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LABORATORY STUDY

Potential of morin and hesperidin in the prevention of cisplatin-induced nephrotoxicity

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ABSTRACT

Oxidative stress is one of the important mechanisms of cisplatin-induced nephrotoxicity. Therefore, this study was designed to explore the potential protective effects of morin and/or hesperidin on oxidative stress in cisplatin-induced nephrotoxicity. This study was performed on 42 Wistar rats. Rats were divided into seven groups: control, morin, hesperidin, cisplatin, cisplatin + morin, cisplatin + hesperidin, and cisplatin + morin + hesperidin. Morin and/or hesperidin were given for 10 consecutive days by oral gavage and on the 4th day a single dose of cisplatin (7 mg/kg) was injected intraperitoneally. After administrations, on the 11th day of the experiment the animals were killed, and malondialdehyde (MDA), nitric oxide (NOx), glutathione (GSH) levels and myeloperoxidase (MPO), catalase (CAT), superoxide dismutase (SOD) activity were measured. Cisplatin-treated rats showed increased levels of MDA, and decreased levels of NOx also activity of CAT. Morin and/or hesperidin pretreatment prevent oxidative stress in kidney tissue, while they increase the NOx level, CAT activity, and decrease MPO activity. In conclusion, morin + hesperidin pretreatment may have a significant potential for protection of cisplatin-induced nephrotoxicity.

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Cisplatin; hesperidin; morin; nephrotoxicity; oxidative stress

Introduction

Cancer, which is characterized by uncontrolled cell division, is a leading cause of death worldwide and is being investigated for the development of new approaches for therapy.[1,2] Surgery, and/or radiotherapy, and/or chemotherapy are the most common types of cancer treatment.[3] Although chemotherapy is a highly effective method for cancer treatment, it causes side effects such as nephrotoxicity and hepatotoxicity.[4]

Cisplatin (*cis*-diamminedichloroplatinum (II)) is one of the most widely used chemotherapeutic agents for the treatment of various human cancers.[5] Cisplatin exerts its effects by inducing the formation of DNA interstrand crosslinks. Cisplatin–DNA crosslinks cause cytotoxic lesions in tumors and other dividing cells.[6,7] Besides the positive effects of cisplatin on cancer treatment, it can lead to many different complications including nephrotoxicity, neurotoxicity, ototoxicity, and emetogenicity.[8,9]

Cisplatin-treated patients show nephrotoxicity (25–42%).[8] Nephrotoxicity occurs by renal toxic effects of cisplatin, but the underlying mechanism is not fully understood.[6] It is proposed that renal cell injury is related to the drug accumulated in kidneys (renal tubular cells), causing direct inflammation, generation of reactive oxygen species (ROS), DNA damage, mitochondrial dysfunction, and apoptosis.[5,6,9,10]

Despite large number of studies about this topic, none of them is proved to be completely effective. For example, selenium, melatonin, vitamins C and E, mannitol, and arjunolic acid may be used to prevent or attenuate cisplatin-induced nephrotoxicity.[6,9–13] Additionally, Kamel et al.[5] reported that hesperidin and rutin showed protective effects against cisplatin-induced nephrotoxicity in male rats. Hesperidin or rutin administration ameliorated the cisplatin-induced nephrotoxicity as indicated by the restoration of kidney function and oxidative stress biomarkers. In different studies, Wei et al.[14] suggested that morin may have renoprotective effects on cisplatin-induced kidney injury.

Morin (3,2′,4′,5,7-pentahydroxyflavone) is one of the most common flavonols and it occurs in *Morus alba* L. and many other herbs and fruits.[15] It is reported that morin shows protective effects against oxidative stress-induced damage on cardiovascular cells,[16,17] glomerular mesenchymal cells,[18] hepatocyte,[19] oligodendrocytes,[20] and neurons.[21] Hesperidin (5,7,3′-trihydroxy-4′-methoxy-flavanone7-rhamnoglucoside) is one of the most abundant natural flavonoids and it is present in a large number of fruits and vegetables.[22,23] Cholesterol[24] and

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blood pressure[25] were decreased by administration of hesperidin in rats. In addition, hesperidin administration increased antioxidant capacity.[26] Although there have been many studies on the effects of various flavonoids administration on nephrotoxicity, effects of morin + hesperidin combinations have not been studied yet. The present study was designed to explore the potential protective effects of morin and hesperidin on oxidative stress in cisplatin-induced nephrotoxicity.

