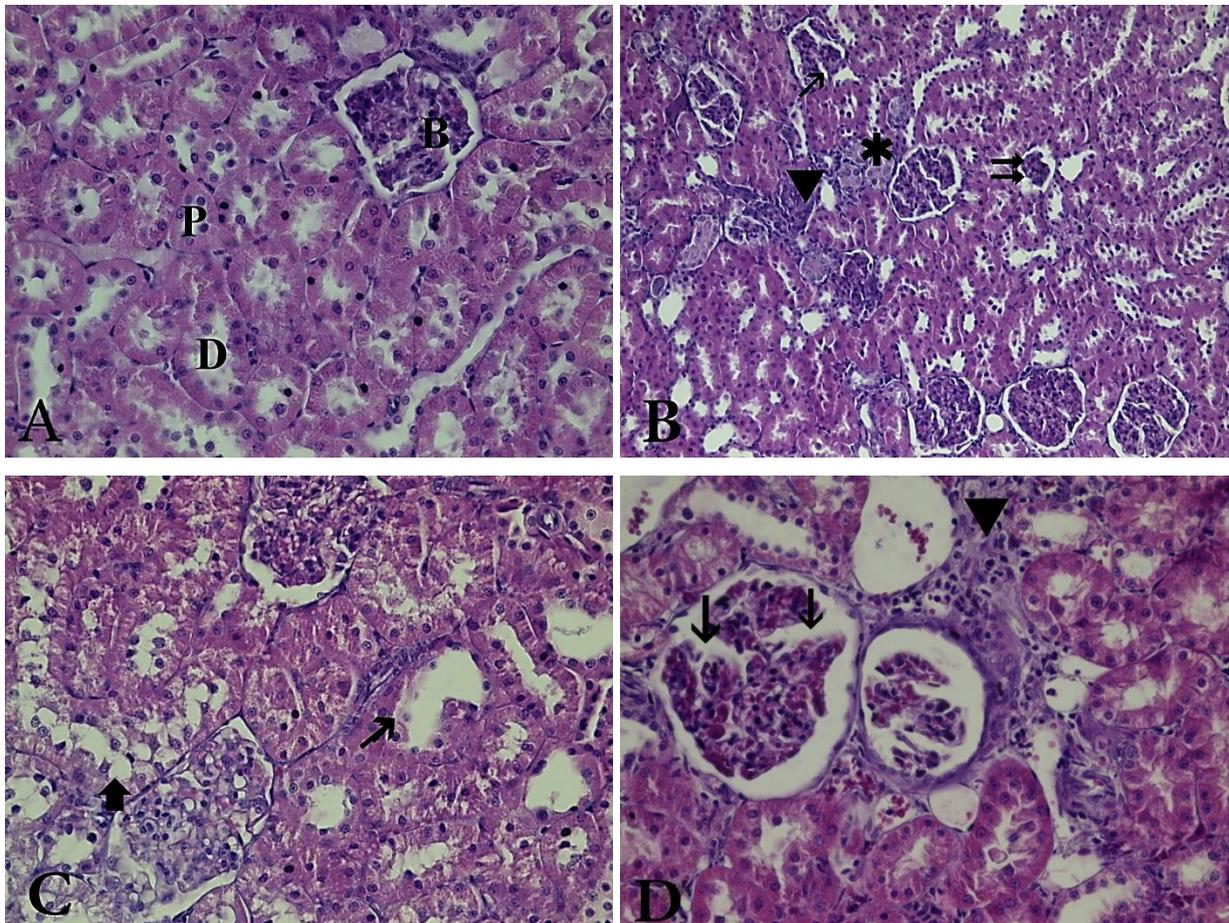
Rats were dissected and small portions of the kidney and livers were removed and placed into the fixative Bouin. After, tissue samples were dehydrated in ascending grades of ethanol and embedded in paraffin and paraffin block of the tissues were prepared. The specimens were cut in 6-7  $\mu$ m thick by using a microtome. Liver and kidney sections were stained with Hematoxylin-Eosin (H & E) by standard histological methodology. Then the tissues were evaluated under a light microscope (Olympus BX51, Tokyo, Japan) and photographed with a camera (Olympus E-330, Olympus Optical Co., Ltd., Japan). Ten slides were prepared from each kidney and liver tissues. Each kidney and liver slides were examined and assigned for severity of changes using scores on a scale of none (-), mild (+), moderate (++) and severe (+++) damage (Table 1 and 2).

