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Original article

Alleviation of Cisplatin-Induced Hepatotoxic Damage: the Synergistic Effect of Morin and Hesperidin against Oxidative Stress

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Abstract

Background and objectives: A key aspect of cisplatin-induced hepatotoxicity is oxidative stress. The current study was conducted to show, for the first time, the restoring and synergistic effects of morin and hesperidin against oxidative stress in hepatotoxicity. **Methods:** Fourty-two Wistar albino rats were randomly divided into seven groups: group A (control), group B (morin), group C (hesperidin), group D (cisplatin), group E (cisplatin+morin), group F (cisplatin+hesperidin), group G (cisplatin+morin+hesperidin). Throughout ten consecutive days, morin and/or hesperidin were given to rats and cisplatin was injected as a single dose (7 mg/kg) on the 4th day, and then the rats were sacrificed on the 11th day. Liver tissue samples collected from the rats were used for the measurement of malondialdehyde, nitric oxide, glutathione levels as well as myeloperoxidase, catalase and superoxide dismutase activities. **Results:** Administration of cisplatin elevated the malondialdehyde and nitric oxide levels and also reduced the glutathione levels and catalase activity in the liver. However, in the morin and/or hesperidin groups, glutathione level and catalase activity were higher but malondialdehyde and nitric oxide levels and myeloperoxidase activity were lower than the cisplatin-induced group. **Conclusion:** Our results indicated that pretreatment with these flavonoids can be used as protective treatment for cisplatin-induced hepatotoxicity.

Keywords: Cisplatin; hepatotoxicity; hesperidin; morin; oxidative stress

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Introduction

Chemotherapy is an important type of the cancer treatment, and is very effective; however, chemotherapeutic agents cause adverse effects [1]. Cisplatin ,(cis-diamminedichloroplatinum (II)), is a common chemotherapeutic agent which acts by triggering the formation of interstrand crosslinks in DNA and can cause various toxicity in tissues of liver (hepatotoxicity) and kidney (nephrotoxicity), and nervous system (neurotoxicity) [2,3].

Oxidative stress plays a serious role in cisplatin-induced hepatotoxicity. The increase in generation of reactive oxygen species (ROS) or decrease in antioxidant activity leads to cell death and toxicity. Various studies have reported that cisplatin treatment increases malondialdehyde (MDA) levels, and decrases antioxidant capacity such as level of glutathione (GSH), and activities of catalase (CAT), glutathione peroxidase (GSH-Px) and superoxide dismutase (SOD) in liver [4-6]. Therefore, reduction of oxidative stress is very important for attenuation of cisplatin-induced hepatotoxicity.

In recent years, there has been an increasing interest in studies on the prevention of adverse effects of cisplatin. Various chemicals such as thymoquinone, vitamin C, molsidomine and zerumbone were tested for attenuation of

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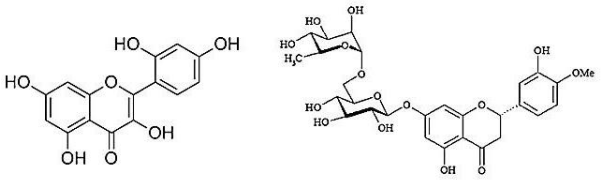
cisplatin-induced hepatotoxicity [6-10]. In addition, many natural products have shown hepatoprotective activity [11,12].

Morin (figure 1) is a naturally occurring flavonoid that occurs in various plants including white mulberry (*Morus alba*), golder (*Otostegia persica*), old fustic (*Maclura tinctoria*), osage orange (*Maclura pomifera*), onion and apple [13], which can protect numerous cell types such as cardiovascular cells [14,15], glomerular mesenchymal cells [16], hepatocytes [17], and neurons from damage caused by oxidative stress [18]. Morin shows various biological activities including antioxidant, antiinflammatory, and antiangiogenic properties [13].



Morin **Hesperidin**

Figure 1. The chemical structures of morin and hesperidin

In a study investigating the impact of morin, it was indicated that morin may exert preventive effect on cyclophosphamide-induced changes in the oxidative status in rat livers [19]. Hesperidin (figure 1) is one of the most important naturally occurring flavonoids in *Citrus* species [20] and its administration can decrease cholesterol level [21] and blood pressure [22] in rats. It has several pharmacological properties such as anticancer, antioxidant, anticarcinogenic and anti-inflammatory activities [20]. Additionally, Omar et al. [23] have suggested that hesperidin may alleviate cisplatin-induced hepatotoxicity in rats. In another study about hesperidin, it was stated that this flavonoid could have a protective effect on CCl_4-induced oxidative stress in the rat liver [24].

The aim of the present study was to explore, for the first time, the restoring and synergistic effects of morin+hesperidin combinations against oxidative stress in hepatotoxicity.

Material and Methods

Ethical considerations

Fourty-two male, Wistar albino rats (200-250 g) were prepared for the current study after receiving approval from the Local Ethics Commitee for Animal Experiments, Gazi University (2012, G.U.ET-12.070).

Drugs and chemicals

Morin, hesperidin, and cisplatin were bought from Sigma (St. Louis, USA). Other chemicals (analytical grade or higher) used in experiment were bought from Sigma (St. Louis, USA) or Merck (Germany).