Materials and methods

Chemicals

Cisplatin, morin, and hesperidin were purchased from Sigma-Aldrich (St. Louis, MO). All other chemicals used in experiment were of the highest analytical grade and purchased from Merck (Darmstadt, Germany) or Sigma-Aldrich (St. Louis, MO).

Animals and experimental design

This work was approved by the Gazi University Local Ethics Committee for Animal Experiments (G.Ü.ET-12.070). Healthy adult 42 male Wistar albino rats (200–250 g) were used. Rats were housed one per cage and given standard rat chow and water *ad libitum* (at room temperature (25 ± 3 °C) and in a 12-h light/12-h dark cycle). Rats were classified into the following groups, with 6 rats in each:

- Group I (Control): Received only distilled water for 10 consecutive days by oral gavage (intragastrically) ($n = 6$).
- Group II (Morin): Received a single daily dose morin (50 mg/kg) prepared in distilled water for 10 consecutive days by oral gavage (intragastrically) ($n = 6$).
- Group III (Hesperidin): Received a single daily dose hesperidin (200 mg/kg) prepared in distilled water for 10 consecutive days by oral gavage (intragastrically) ($n = 6$).
- Group IV (Cisplatin): Received a single dose of cisplatin (7 mg/kg) prepared in distilled water on the 4th day of the experiment by intraperitoneally (i.p.) ($n = 6$).
- Group V (Cisplatin + Morin): Received a single daily dose morin (50 mg/kg) prepared in distilled water for 10 consecutive days by oral gavage (intragastrically) and on the 4th day a single dose of cisplatin (7 mg/kg) was injected intraperitoneally ($n = 6$).
- Group VI (Cisplatin + Hesperidin): Received a single daily dose hesperidin (200 mg/kg) prepared in distilled water for 10 consecutive days by oral gavage (intragastrically) and on the 4th day a single dose of cisplatin (7 mg/kg) was injected intraperitoneally ($n = 6$).
- Group VII (Cisplatin + Morin + Hesperidin): Received a single daily dose morin (50 mg/kg) + hesperidin (200 mg/kg) prepared in distilled water for 10 consecutive days by oral gavage (intragastrically) and on the 4th day a single dose of cisplatin (7 mg/kg) was injected intraperitoneally ($n = 6$).

One day after the last treatment (11th day), all animals were killed with intracardiac blood aspiration under anesthesia (intramuscularly with 50 mg/kg of ketamine-HCl and 5 mg/kg xylazine-HCl). The kidney tissue samples were harvested and transported immediately frozen in liquid nitrogen, and then the samples were kept at −30 °C until assay.

Biochemical analyses

Determination of MDA levels

Thiobarbituric acid-reactive substances (TBARs) level as a lipid peroxidation indicator. Lipid peroxidation was quantified by measuring the formation of malondialdehyde (MDA). Briefly, tissue samples were homogenized in 0.15 M ice-cold potassium chloride, and 15% trichloroacetic acid added to 1 ml homogenate for deproteinization. Following centrifugation at 2000 × *g* for 10 min, 1 ml of supernatant was added to 0.67% thiobarbituric acid and 1% butylated hydroxytoluene, and heated at 100 °C for 10 min. The absorbance of the samples was measured at 535 nm (UV Mini 1240, Shimadzu, Tokyo, Japan).[27] A 1 mm stock tetraethoxypropane solution was used as standard. Results were expressed as nmol/g tissue.

Determination of nitric oxide (NOx) levels

The reactive nitrogen oxide species level, which are stable end products of NO in tissue homogenates, were determined by Griess reaction. Tissue samples were homogenized in phosphate buffer (pH 7) and centrifuged at 3500 rpm for 15 min; to supernatants (0.5 ml), 0.25 ml of 0.3 M NaOH was added. After incubation for 5 min at room temperature, supernatants were used for Griess assay. Vanadium trichloride (VCl_3) was added to supernatants and this mixture was incubated for 30 min at 37 °C. After incubation, Griess reagents (sulphanilamide (SULF) and N-(1-naphtyl) ethylenediamine dihydrochloride (NEDD)) were added and again incubated. After incubation, samples were measured at 540 nm (UV Mini 1240, Shimadzu, Tokyo, Japan).[28]

Sodium nitrite (1, 10, 50, and 100 μM) was used as standard. Results were expressed as μmol/g tissue.