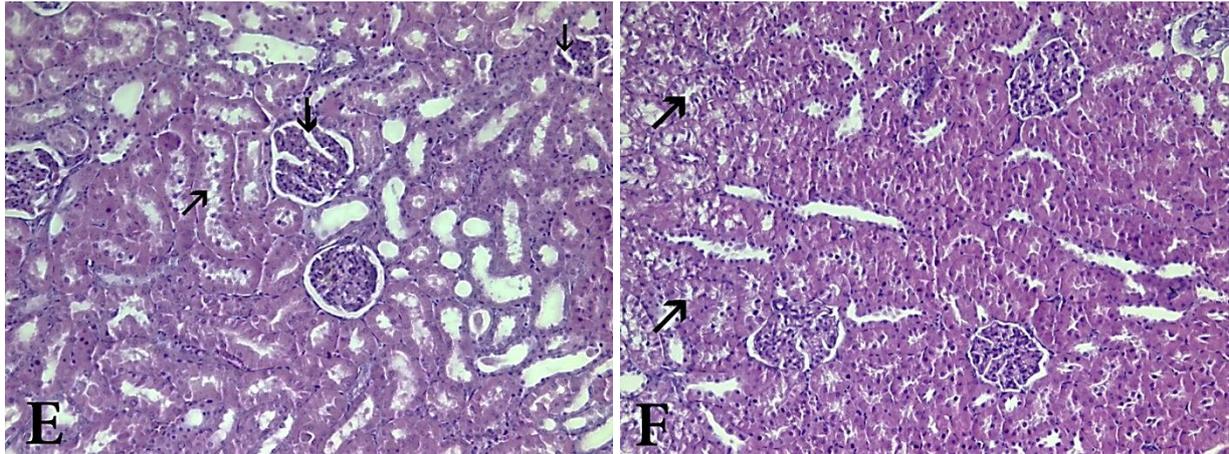
### 3. RESULTS

#### 3.1. Histopathological Results of Kidney and Liver Tissues

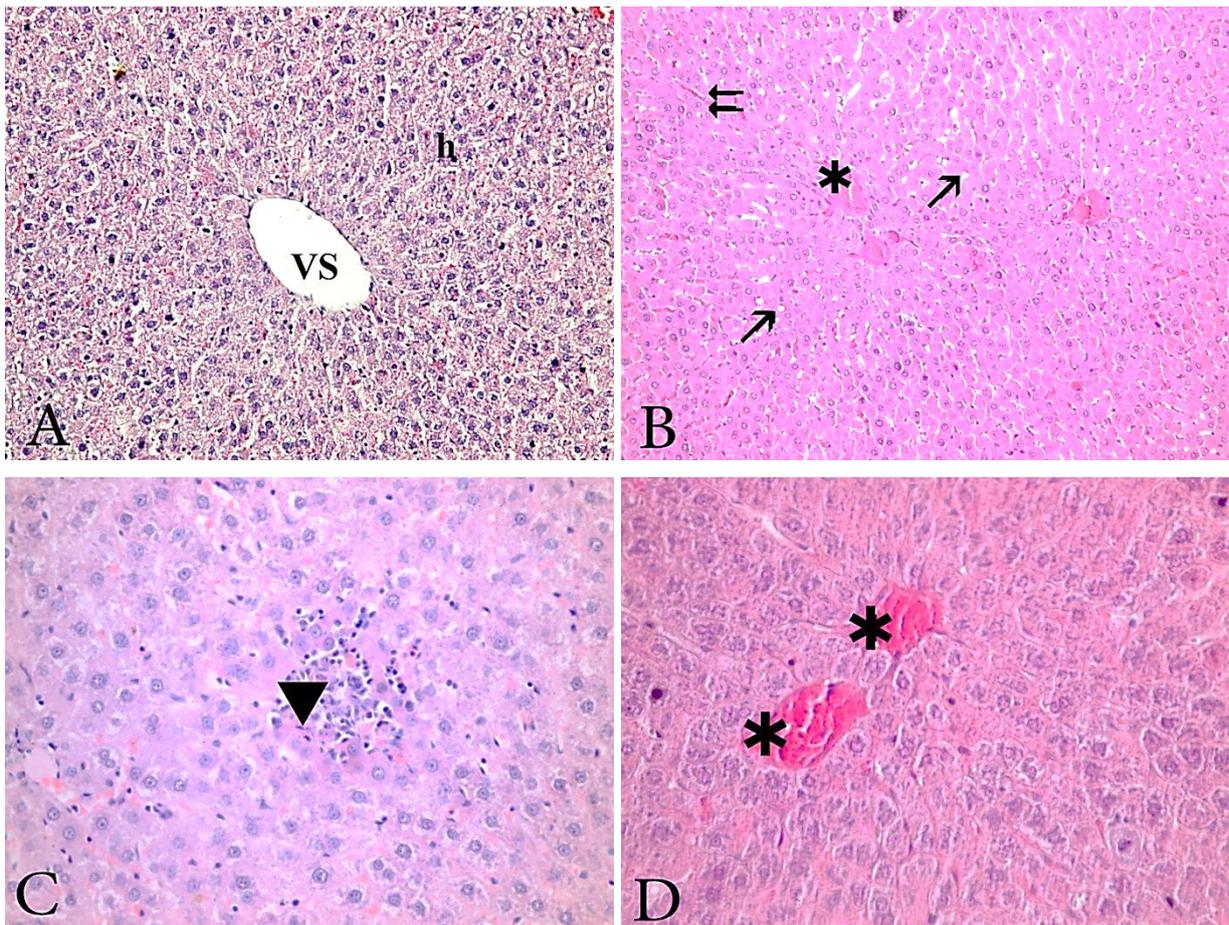
In this study histopathological examination of rat kidney and livers were investigated using light microscope. The histological examination of the kidney and liver tissues of the control, hesperidin and morin treated rats showed normal histological structure. Rats treated with cisplatin alone exhibited tubular degeneration, glomerular atrophy, glomerular lobulation and mononuclear cell infiltration in kidney tissues. In cisplatin+morin and cisplatin+hesperidin treated groups we showed that tubular degeneration in kidney tissues. We determined only tubular degeneration in cisplatin+morin+hesperidin treated rats (Figure 1 A-D).