Experimental design

The rats were singly housed per cage at room temperature with free access to food and water (in a 12/12 h cycle). They were fed with standard rat pellet. The rats were divided into seven groups (table 1).

Table 1. Experimental groups

	Cisplatin	Morin	Hesperidin
Group A (control) (n=6)	-	-	-
Group B (morin) (n=6)	-	+	-
Group C (hesperidin) (n=6)	-	-	+
Group D (cisplatin) (n=6)	+	-	-
Group E (cisplatin+morin) (n=6)	+	+	-
Group F (cisplatin+hesperidin) (n=6)	+	-	+
Group G (cisplatin+morin+hesperidin) (n=6)	+	+	+

All rats were divided into the seven groups. The administrations in the groups are indicated with the "+"

Control group was given distilled water only for ten consecutive days by oral gavage (group A). In flavonoid administration groups, throughout 10 consecutive days, morin (50 mg/kg) and/or hesperidin (200 mg/kg) were prepared in distilled water and given to the rats in single daily dose by oral gavage (group B, C, E, F, G) (table 1). The doses were determined considering the previous studies of Omar et al. [23], Lim et al. [25], and Shu et al. [26]. Cisplatin (prepared in distilled water) was injected intraperitoneally in single dose (7 mg/kg) on the 4^{th} day in the cisplatin-induced groups (group D, E, F, G). On the 11^{th} day, all rats were sacrificed with intracardiac blood aspiration (intracardiac puncture) under ketamine/xylazine anesthesia (intramuscularly, 50 and 5 mg/kg, respectively) [27,28]. The liver tissue samples were collected, frozen and kept at -30° C until assay.

Determination of biochemical parameters

Lipid peroxidation was determined spectrophotometrically according to the method detailed before [29] at 535 nm. This method is based on the production of malondialdehyde (MDA) which is an end product of lipid peroxidation. The production of MDA is determined by the thiobarbituric acid reactive

substances (TBARs) assay (MDA is reacted with thiobarbituric acid). Tetraethoxypropane solution was used as a standard for this assay. The reactive nitrogen oxide species (NOx) levels were determined spectrophotometrically at 540 nm by Griess reaction [30]. NOx are stable end products of nitric oxide. Sodium nitrite was used as the standard. Levels of glutathione (GSH) were measured spectrophotometrically according to a modified version of the Ellman method [31] at 412 nm. In this method, 5,5′-dithiobis (2-nitrobenzoic acid) (DTNB) reacts with reduced GSH to form a yellow-colored compound. Myeloperoxidase (MPO) activity was studied spectrophotometrically according to the procedure used by Schierwagen et al. [32] at 410 nm. The MPO activity towards O-dianisidine was assessed in the tissue samples. One unit of enzyme activity was expressed as the quantity of MPO activity in tissue that led to a difference in absorbance of 1/min at 37 °C. Catalase (CAT) activity was measured spectrophotometrically as detailed previously by Aebi [33] at 240 nm. H_2O_2 dismutation at 20 °C in phosphate buffer was assayed. One unit is expressed as the quantity of enzyme that reduces 1 µ/mol of H_2O_2/min. Superoxide dismutase (SOD) activity was detected spectrophotometrically according to the procedure used by Sun *et al*. [34] at 560 nm. In this procedure, the reduction of nitro blue tetrazolium (NBT) is generated by the xanthine/xanthine oxidase system. NBT reduction is used as an indicator of superoxide radical. One unit of SOD is expressed as the quantity of enzyme causing 50% inhibition of NBT reduction.

Statistical analysis

All results were expressed as the mean±standard deviation. One-way ANOVA (with post-hoc Tukey test) was used to compare mean differences (IBM SPSS Statistics ver 22). Results of $p<0.05$ were considered to be statistically significant.

Results and Discussion

Morin and hesperidin, naturally occurring flavonoids, are available in fruits and vegetables. The results of this study indicated that these flavanoids could be used as protective agents for cisplatin-induced hepatotoxicity.

The measurement of MDA is used as a marker of oxidative stress that is induced by lipid peroxidation in cells and tissues [35]. Our results showed that the level of MDA significantly increased in the cisplatin group (group D) compared with the control group (group A) ($p<0.05$) (table 2 and figure 2). These result support previous studies which were reported that MDA level increased by cisplatin in the rat liver [36,37]. In our study, administration of morin and/or hesperidin (group E, F, G) statistically decreased MDA levels when compared with the single use of cisplatin administration (group D) ($p<0.05$) (table 2 and figure 2). This result is in parallel with those of previous studies in terms of the effects of morin or hesperidin on MDA in liver and kidney [23,26,38,39]. Dual administration of morin+hesperidin (group G) statistically decreased MDA levels when compared with morin or hesperidin (group E and F) (table 2 and figure 2). Furthermore, there seems to be a synergistic effect of these flavonoids compared with single use of them. It can be an important result which indicates that morin and/or hesperidin pretreatment attenuate cisplatin-induced hepatotoxicity triggered by ROS. Also, our results revealed that cisplatin elevated NOx level in liver tissue ($p<0.05$) (table 2 and figure 3). In recent years, there has been increasing studies about toxicity of cisplatin in literature. It is proposed that nitric oxide is related to cisplatin-induced hepatotoxicity [40,41]. Nitric oxide is a highly reactive molecule synthesized by liver parenchymal and nonparenchymal cells from L-arginine via the induction of the inducible form of nitric oxide synthase (iNOS) [39]. Furthermore, several researchers have determined that cisplatin treatment causes a significant increase in the level of NO in liver tissue [4,23,42]. Our results are consistent with these studies. Additionally, increase in NOx and MDA happened simultaneously. Findings of the present study (elevations in the MDA and NOx levels) suggest a role for nitric oxide in promoting cisplatin-induced toxicity of liver. In our study, administration of morin and/or hesperidin significantly reduced NOx level in cisplatin-induced rats ($p<0.05$) (table 2 and figure 3). Xiaoting et al. reported that hesperidin treatment decreased iNOS expression in cultured rabbit retinal pigment epithelial cells [43]. In addition,Sakata *et al.* showed that hesperidin suppressed the expression of iNOS protein in mouse macrophage cells [44].