Determination of GSH levels

Levels of glutathione (GSH) were determined by a modified Ellman method.[29] Briefly, tissue samples were homogenized in 0.15 M ice-cold potassium chloride, and the mixture of meta-phosphoric acid/ethylenediaminetetraacetic acid/sodium chloride added to 0.5 ml homogenate for deproteinization. Following centrifugation at 4000 rpm for 20 min, 0.5 ml of supernatant was added to the 2 ml of 0.3 M disodium hydrogen phosphate and 0.2 ml solution of dithiobisnitrobenzoate (0.4 mg/mL 1% sodium citrate). The absorbance of each sample was determined at 412 nm (UV Mini 1240, Shimadzu, Tokyo, Japan). Results were expressed as μmol/g tissue.

Determination of MPO activity

Schierwagen et al.[30] method was used to detect myeloperoxidase (MPO) activity in kidney tissue. Briefly, tissue samples were homogenized in 20 mmol/L potassium phosphate buffer (pH 7.4) and centrifuged for 5 min at 10,000 × *g* at 4 °C. The supernatant was discarded, and the pellet was resuspended in 50 mmol/L potassium phosphate buffer (pH 6.0) containing 0.5% hexadecyltrimethylammonium bromide. The suspension was frozen, and it was then sonicated once only for 10 s, incubated for 2 h in a water bath at 60 °C, and centrifuged at 10,000 × *g* for 5 min. The supernatants were used for MPO assay. The MPO activity was assessed by measuring H_2O_2-dependent oxidation of O-dianisidine. One unit of enzyme activity was defined as the amount of MPO activity in tissue that caused a change in absorbance of 1/min at 410 nm and 37 °C (UV Mini 1240, Shimadzu, Tokyo, Japan). Results were expressed as U/mg protein.

Determination of CAT activity

Kidney catalase (CAT) activity was assayed according to the method of Aebi[31] Briefly, H_2O_2 was used as the substrate and the decrease of H_2O_2 concentration at 20 °C in phosphate buffer was assayed by spectrophotometry at 240 nm (UV Mini 1240, Shimadzu, Tokyo, Japan). One unit of CAT activity is the amount of enzyme that degrades 1 μmol of H_2O_2/min; CAT activity was expressed as U/mg protein.

Determination of SOD activity

Sun et al.[32] method was used to detect superoxide dismutase (SOD) activity. Tissue samples were homogenized in ice-cold 0.9% NaCl and centrifuged at 7000 rpm and stayed +4 °C for 30 min. After homogenization, ethanol–chloroform mixture (3:5, v/v) was added to 1 ml supernatant. Supernatants were homogenized at 7000 rpm for 60 min. A 2.45 ml of assay reagent (containing 3 mM xanthine, 0.6 mM ethylenediaminetetra acetic acid (EDTA), 150 μM NBT, 400 mM Na_2CO_3 and 1 g/L BSA) was combined with 500 μl of supernatant sample. Xanthine oxidase (50 μl) was added to initiate the reaction and the reduction of nitro blue tetrazolium (NBT) by O_2^- After incubation for 20 min at 25 °C, $CuCl_2$ was added to mixture. Absorbance of samples was measured at 560 nm (UV Mini 1240, Shimadzu, Tokyo, Japan). One unit of SOD is defined as that amount of enzyme causing half-maximal inhibition of NBT reduction. Results were expressed as U/g tissue.

Statistical analysis

Mean differences were compared by one-way analysis of variance (ANOVA) followed by Tukey's test for post-hoc analysis. Values of $p < 0.05$ were considered to be significant. Results were expressed as the mean ± standard deviation.

Results

The overall measurement results are summarized in Table 1 and Figures 1–6 with their statistical significance.

MDA levels

The level of MDA was significantly increased in the cisplatin group (Group IV) compared with control group (Group I) ($p < 0.05$). In the cisplatin-induced rat groups, the increased MDA levels were decreased with the administration of cisplatin + morin and cisplatin + morin + hesperidin (Groups V and VII) ($p < 0.05$) (Figure 1).

NOx levels

The kidney tissue NOx levels statistically decreased with the administration of cisplatin (Group IV) compared with control group (Group I) ($p < 0.05$). On the contrary, administration of cisplatin-induced rats with morin and/or hesperidin (Groups V, VI, VII) significantly restored the normal level of NOx (Figure 2).

