



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:** Forty-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 decreases 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*), goldler (*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].

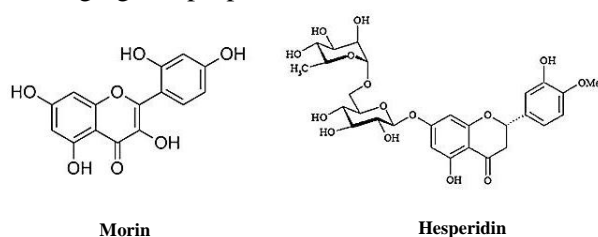


Figure 1. The chemical structures of morin and hesperidin

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

Forty-two male, Wistar albino rats (200-250 g) were prepared for the current study after receiving approval from the Local Ethics Committee 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 4th day in the cisplatin-induced groups (group D, E, F, G). On the 11th 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 (NO_x) levels were determined spectrophotometrically at 540 nm by Griess reaction [30]. NO_x 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. H₂O₂ dismutation at 20 °C in phosphate buffer was assayed. One unit is expressed as the quantity of enzyme that reduces 1 μmol of H₂O₂/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 NO_x 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 NO_x and MDA happened simultaneously. Findings of the present study (elevations in the MDA and NO_x 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 NO_x 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 ^{ab}	1146.82±83.70 ^{ab}
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)

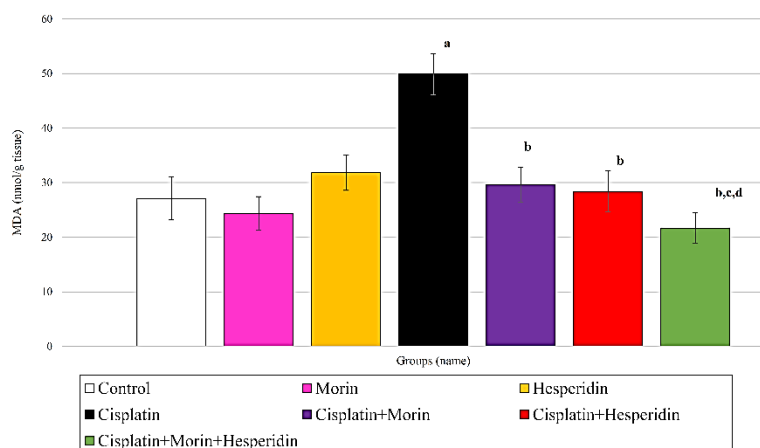


Figure 2. Levels of MDA in liver tissues. The administration of morin and/or hesperidin statistically decreased MDA levels when compared with the single use of cisplatin administration; a: p<0.05 as compared with the control group; b: p<0.05 as compared with the cisplatin group; c: <0.05 as compared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

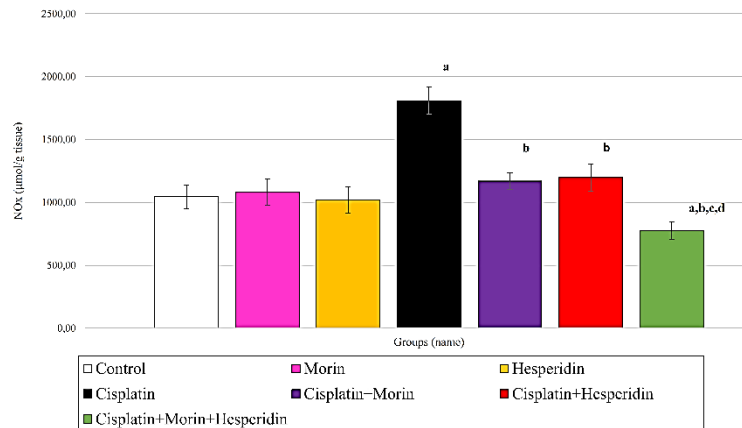


Figure 3. Levels of NOx in liver tissues. The administration of morin and/or hesperidin significantly reduced NOx level in cisplatin-induced rats; a: $p < 0.05$ as compared with the control group; b: $p < 0.05$ as compared with the cisplatin group; c: $p < 0.05$ as compared with the cisplatin+morin group;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

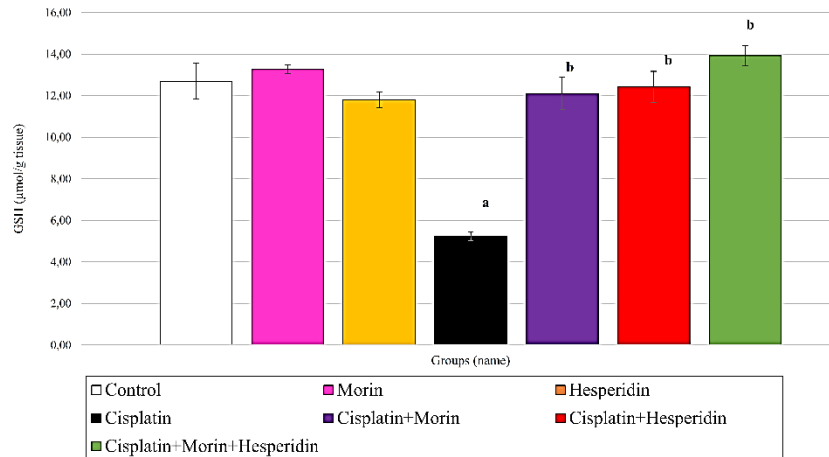


Figure 4. Levels of GSH in liver tissues. The administration of morin and/or hesperidin significantly increased the level of GSH; a: $p < 0.05$ as compared with the control group; b: $p < 0.05$ as compared with the cisplatin group; c: $p < 0.05$ as compared with the cisplatin+morin group; d: $p < 0.05$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