Table 2. Oxidant and antioxidant markers

	Control	Morin	Hesperidin	Cisplatin	Cisplatin+morin	Cisplatin+hesperidin	Cisplatin+morin+ hesperidin
MDA (nmol/g tissue)	27.09±3.95	24.39±3.06	31.80±3.21	49.88±3.72[a]	29.58±3.15[b]	28.37±3.77[b]	21.68±2.82[b,c,d]
NOx (µmol/g tissue)	1045.76±94.24	1082.88±104.69	1017.99±103.14	1808.01±106.97[a]	1169.67±67.62[b]	1197.31±110.21[b]	776.45±68.14[a,b,c,d]
GSH (µmol/g tissue)	12.70±0.86	13.27±0.22	11.80±0.38	5.24±0.21[a]	12.10±0.78[b]	12.42±0.75[b]	13.93±0.49[b]
MPO (U/mg protein)	31.66±3.81	41.55±3.89	50.72±4.72[a]	61.50±6.59[a]	58.67±8.49[a]	45.00±6.73[a,b,c]	62.22±6.79[a,c]
CAT (U/mg protein)	949.69±81.41	1018.12±75.09	1015.53±102.11	718.52±64.20[a]	902.22±88.34[b]	1255.86±22.86[a,b]	1146.82±83.70[a,b]
SOD (U/g tissue)	259.15±11.20	261.75±4.69	234.19±23.67	232.97±13.42	228.54±20.05	213.77±11.28	255.73±14.80

Each value represents the mean ± SD. One-way ANOVA (with post-hoc Tukey test) was used to compare mean differences. (a: p<0.05 as compared with the control group; b: p<0.05 as compared with the cisplatin group; c: <0.05 as compared with the cisplatin+morin group; d: p<0.05 as compared with the cisplatin+hesperidin group)

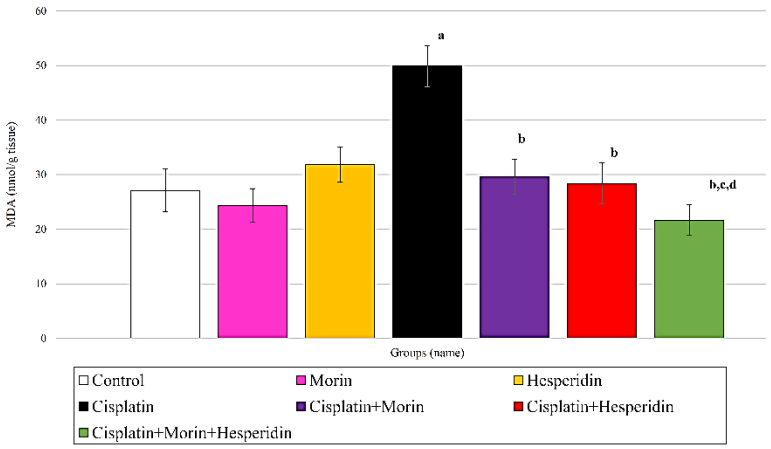


Figure 2. Levels of MDA in liver tissues. The administration of morin and/or hesperidin statistically decreased MDA levels when compared with the single use of cisplatin administration; a:p<0.05 as compared with the control group; b: p<0.05 as compared with the cisplatin group; c:-<0.05 as compared with the cisplatin+morin group; d: p<0.05 as compared with the cisplatin+hesperidin group

Similarly, Chen *et al*. determined that the production of NO and also the expression of iNOS were suppressed by the administration of morin in chondrocytes [45]. In the same way, Dishara *et al*. reported that morin downregulates nitric oxide in microglial cells [46]. Moreover, dual supplementation of morin and hesperidin (group G) has become more effective than single use of them (group E and F) (p<0.05) (table 2 and figure 3). Therefore, it can be concluded that these flavonoids suppress the production of NOx in liver tissue.