GSH levels

As shown in Figure 3, hesperidin administration (Group III) significantly increased GSH levels compared with control group (Group I) ($p < 0.05$) (Figure 3).

Table 1. Oxidant and antioxidant markers.

	Control	Morin	Hesperidin	Cisplatin	Cisplatin + morin	Cisplatin + hesperidin	Cisplatin + morin + hesperidin
MDA (nmol/g tissue)	30.68 ± 1.89	30.74 ± 1.84[b]	32.68 ± 0.93	35.40 ± 1.29[a]	30.99 ± 0.68[b]	37.30 ± 2.74[a]	32.00 ± 0.88[b]
NOx (µmol/g tissue)	654.99 ± 80.17	661.52 ± 134.47	421.28 ± 88.76[a]	525.45 ± 52.58[a]	887.54 ± 83.67[a,b]	813.35 ± 114.01[a,b]	903.30 ± 151.09[a,b]
GSH (µmol/g tissue)	7.38 ± 0.38	7.51 ± 0.49	8.65 ± 0.21[a]	7.51 ± 0.51	7.72 ± 0.62	7.66 ± 0.47	7.98 ± 0.61
MPO (U/mg protein)	59.61 ± 5.92	64.83 ± 8.79	65.11 ± 9.08	76.33 ± 8.28[a]	67.61 ± 9.57	28.59 ± 2.64[a,b]	27.14 ± 2.29[a,b]
CAT (U/mg protein)	301.78 ± 35.36	293.64 ± 73.53	293.97 ± 82.63	201.90 ± 39.05[a]	258.78 ± 49.24	358.39 ± 45.37[b]	279.06 ± 78.09
SOD (U/g tissue)	280.01 ± 10.99	275.12 ± 6.35	281.26 ± 5.85	283.24 ± 8.31	277.79 ± 2.97	273.92 ± 15.31	273.41 ± 7.25

Data are expressed as means ± standard deviations of six rats per group.
[a]$p < 0.05$ as compared with the control group.
[b]$p < 0.05$ as compared with the cisplatin group.

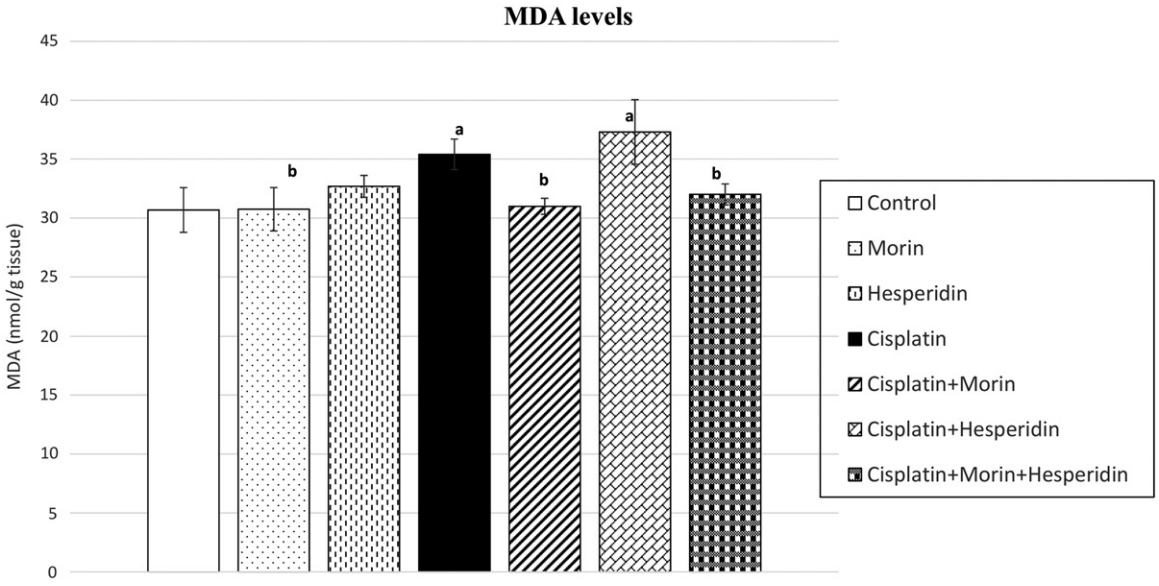


Figure 1. Levels of MDA in kidney tissues.