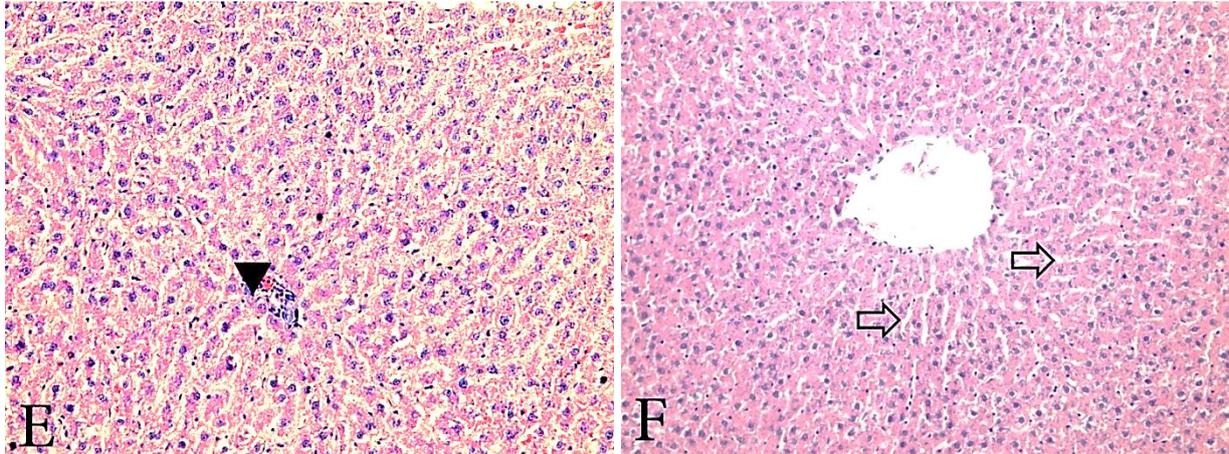
There were showed that vascular congestion, lipidosis, sinusoidal congestion, mononuclear cell infiltration in liver tissues cisplatin treated rats. In cisplatin+morin and cisplatin+hesperidin mononuclear cell infiltration and dilatation of sinusoids were shown. It was shown that only some dilatation of sinusoids in cisplatin+morin+hesperidin treated rats (Figure 2 A-D).