The GSH levels statistically decreased with the administration of cisplatin (group D) compared with the control group (group A) (p<0.05) (table 2 and figure 4). GSH is an essentials non-enzymatic antioxidant decreased by cisplatin administration in liver tissue in our study and also in several others [6,23]. However, administration of morin and/or hesperidin (group E, F, G) significantly increased the level of GSH (table 2 and figure 4). On the other hand, this cisplatin-induced decrease was restored by morin and/or hesperidin pretreatment in our study. Morin and hesperidin may increase the level of GSH in various cell and tissues [19,23,24,47]. Our results indicated that pretreatment of cisplatin-induced rats with morin and/or hesperidin increased the GSH level in liver.

As shown in figure 4, the activity of MPO significantly increased in the cisplatin group (group D) compared with control group (group A) (p<0.05) (table 2 and figure 5). MPO catalyses the formation of ROS and is produced by neutrophils and shows antimicrobial activity [48]. On the contrary, our hesperidin pretreatment (group F) reduced MPO activity compared with cisplatin-induced group (group D) (p<0.05) (table 2 and figure 5). Sing et al. and Sahu *et al.* reported that morin hydrate or hesperidin may inhibit MPO activity in liver and kidney tissues, respectively [26,38].

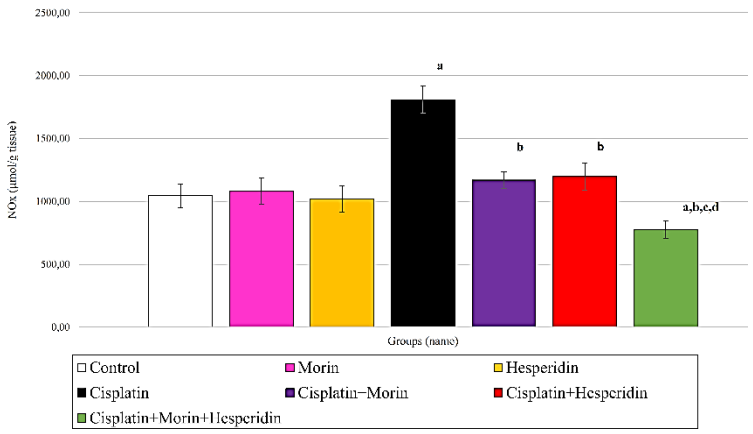


Figure 3. Levels of NOx in liver tissues. The administration of morin and/or hesperidin significantly reduced NOx level in cisplatin-induced rats; a: p<0.05 as compared with the control group; b: p<0.05 as compared with the cisplatin group; c:<0.05 as compared with the cisplatin+morin group; d: p<0.05 as compared with the cisplatin+hesperidin group

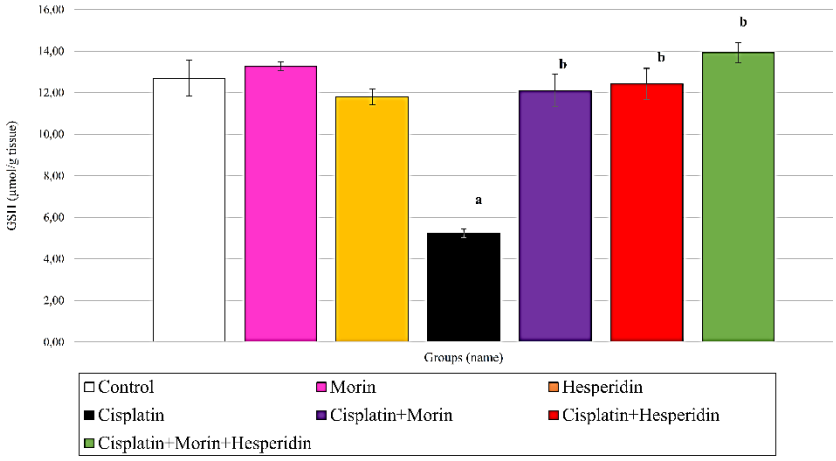


Figure 4. Levels of GSH in liver tissues**.** The administration of morin and/or hesperidin significantly increased the level of GSH; a: p<0.05 as compared with the control group; b: p<0.05 as compared with the cisplatin group; c: <0.05 as compared with the cisplatin+morin group; d: p<0.05 as compared with the cisplatin+hesperidin group

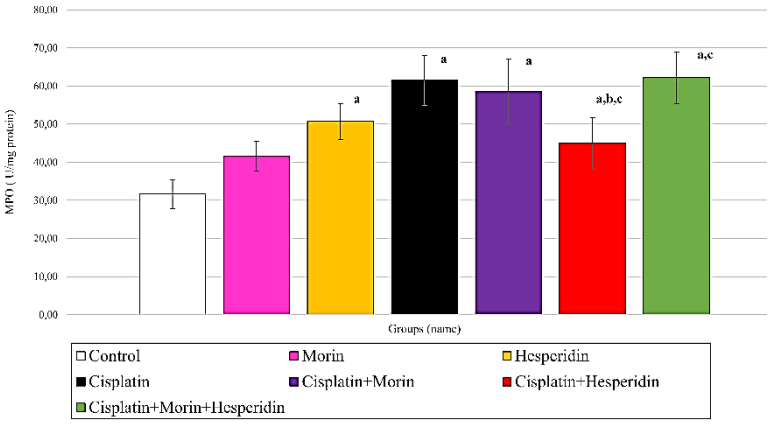


Figure 5. Activity of MPO in liver tissues. The hesperidin administration reduced MPO activity compared with cisplatin-induced group ; a: p<0.05 as compared with the control group; b: p<0.05 as compared with the cisplatin group, c: <0.05 as compared with the cisplatin+morin group; d: p<0.05 as compared with the cisplatin+hesperidin group