MPO activity

The activity of MPO was significantly increased in the cisplatin group (Group IV) compared with control group (Group I) ($p < 0.05$). Administration of cisplatin-induced rats with hesperidin and morin + hesperidin (Groups VI, VII) significantly decreased the activity of MPO compared with control and cisplatin group (Groups I, IV) (Figure 4).

CAT activity

CAT activity was assessed in the kidney tissue in rats. There was a significant ($p < 0.05$) decline in the CAT activity of cisplatin-induced group (Group IV) compared to the control group (Group I). Administration of hesperidin (Group VI) significantly increased the cisplatin-induced changes in CAT activity (Group IV) ($p < 0.05$) (Figure 5).

SOD activity

No statistically significant difference was detected in SOD activity among the all groups ($p > 0.05$) (Figure 6).

Discussion

The current study showed that morin and/or hesperidin has protective effect on oxidant and antioxidant parameters in the kidney of cisplatin-induced rats. Our findings suggested that morin and hesperidin may serve as promising preventive flavonoids against cisplatin-induced nephrotoxicity.

Tissue MDA levels statistically decreased due to the application of cisplatin + morin and cisplatin + morin + hesperidin (Group V and VII) when it is compared with cisplatin group (Group IV) ($p < 0.05$). Morin could be evaluated more effective than hesperidin in terms of reducing lipid peroxidation at renal tissue. MDA is an end product of lipid peroxidation, and lipid peroxidation products are also markers for oxidative stress. Oxidative stress is one of the important mechanisms of cisplatin-induced nephrotoxicity. Various ROS are produced by cisplatin induction in cultured renal tubular cells and the kidney,[33,34] and they can damage cell through tampering lipids, proteins, and DNA.[35] In the literature, it has been reported that MDA levels were significantly increased by cisplatin in rats.[5,6] The obtained results indicate that the pretreatment of

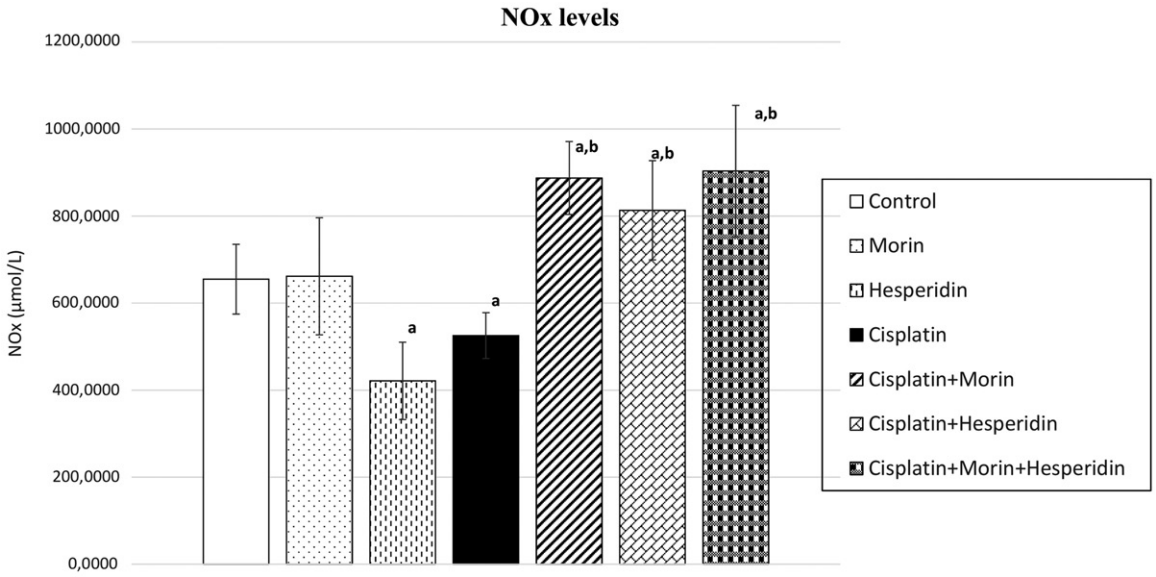


Figure 2. Levels of NOx in kidney tissues.