**Figure 1.** (A) Kidney sections of control rats P: proximal tubules, D: distal tubules, B: bowman capsule. (B-D) Kidney sections of cisplatin treated rats: glomerular lobulation (↓), glomerular atrophy (↓↓), necrosis (\*), mononuclear cell infiltration (▲), tubular degeneration (→), vacuolar degeneration (♣), (E) Kidney sections of cisplatin+morin/hesperidin treated rats: Tubular degeneration (→), glomerular lobulation (↓). (F) Kidney sections of cisplatin+morin+hesperidin treated rats, Tubular degeneration (→) X200, H&E.





**Figure 2.** (A) Liver sections of control rats VS: vena centralis, h:hepatosit. (B-D) Liver sections of cisplatin treated rats: vascular congestion (\*), lipidosis (→), sinusoidal congestion (↑↑), mononuclear cell infiltration (▲). (E) Liver sections of cisplatin+morin/hesperidin treated rats: Mononuclear cell infiltration (▲) (F) Liver sections of cisplatin+morin+hesperidin treated rats, dilatation of sinuzoids (⇨) X200, H&E.

**Table 1:** Grading of the histopathological changes in the kidney sections

Groups Pathology	Control	M	H	CP	CP+M	CP+H	CP+M+H
Glomerular atrophy	-	-	-	++	-	-	-
Glomerular lobulation	-	-	-	+++	++	++	-
Tubular degeneration	-	-	-	+++	++	++	+
Infiltration	-	-	-	++	-	-	-
Vacuolar degeneration	-	-	-	+	-	-	-
Necrosis	-	-	-	+	-	-	-

none (-), mild (+), moderate (++) and severe (+++) damage

**Table 2:** Grading of the histopathological changes in the liver sections

Groups Pathology	Control	M	H	CP	CP+M	CP+H	CP+M+H
Vascular congestion	-	-	-	+++	-	-	-
Lipidosis	-	-	-	+++	-	-	-
Sinusoidal congestion	-	-	-	+++	-	-	-
Infiltration	-	-	-	++	++	++	-
Dilatation of sinuzoids	-	-	-	+	-	-	++

none (-), mild (+), moderate (++) and severe (+++) damage

#### 4. DISCUSSION

Histopathological studies have been widely used as biomarkers for the toxicological investigations and also chemical toxicities [15]. It is reported that cisplatin causes many toxicological changes in experimental animals [2,6]. Similar to other studies, in this study we revealed that several histopathological changes such as mononuclear cell infiltration, dilatation of sinusoids in liver and also necrosis and glomerular atrophy in kidney by CP-induced. This evidence is in parallel with another study [5].

Tilyek *et al.* (2016) reported that CP is dose dependent and involves both apoptotic and necrotic cell death [22]. In the current study similarly we revealed that several histopathological changes because of the cellular damage. These effects of cisplatin on histopathological changes are in accordance with the results obtained by Omar *et al.*, 2016 who explained that liver morphology and also kidney morphology in cisplatin treated-rats was characterized [5, 22].

Oxidative stress occurs from an imbalance between the antioxidant defense systems and the formation of ROS which may be the most important reason of cisplatin-induced hepatotoxicity and nephrotoxicity [13]. Hence, in current study, oxidative stress may play a crucial role in cisplatin-mediated nephrotoxicity and hepatotoxicity. It is well known that lipid peroxidation is one of the main processes of oxidative damage, which plays a critical role in the toxicity of many xenobiotic [23]. These toxic effects probably occur through the generation of ROS causing damage to various membranous components of the cell which explains the mechanism of histopathological observation in this study [24]. It may have caused oxidative damage to disrupt the structure of cisplatin cells. Similarly, it is reported that increased oxidative stress in the kidney leads to deterioration of the renal function, inflammation, tubular degenerations and apoptosis [25, 26, 27].

Flavonoids have been shown to act as scavengers of various reactive oxygen species and also antioxidant activity. It is well known that flavonoids are naturally present in fruits and vegetables [28]. Morin and hesperidin have a variety beneficial effect, including anti-fibrotic, anti-inflammatory, ROS scavenging [16, 29]. It is demonstrated that flavonoids have protective effects against oxidative stress induced by xenobiotic [13,15]. Moreover, we observed that in this study, the morin and/or hesperidin pre- and co-treatment with cisplatin groups were mild hepatotoxic and nephrotoxic effects. These protective effects may be due to their anti-oxidative ability on the kidney and liver tissues.