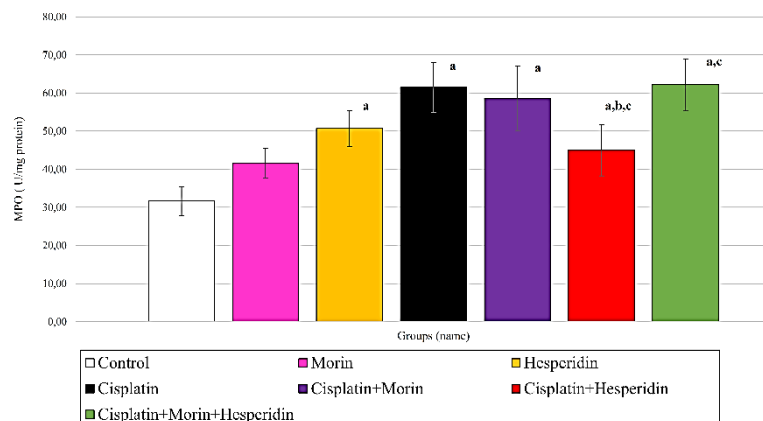


Figure 5. Activity of MPO in liver tissues. The hesperidin administration reduced MPO activity compared with cisplatin-induced group ; a: $p < 0.05$ as compared with the control group; b: $p < 0.05$ as compared with the cisplatin group, c: $p < 0.05$ as compared with the cisplatin+morin group; d: $p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

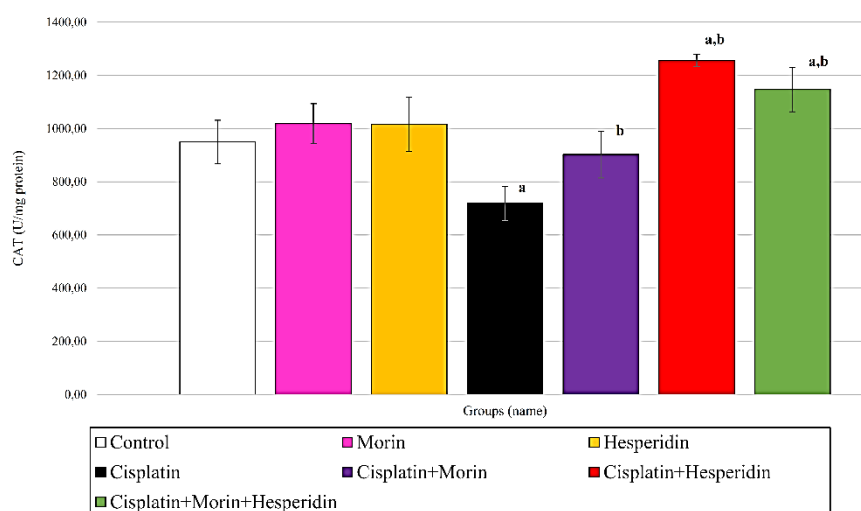


Figure 6. Activity of CAT in liver tissues. The activities of CAT significantly increased in the morin and/or hesperidin groups compared with cisplatin group; a: $p < 0.05$ as compared with the control group; b: $p < 0.05$ as compared with the cisplatin group; c: $p < 0.05$ as compared with the cisplatin+morin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

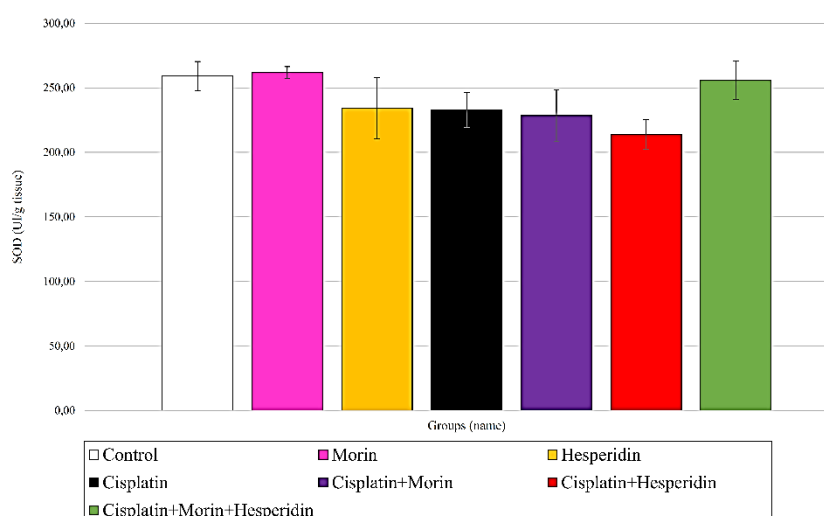


Figure 7. Activity of SOD in liver tissues. No significant changes were detected among all groups in terms of SOD activity; a: $p < 0.05$ as compared with the control group; b: $p < 0.05$ as compared with the cisplatin group; c: $p < 0.05$ as compared with the cisplatin+morin group; d: $p < 0.05$ as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

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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