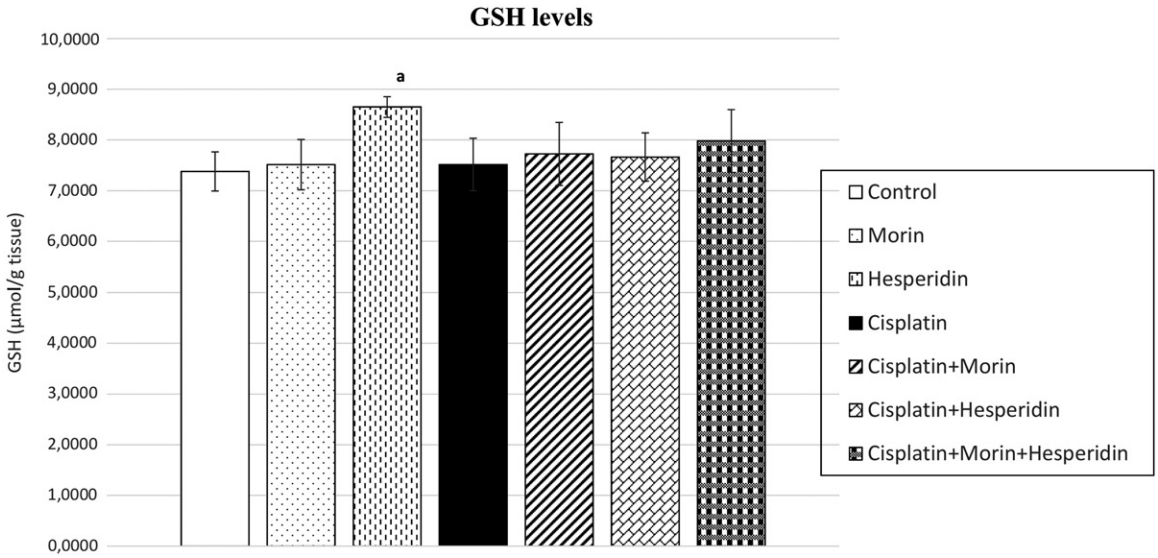


Figure 3. Levels of GSH in kidney tissues.

flavonoids used in our study, especially on morin and morin + hesperidin combination, may inhibit the cisplatin-induced oxidative stress in rats.

It is suggested in several studies that NOx is play an important role in cisplatin-induced nephrotoxicity.[36–39] Moreover, NO production is also reported to be low in chronic kidney disease (CKD) patients, and NO deficiency may play a role in CKD progression.[39–41] Saleh et al.[37] reported that cisplatin alone induced a significant decrease in male rat kidney total nitrate/nitrite level. They argued that, this decrease can be explained by the damage of glomerular endothelial cells. Furthermore, L-NAME, inhibitor of NO synthase, exacerbates the cisplatin-induced nephrotoxicity. In the same way, Saad et al.[38] determined that cisplatin treatment induced reductions in the male rat kidney total nitrate/nitrite (NOx). In addition, Moslemi et al.[39] reported that L-NAME increases cisplatin-induced nephrotoxicity, depending on gender. In our study, cisplatin decreased NOx levels in kidney tissue ($p < 0.05$). In response to cisplatin + morin and/or + hesperidin administration, the level of NOx was observed to be significantly increased ($p < 0.05$). According to these results, NOx levels elevated to normal level by morin and/or hesperidin pretreatment in the kidney tissue of cisplatin-induced rats. Both hesperidin and morin increased NO levels of renal tissue in rats. When these two flavonoids (morin and hesperidin) were used together, they increased tissue