Catalase (CAT) activities in the liver tissue in rats were assessed. There was a significant ($p<0.05$) decline in the CAT activity of cisplatin-induced group (group D) compared to the control group (group A) (table 2 and figure 6). Catalase is an enzyme found in various tissues, especially in liver and kidney, and it degrades H_2O_2. It plays an important role in protecting the cell from ROS-induced damage [49]. Cetin *et al.* have reported that cisplatin treatment decreases CAT activity in liver tissue [50]. Our results showed that pretreatment of these flavonoids increases the activities of CAT in cisplatin-induced rats. The activities of CAT significantly increased in the morin and/or hesperidin groups (group E, F, G) compared with cisplatin group (group D) ($p<0.05$) (table 2 and figure 6). Several studies have reported that morin or hesperidin may increase CAT activity in kidney [39,51]. According to our results, morin and/or hesperidin increase the CAT activity in cisplatin-induced rats.

No significant changes were detected among all groups in terms of SOD activity ($p>0.05$) (table 2 and figure 7).

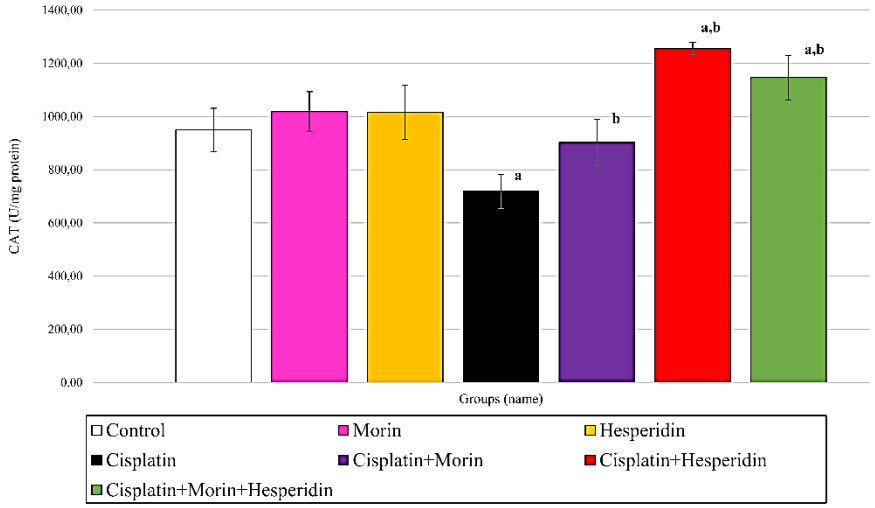


Figure 6. Activity of CAT in liver tissues.The activities of CAT significantly increased in the morin and/or hesperidin groups compared with cisplatin group; a: $p<0.05$ as compared with the control group; b: $p<0.05$ as compared with the cisplatin group; c: <0.05 as compared with the cisplatin+morin group; d:$p<0.05$ as compared with the cisplatin+hesperidin group

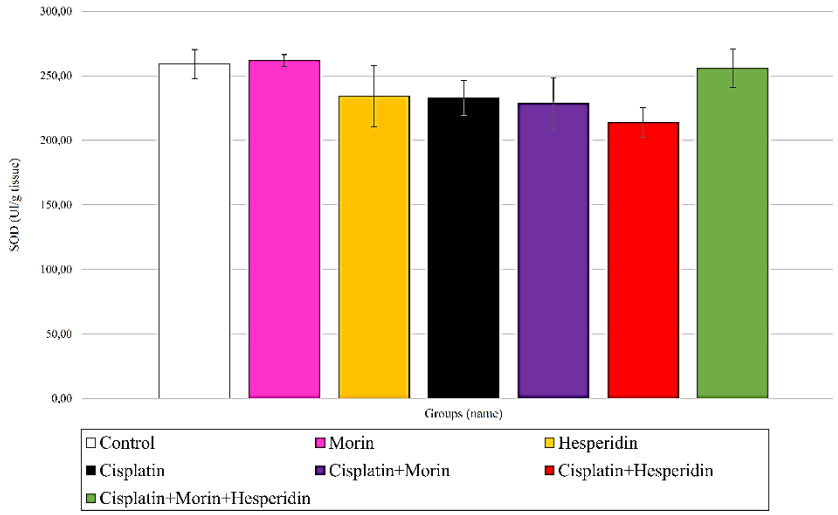


Figure 7. Activity of SOD in liver tissues No significant changes were detected among all groups in terms of SOD activity; a: $p<0.05$ as compared with the control group; b: $p<0.05$ as compared with the cisplatin group; c: <0.05 as compared with the cisplatin+morin group; d: $p<0.05$ as compared with the cisplatin+hesperidin group

Several reports have shown that many bioactive molecule acts as potent antioxidants in hepatotoxicity. For example, Niu et al. [52] reported that application of hyperine, a flavonoid occurring in plants, decreased lipid peroxidation in the cisplatin-induced liver. In the same way, Omar et al. showed that hesperidin significantly reduced MDA and NO and increased GSH in liver [23]. Similarly, Omar et al. determined that tangeretin, a pentamethoxyflavone flavone, decreased MDA and NO, and as a result it alleviated cisplatin-induced acute hepatic injury [53]. Considering our study and the above mentioned toxicology studies, it can be concluded that phenolics obtained from natural plants are beneficial for alleviation of hepatotoxicity.