Our results demonstrated that, intoxication with cisplatin-induced significant damage of the kidney and liver tissues. Besides, supplementation with hesperidin and morin during our experimental period partially ameliorates the toxic effect of cisplatin and its functions but not protect completely.

#### CONFLICTS OF INTEREST

No conflict of interest was declared by the authors.

#### REFERENCES

- [1] Radwan, R.R., and Abdel Fattah, S.M., "Mechanisms involved in the possible nephroprotective effect of rutin and low dose  $\gamma$  irradiation against cisplatin-induced nephropathy in rats", *Journal of Photochemistry and Photobiology B Biology*, 169: 56–62, (2017).
- [2] Shaik, A.N., Altomare, D.A., Lesko, L.J., and Trame, M.N., "Development and validation of a LC–MS/MS assay for quantification of cisplatin in rat plasma and urine", *Journal of Chromatography B*, 1046: 243–249, (2017).
- [3] 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", *Toxicology Reports*, 1: 682–691, (2014).

- [4] Yang, Z., Schumake, L.M., Egorin, M.J., E.G., Zuhowski, Z., and Guo, K.J., "Cisplatin preferentially binds mitochondrial DNA and voltage-dependent anion channel protein in the mitochondrial membrane of head and neck squamous cell carcinoma: possible role in apoptosis", *Clinical Cancer Research*, 12: 5817–5825, (2006).
- [5] Omar, H.A., Mohameda, W.R., Arafa, E.A., Shehata, B.A., El Sherbincy, G.A., Arab, H.H., and Elgendy, A.N., "Hesperidin alleviates cisplatin-induced hepatotoxicity in rats without inhibiting its antitumor activity", *Pharmacological Reports*, 68: 349–356, (2016).
- [6] Callejo, A., Durochat, A., Bressieux, S., Saleur, A., Chabbert, C., Juan, I.D., Llorens, J., and Gaboyard-Niay, S., "Dose-dependent cochlear and vestibular toxicity of trans-tympanic cisplatin in the rat", *NeuroToxicology*, 60: 1–9, (2017).
- [7] Heeba, G.H., Hamza, A.A., and Hassanin, S.O., "Induction of heme oxygenase-1 with hemin alleviates cisplatin-induced reproductive toxicity in male rats and enhances its cytotoxicity in prostate cancer cell line", *Toxicological Letters*, 264: 38–50, (2016).
- [8] Reddy, K.P., Madhu, P., and Reddy, P.S., "Protective effects of resveratrol against cisplatin-induced testicular and epididymal toxicity in rats", *Food and Chemical Toxicology*, 91: 65-72, (2016).
- [9] El-Awady, E.E., Moustafa, Y.M., Abo-Elmatty, D.M., and Radwan, A., "Cisplatin-induced cardiotoxicity: Mechanisms and cardioprotective strategies", *European Journal of Pharmacology*, 650: 335–341, (2011).
- [10] Hassan, M.S., Morgan, A.M., Mekawy, M.M., Zaki, A.R., and Ghazi, Z.M., "Teratogenic effect of cisplatin in rats and the protective role of sodium selenite", *Experimental Toxicology and Pathology*, 68: 277–287, (2016).
- [11] Szentmihályi, K., Maya, Z., Szénási, G., Máthé, C., Sebestény, A., Albert, M., Hegyi, G., and Blázovics, A., "Changes of essential element content and prevention of cisplatin induced oxidative stress in rat liver by CV247 administration", *European Journal of Integrative Medicine*, 8: 36–41, (2016).
- [12] Kılıc, C., Gülec-Peker, E.G., Acartürk, F., Sarı Kılıcaslan, S.M., and Coskun-Cevher, S., "Investigation of the effects of local glutathione and chitosan administration on incisional oral mucosal wound healing in rabbits", *Colloid Surface, B* 112: 499–507, (2013).
- [13] Kaltalioglu, K., and Coskun-Cevher, S., "Potential of morin and hesperidin in the prevention of cisplatin-induced nephrotoxicity", *Renal Failure*, 38: 1291–1299, (2016).
- [14] Apaydın, F.G., Bas, H., Kalender, S., and Kalender, Y., "Bendiocarb induced histopathological and biochemical alterations in rat liver and preventive role of vitamins C and E". *Environmental Toxicology and Pharmacology*, 49: 148–155, (2017).
- [15] Uzun, F.G., and Kalender, Y., "Chlorpyrifos induced hepatotoxic and hematologic changes in rats: The role of quercetin and catechin", *Food and Chemical Toxicology*, 55: 549-556, (2013).
- [16] Shehata, A.S., Amera, M.G., Abd El-Haleem, M.R., and Karam RA., "The ability of hesperidin compared to that of insulin for preventing osteoporosis induced by type I diabetes in young male albino rats: A histological and biochemical study", *Experimental Toxicology and Pathology*, 69: 203–212, (2017).
- [17] Zareei, S., Boojar, M.M.A., and Amanlou, M., "Inhibition of liver alanine aminotransferase and aspartate aminotransferase by hesperidin and its aglycone hesperetin: An in vitro and in silico study", *Life Sciences*, 178: 49–55, (2017).