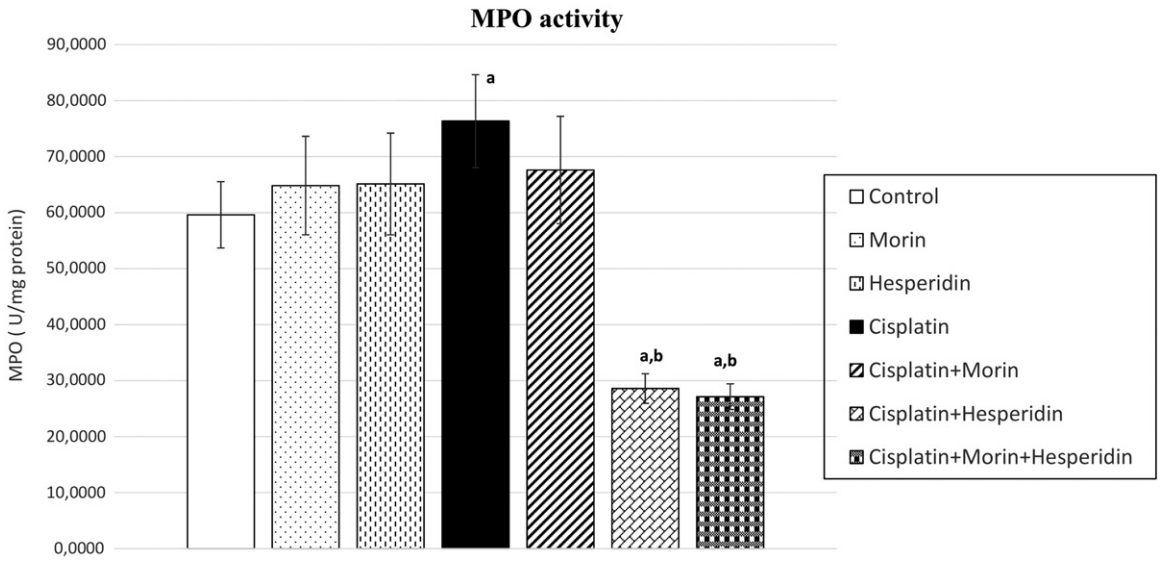


Figure 4. Activity of MPO in kidney tissues.

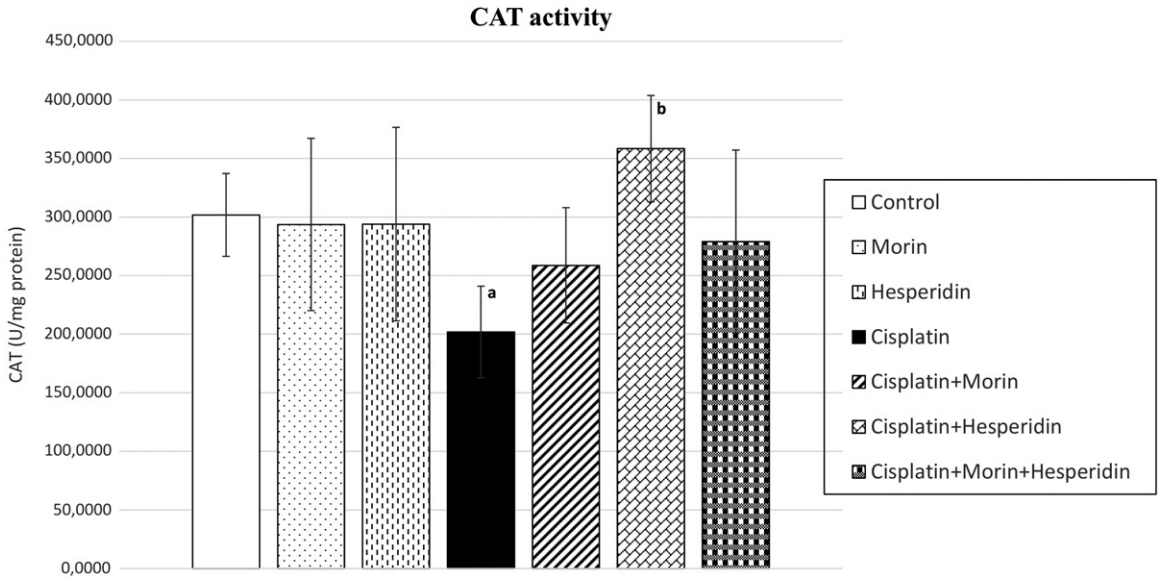


Figure 5. Activity of CAT in kidney tissues.

NOx levels much more then when it is compared with the single pretreatments of morin or hesperidin. However, this increase was not statistically significant ($p > 0.05$). Additionally, hesperidin pretreatment (Group III) significantly decreases NOx level compared to the control (Group I). This scenario may be occurring due to dose of hesperidin.

It has been known that GSH and ascorbic acid are non-enzymatic antioxidant as second line of defense against free radicals. In our study, hesperidin application significantly increased GSH levels in normal rats. It can be said that hesperidin supplementation increases the antioxidant capacity of renal tissue. Both hesperidin and morin pretreatment increased tissue GSH levels when compared to control rat and cisplatin treated rats, but this increase is not statistically significant. This result bring to mind, GSH will be used or consumed to eliminate the increased lipid peroxidation with cisplatin application. The alteration of MDA levels in groups supports our present findings related to GSH.