Overall, the present study strengthens the idea that flavonoids, especially morin and hesperidin, can protect cisplatin-induced liver from damage caused by oxidative stress. The MDA and NOx levels and MPO activity of the cisplatin-induced liver tissues significantly decreased while the GSH level and CAT activity significantly increased as a result of morin and/or hesperidin pretreatment which suggest them to be used as protective agents for cisplatin-induced hepatotoxicity.

Author contributions

Kaan Kaltalioglu and Sule Coskun-Cevher conceived and designed the study; Kaan Kaltalioglu and Sule Coskun-Cevher performed the experiments; Kaan Kaltalioglu, Barbaros Balabanli and Sule Coskun-Cevher analyzed the data and wrote the paper.

Declaration of interest

The authors declare that there is no conflict of interest. The authors alone are responsible for the content of the paper.

References

[1] Gehdoo RP. Anticancer chemotherapy and it's anaesthetic implications (Current Concepts). *Indian J Anaesth.* 2009; 53(1): 18-29.

[2] Kidera Y, Kawakami H, Sakiyama T, Okamoto K, Tanaka K, Takeda M, Kaneda H, Nishina S, Tsurutani J, Fujiwara K, Nomura M, Yamazoe Y, Chiba Y, Nishida S, Tamura T, Nakagawa K. Risk factors for cisplatin-induced nephrotoxicity and potential of magnesium supplementation for renal protection. *PLoS One.* 2014. Article ID e101902.

[3] Ruggiero A, Rizzo D, Trombatore G, Maurizi P, Riccardi R. The ability of mannitol to decrease cisplatin-induced nephrotoxicity in children: real or not?. *Cancer Chemother Pharmacol.* 2015; 77(1): 19-26.

[4] Yilmaz HR, Sogut S, Ozyurt B, Ozugurlu F, Sahin S, Isik B, Uz E, Ozyurt H. The activities of liver adenosine deaminase, xanthine oxidase, catalase, superoxide dismutase enzymes and the levels of malondialdehyde and nitric oxide after cisplatin toxicity in rats: protective effect of caffeic acid phenethyl ester. *Toxicol Ind Health.* 2005; 21(3-4): 67-73.

[5] Lu Y, Cederbaum AI. Cisplatin-induced hepatotoxicity is enhanced by elevated expression of cytochrome P450 2E1. *Toxicol Sci.* 2006; 89(2): 515-523.

[6] Al-Malki AL, Sayed AAR. Thymoquinone attenuates cisplatin-induced hepatotoxicity via nuclear factor kappa- β. *BMC Complement Altern Med.* 2014; Aricle ID 282.

[7] De Martinis BS, Bianchi MD. Effect of vitamin C supplementation against cisplatin-induced toxicity and oxidative DNA damage in rats. *Pharmacol Res*. 2001; 44(4): 317-320.

[8] Ibrahim MY, Abdul AB, Wahab SIA, Elhassan MM, Alzubairi AS, Syam MM. Attenuation of cisplatin induced hepatotoxicity in rats using zerumbone. *Res J Biol Sci.* 2009; 4(7): 777-784.

[9] Ahmad RM, Al-Jawary AH. Effect of vitamin C on the hepatotoxicity induced by cisplatin in rats. *Raf J Sci*. 2012; 23(2): 23-33.

[10] Bentli R, Parlakpinar H, Polat A, Samdanci E, Sarihan ME, Sagir M. Molsidomine prevents cisplatin-induced hepatotoxicity. *Arch Med Res.* 2013; 44(7): 521-528.

[11] Ibrahim NA, El-Seedi HR, Mohammed MMD. Phytochemical investigation and hepatoprotective activity of *Cupressus sempervirens* L. leaves growing in Egypt. *Nat Prod Res.* 2007; 21(10): 857-866.

[12] Madrigal-Santillán E, Madrigal-Bujaidar E, Álvarez-González I, Sumaya-Martínez MT, Gutiérrez-Salinas J, Bautista M, Morales-

González A, García-Luna Y, González-Rubio M, Aguilar-Faisal JL, Morales-González JA. Review of natural products with hepatoprotective effects. *World J Gastroenterol*. 2014; 20(40): 14787-14804.

[13] Yue M, Zeng N, Xia Y, Wei Z, Dai Y. Morin exerts anti-arthritic effects by attenuating synovial angiogenesis via activation of peroxisome proliferator activated receptor-gama. *Mol Nutr Food Res*. 2018; Article ID e1800202.

[14] Wu TW, Zeng LH, Wu J, Fung KP. Morin: a wood pigment that protects three types of human cells in the cardiovascular system against oxyradical damage. *Biochem Pharmacol*. 1994; 47(6): 1099-1103.