- [18] Sultana, F., Neog, M.K., and Rasool, M.K., “Targeted delivery of morin, a dietary bioflavanol encapsulated mannosylated liposomes to the macrophages of adjuvant-induced arthritis rats inhibits inflammatory immune response and osteoclastogenesis”, *European Journal of Pharmaceutics and Biopharmaceutics*, 115: 229–242, (2017).
- [19] Khan, M.H.A., and Parvez, S., “Hesperidin ameliorates heavy metal induced toxicity mediated by oxidative stress in brain of Wistar rats”, *Journal of Trace Elements in Medicine and Biology*, 31: 53–60, (2015).
- [20] Mahmoud, A..M, Ashour, M.B., Abdel-Moneim, A., and Ahmed, O.M., “Hesperidin and naringin attenuate hyperglycemia-mediated oxidative stress and proinflammatory cytokine production in high fat fed/streptozotocin-induced type 2 diabetic rats”, *Journal of Diabetes and its Complications*, 26: 483–90, (2012).
- [21] Jung, K., An, J.M., Eom, D., Kang, K.S., and Kim, S., “Preventive effect of fermented black ginseng against cisplatin-induced nephrotoxicity in rats”, *Journal of Ginseng Research*, 41: 188-194, (2017).
- [22] Tilyek, A., Chai, C., Hou, X., Zhou, B., Zhang, C., Cao, Z., and Yu, B., “The protective effects of *Ribes diacanthum* Pallon cisplatin-induced nephrotoxicity in mice”, *Journal of Ethnopharmacology*, 178: 297–306, (2016).
- [23] Ongjanović, B.I., Marković, S.D., Đoršđević, N.Z., Trbojević, I.S., Štajn, A.Š., and Saičić, Z.S., “Cadmium-induced lipid peroxidation and changes in antioxidant defense system in the rat testes: Protective role of coenzyme Q10 and Vitamin E”, *Reproductive Toxicology*, 29: 191-197, (2010).
- [24] Elhalwagy, M.E.A., Darwish, N.S., and Zaher, E.M., “Prophylactic effect of green tea polyphenols against liver and kidney injury induced by fenitrothion insecticide”, *Pesticide Biochemistry and Physiology*, 91: 81-89, (2008).
- [25] Manucha, W., and Vallés, P.G., “Apoptosis modulated by oxidative stress and inflammation during obstructive nephropathy”, *Inflammation and Allergy - Drug Targets*, 11: 303-12, (2012).
- [26] Ozbek, E., “Induction of oxidative stress in kidney”, *International Journal of Nephrology*, 2012: 465-897, (2012).
- [27] Apaydin, F.G, Bas, H., Kalender, S., Kalender, Y., “Subacute effects of low dose lead nitrate and mercury chloride exposure on kidney of rats”, *Environmental Toxicology and Pharmacology*, 2016: 41:219-224.
- [28] Harborne, J.B., and Williams, C.A., “Advantages in flavonoid research since 1992”, *Phytochemistry*, 55: 481-504, (2000).
- [29] Ke, Y., Liu, C., Hao, J., Lu, L., Lu, N., Wu, Z., Zhu, S., and Chen, X., “Morin inhibits cell proliferation and fibronectin accumulation in rat glomerular mesangial cells cultured under high glucose condition”, *Biomedicine and Pharmacotherapy*, 84: 622–627, (2016).