Cisplatin administration triggered inflammatory response in rat renal tissue by inducing TNF-α with the increased expression of MPO.[42] Sahu et al.[42] reported that hesperidin ameliorated neutrophil infiltration as evidenced by inhibition of MPO levels in kidney tissues. Also Kamel et al.[5] suggested that hesperidin administration decreased inflammatory cell infiltration in kidney tissues of cisplatin-induced rats. Singh et al.[43] pointed

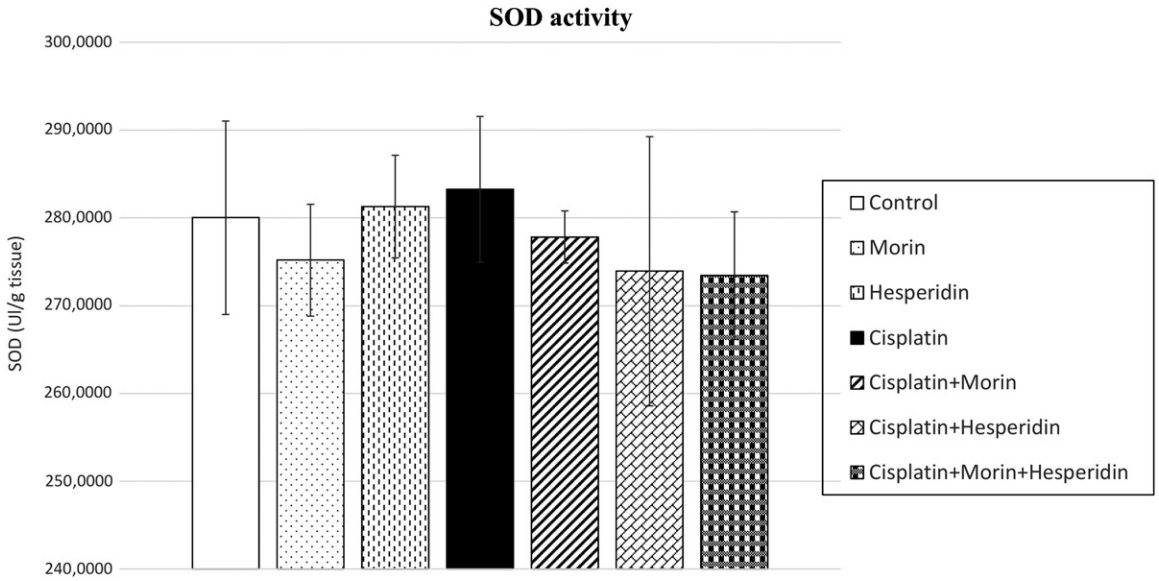


Figure 6. Activity of SOD in kidney tissues.

that morin hydrate attenuates the acrylamide-induced imbalance in MPO activity at liver homogenate and serum in a murine model. In consistent with above studies, our results indicated that both morin and hesperidin administration decreased MPO levels of renal tissues in cisplatin-induced groups. When morin and hesperidin applied together, they decreased inflammatory events in renal tissue of cisplatin-induced rat.

Our hesperidin pretreatment increases the activities of CAT in cisplatin-induced rats. CAT is an enzyme found in various tissues and cells. In general, it is abundant in erythrocytes, liver, and kidneys and degrades the hydrogen peroxide. It is a very important enzyme in protecting the cell from ROS-mediated damage.[44] Previous studies have shown that cisplatin treatment decreases CAT activity in kidney tissue.[45,46] Our results are supported in another study by Siddiqi et al.[47] They reported that in trichloroethylene-induced nephrotoxicity, kidney CAT activity was significantly increased by hesperidin administration in Wistar rats. Ma et al.[44] suggest that CAT and its derivatives inhibit cisplatin-induced nephrotoxicity.

SOD activities were not significantly changed in the morin and/or hesperidin administrations groups compared with another groups ($p > 0.05$). Although the MDA levels decrease, the activity of SOD does not change. This situation brings into mind that alternative antioxidant defense mechanisms (such as CAT, etc.) are used to scavenge increased ROS formation.

Conclusion

In conclusion, morin and/or hesperidin pretreatment prevent oxidative stress in kidney tissue of cisplatin-induced rats, while they increase the NOx level, CAT activity, and decrease MPO activity. Morin + hesperidin pretreatment may have a significant potential for protection of cisplatin-induced nephrotoxicity.

Disclosure statement

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

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