[15] Kok LDS, Wong YP, Wu TW, Chan HC, Kwok TT, Fung KP. Morin hydrate: a potential antioxidant in minimizing the free-radicals- mediated damage to cardiovascular cells by anti-tumor drugs. *Life Sci*. 2000; 67(1): 91-99.

[16] Zeng LH, Fung KP, Wu TW. Morin hydrate protects cultured rat glomerular mesangial cells against oxyradical damage. *Life Sci*. 1994; 55(18): 351-357.

[17] Sivaramakrishnan V, Shilpa PNM, Praveen Kumar VR, Niranjali Devaraj S. Attenuation of N-nitrosodiethylamine-induced hepatocellular carcinogenesis by a novel flavonol-Morin. *Chem Biol Interact*. 2008; 171(1): 79-88.

[18] Gottlieb M, Leal-Campanario R, Campos-Esparza MR, Sanchez-Gomez MV, Alberdi E, Arranz A, Delgado-García JM, Gruart A, Matute C. Neuroprotection by two polyphenols following excitotoxicity and experimental ischemia. *Neurobiol Dis*. 2006; 23(2): 374-386.

[19] Merwid-Lad A, Trocha M, Chlebda-Sieragowska E, Sozański T, Szandruk M, Magdalan J, Ksiadzyna D, Piesniewska M, Fereniec-Golebiewska L, Kwiatkowska J, Szelag A. The impact of morin, a natural flavonoid, on cyclophosphamide-induced changes in the oxidative stress parameters in rat livers. *Adv Clin Exp Med*. 2014; 23(4): 505-509.

[20] Devi KP, Rajavel T, Nabavi SF, Setzer WN, Ahmadi A, Mansouri K, Nabavi SM. Hesperidin: a promising anticancer agent from nature. *Ind Crops Prod*. 2015; 76: 582-589.

[21] Kim HK, Jeong TS, Lee MK, Park YB, Choi MS. Lipid-lowering efficacy of hesperetin metabolites in high-cholesterol fed rats. *Clin Chim Acta*. 2003; 327(1): 129-137.

[22] Yamamoto M, Suzuki A, Jokura H, Yamamoto N, Hase T. Glucosyl hesperidin prevents endothelial dysfunction and oxidative stress in spontaneously hypertensive rats. *Nutrition*. 2008; 24(5): 470-476.

[23] Omar HA, Mohamed WR, Arafa ESA, Shehata BA, El Sherbiny GA, Arab HH, Nasser A, Elgendy AM. Hesperidin alleviates cisplatin-induced hepatotoxicity in rats without inhibiting its antitumor activity. *Pharmacol Reports*. 2016; 68(2): 349-356.

[24] Tirkey N, Pilkhwal S, Kuhad A, Chopra K. Hesperidin, a citrus bioflavonoid, and decreases the oxidative stress produced by carbon tetrachloride in rat liver and kidney. *BMC Pharmacol*. 2005; Article ID 15683547.

[25] Lim SC, Im YB, Bae CS, Han SI, Kim SE, Han HK. Protective effect of morin on the imipenem-induced nephrotoxicity in rabbits. *Arch Pharm Res*. 2008; 31(8): 1060-1065.

[26] Sahu BD, Kuncha M, Sindhura GJ, Sistla R. Hesperidin attenuates cisplatin-induced acute renal injury by decreasing oxidative stress, inflammation and DNA damage. *Phytomedicine*. 2013; 20(5): 453-460.

[27] Éboli LP, Netto AA, Azevedo RA, Lanzoni VP, Paula TS, Goldenberg A, Gonzalez AM. Evaluating the best time to intervene acute liver failure in rat models induced by d-galactosamine. *Acta Cirurgica Brasileira*. 2016; 31(12): 783-792.

[28] Dindar B, Kaltalioglu K, Coskun-Cevher S. Effect of dual growth factor administration on oxidative markers during acute stage wound healing in rats. *Turk J Zool*. 2017; 41: 841-847.

[29] Casini AF, Ferrali M, Pompella A, Maellaro E, Comporti M. Lipid peroxidation and cellular damage in extrahepatic tissues of bromobenzene-intoxicated mice. *Am J Pathol*. 1986; 123(3): 520-531.

[30] Miranda KM, Espey MG, Wink DA. A rapid, simple spectrophotometric method for simultaneous detection of nitrate and nitrite. *Nitric Oxide*. 2001; 5(1): 62-71.

[31] Aykac G, Uysal M, Yalcin AS, Kocak-Toker N, Sivas A, Oz H. The effect of chronic ethanol ingestion on hepatic lipid

peroxide, glutathione, glutathione peroxidase and glutathione transferase in rats. *Toxicol.* 1985; 36(1): 71-76.
[32] Schierwagen C, Bylund-Fellenius AC, Lundberg C. Improved method for quantification of tissue PMN accumulation measured by myeloperoxidase activity. *J Pharmacol Methods.* 1990; 2(3): 179-186.
[33] Aebi H. Catalase in vitro. *Methods Enzymol.* 1984; 105: 121-126.
[34] Sun Y, Oberley LW, Li Y. A simple method for clinical assay of superoxide dismutase. *Clin Chem.* 1988; 34(3): 497-500.
[35] Siddique YH, Ara G, Afzal M. Estimation of lipid peroxidation induced by hydrogen peroxide in cultured human lymphocytes. *Dose Response.* 2012; 10(1): 1-10.
[36] Avci A, Çetin R, Ergüder IB, Devrim E, Kiliçoglu B, Çandir Ö, Ozturk HS, Durak I. Cisplatin causes oxidation in rat liver tissues: possible protective effects of antioxidant food supplementation. *Turk J Med Sci.* 2008; 38(2): 117-120.
[37] Yu YN, Chen H, Li Y. Effect of Bicyclol on cisplatin-induced hepatotoxicity in the hepatocarcinoma 22 tumour-bearing mice. *Basic Clin Pharmacol Toxicol.* 2009; 104(4): 300-305.
[38] Singh MP, Jakhar R, Kang SC. Morin hydrate attenuates the acrylamide-induced imbalance in antioxidant enzymes in a murine model. *Int J Mol Med.* 2015; 36(4): 992-1000.
[39] Kaltalioglu K, Coskun-Cevher S. Potential of morin and hesperidin in the prevention of cisplatin-induced nephrotoxicity. *Ren Fail.* 2016; 38(8): 1291-1299.
[40] Gardner CR, Heck DE, Yang CS, Thomas PE, Zhang XJ, DeGeorge GL, Laskin JD, Laskin DL. Role of nitric oxide in acetaminophen-induced hepatotoxicity in the rat. *Hepatology*. 1998; 27(3): 748-754.
[41] Carnovale CE, Ronco MT. Role of nitric oxide in liver regeneration. *Ann Hepatol.* 2012; 11(5): 6366-6447.
[42] Srivastava RC, Farookh A, Ahmad N, Misra M, Hasan SK, Husain MM. Evidence for the involvement of nitric oxide in cisplatin-induced toxicity in rats. *Biometals.* 1996; 9(2): 139-142.
[43] Xiaoting L, Xiangyun Z, Shumei L, Minghua D, Liang X. Effect of hesperidin on expression of inducible nitric oxide synthase in cultured rabbit retinal pigment epithelial cells. In: Anderson R, Hollyfield J, LaVail M, Eds. Retinal degenerative diseases: advances in experimental medicine and biology. New York: Springer, 2010.
[44] Sakata K, Hirose Y, Qiao Z, Tanaka T, Mori H. Inhibition of inducible isoforms of cyclooxygenase and nitric oxide synthase by flavonoid hesperidin in mouse macrophage cell line. *Cancer Lett.* 2003; 199(2): 139-145.
[45] Chen WP, Wang YL, Tang JL, Hu PF, Bao JP, Wu LD. Morin inhibits interleukin-1β-induced nitric oxide and prostaglandin E2 production in human chondrocytes. *Int Immunopharmacol.* 2012; 12(2): 447-452.
[46] Dilshara MG, Jayasooriya RGPT, Lee S, Choi YH, Kim GY. Morin downregulates nitric oxide and prostaglandin E2 production in LPS-stimulated BV2 microglial cells by suppressing NF-κB activity and activating HO-1 induction. *Environ Toxicol Pharmacol.* 2016; 44: 62-68.
[47] Subash S, Subramanian P. Morin a flavonoid exerts antioxidant potential in chronic hyperammonemic rats: a biochemical and histopathological study. *Mol Cell Biochem.* 2009; 327(1-2): 153-161.
[48] Klebanoff SJ. Myeloperoxidase: friend and foe. *J Leukoc Biol.* 2005; 77(5): 598-625.
[49] Ma SF, Nishikawa M, Hyoudou K, Takahashi R, Ikemura M, Kobayashi Y, Yamashita F, Hashida M. Combining cisplatin with cationized catalase decreases nephrotoxicity while improving antitumor activity. *Kidney Int*. 2007; 72(12): 1474-1482.
[50] Cetin R, Devrim E, Kilicoglu B, Avci A, Candır O, Durak I. Cisplatin impairs antioxidant system and causes oxidation in rat kidney tissues: possible protective roles of natural antioxidant foods. *J Appl Toxicol.* 2006; 26(1): 42-46.
[51] Siddiqi A, Nafees S, Rashid S, Sultana S. Hesperidin ameliorates trichloroethylene-induced nephrotoxicity by abrogation of oxidative stress and apoptosis in wistar rats. *Mol Cell Biochem*. 2015; 406(1-2): 9-20.
[52] Niu C, Ma M, Han X, Wang Z, Li H. Hyperin protects against cisplatin-induced liver injury in mice. *Acta Cir Bras*. 2017; 32(8): 633-640.
[53] Omar HA, Mohamed WR, Arab HH, Arafa ESA. Tangeretin alleviates cisplatin-induced acute hepatic injury in rats: targeting MAPKs

and apoptosis. *PLoS One*. 2016; Article ID e0151649.

Abbreviations

CAT: catalase; DTNB: 5,5′-dithiobis (2-nitrobenzoic acid); GSH: glutathione; GSH-Px: glutathione peroxidase; MDA: malondialdehyde; MPO: myeloperoxidase; NBT: nitro blue tetrazolium; NOx: nitric oxide; ROS: reactive oxygen species; SOD: superoxide dismutase; TBARs: thiobarbituric-acid reactive substances