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Article

The effects of agomelatine in cisplatin-induced toxicity on the kidney and liver tissues: *In vivo* study

Fatma Nihan Cankara[1*], Caner Günaydın[2], Zülfinaz Betül Çelik[3], Yasemin Şahin[1], Şakir Pekgöz[1], Yalçın Erzurumlu[4], Kanat Gülle[5]

[1]Department of Pharmacology, Faculty of Medicine, Suleyman Demirel University, Isparta, Turkey, [2]Department of Pharmacology, Faculty of Medicine, Ondokuz Mayıs University, Samsun, Turkey, [3]Department of Medical Biology, Faculty of Medicine, Ondokuz Mayıs University, Samsun, Turkey, [4]Department of Biochemistry, Faculty of Pharmacy, Suleyman Demirel University, Isparta, Turkey, [5]Department of Histology and Embryology, Faculty of Medicine, Suleyman Demirel University, Isparta, Turkey

Nephrotoxicity and hepatotoxicity are frequently seen adverse effects during cisplatin chemotherapy. In this study, we investigated the effects of agomelatine on cisplatin-induced toxicity in the kidney and liver. Animals were administered with a single dose of cisplatin (7 mg/kg, i.p.) and treated with agomelatine (20 and 40 mg/kg, p.o) for seven days. Renal and hepatic functions were evaluated by measuring concentrations of creatinine, BUN, AST and ALT in the serum. Oxidative stress and protein peroxidation were assessed by measuring SOD, CAT, GSH and AOPP levels in both tissues. Serum PON-1 levels were also evaluated. Histopathological analysis was performed to determined structural changes in the kidney and liver. Agomelatine (20 mg/kg) treatment approximately halved cisplatin-related increase in serum creatinine, BUN, AST and ALT levels. Agomelatine (20 mg/kg) significantly prevented the cisplatin-induced excessive decrease in SOD, CAT, GSH in both tissues and serum PON-1 levels. Agomelatine (20 and 40 mg/kg) protected the structural integrity of the kidney against cisplatin-insult. Although agomelatine (40 mg/kg) protected the kidney and showed parallel results with 20 mg/kg biochemically, it failed to show the same liver tissue effects in both analyses. Although agomelatine protected against cisplatin-induced toxicity in the kidney and liver, care should be taken with higher doses for possible hepatotoxicity.

Keywords: Agomelatine. Cisplatin. Nephrotoxicity. Hepatotoxicity. Oxidative stress.

INTRODUCTION

Cis-diamminedichloroplatinum (cisplatin) is a platinum-based anti-cancer agent that is the mainstay for the treatment of broad-spectrum malignancies, such as head-neck, bladder, breast, lung, ovarian and testicular cancers. Despite its proven clinical usefulness, severe side effects in other tissues during treatment limits its clinical application (Zhang *et al.,* 2006). In particular, cisplatin-induced nephrotoxicity is the most common dose-limiting effect because of the tendency of cisplatin to accumulate in the proximal renal tubules (Galgamuwa *et al.,* 2016). In addition to the nephrotoxicity, cisplatin also demonstrated to have a toxic effect on hepatic tissue, which was recently ascertained as another significant dose-limiting side effect (Liao *et al.,* 2008).

However, cisplatin's tubule accumulation is generally considered the primary mechanism of cisplatin-induced nephrotoxicity; the reason behind cisplatin-induced hepatotoxicity remains mostly unexplored. These two toxic effects of cisplatin are significant obstacles in cisplatin chemotherapy. Therefore, investigating and uncovering possible mechanisms is necessary to find ways to prevent these toxicities, under continuous cisplatin treatment for aggressive tumors. Although obscurity continues about

***Correspondence:** F. N. Cankara. Department of Pharmacology. Suleyman Demirel University Faculty of Medicine. Isparta, 32260, Turkey. Phone: +90 246 211 36 24. E-mail: nihancankara@yahoo.com. ORCID: https://orcid.org/0000-0002-2367-6412**.** Caner Günaydın – ORCID: https://orcid.org/0000-0002-8304-832X

the primary mechanism, several different underlying mechanisms have been suggested. Oxidative stress and dysregulated oxidant/antioxidant balance have become prominent among these (Chirino, Pedraza-Chaverri, 2009). The generation of free oxygen radical species and the resulting reaction of these species with cellular structures, is known to be a reason for cellular dysfunction. Normally, cells overcome the destructive effects of reactive oxygen and nitrogen derivatives by endogenous enzymatic antioxidants or non-enzymatic antioxidants. Several studies have demonstrated that free radical scavengers, many antioxidants and natural compounds, protect cellular structures against cisplatin's toxic effects (Hajian, Rafieian-Kopaei, Nasri, 2014; Weijl *et al.,* 2004). However, cisplatin's main tumoricidal mechanism is its ability to produce DNA adducts, eliminating side effects without changing its tumoricidal activity's important objective, which possibly contributes to cancer chemotherapy.

Agomelatine is a melatonin analog and has a longer half-life and more significant affinity for melatonin-1 (MT-1) and melatonin-2 (MT-2) receptors than melatonin itself (Aygun, Gul, 2019; Millan *et al.,* 2003). Currently, agomelatine is used in major depressive disorder and sleep problems. Available studies have demonstrated the protective effects of agomelatine against several oxidative-stress mediated pathology (Aguiar *et al.,* 2013; Demirdaş, Nazıroğlu, Ünal, 2016). Additionally, several groups have highlighted the protective effects of agomelatine against xenobiotic-induced toxicity in different tissues. However, knowledge about effects of agomelatine on different tissues simultaneously against these toxicities remains limited.

Therefore, using an analog of the potent endogenous antioxidant melatonin, agomelatine, might exert beneficial effects against cisplatin-induced kidney and liver toxicity. As far as we were able to determine, there are no studies in the literature regarding the effects of agomelatine in cisplatin-induced toxicity. Therefore, we aimed to investigate agomelatine's effects in cisplatin-induced nephrotoxicity and hepatotoxicity, with biochemical and histopathological analysis.

MATERIAL AND METHODS

Animals

Forty adult male Wistar albino rats were obtained from the Suleyman Demirel University vivarium, with permission from the Animals Ethics Committee of Suleyman Demirel University (2019-10/03). Animals were maintained under constant conditions (55% humidity, 22±0.5°C, 12/12 day/night cycle) until the experiments and the latter were carefully conducted to reduce the number and suffering of animals, in line with the Declaration of Helsinki's Guide.

Chemicals

Cisplatin, agomelatine, ketamine and xylazine were obtained from Sigma-Aldrich Inc. (Illinois, US). Cisplatin, ketamine and xylazine were dissolved in saline. Agomelatine was dissolved in dimethyl sulfoxide (DMSO) and further diluted with saline (5%, v:v) to avoid DMSO treatment's toxic effects.

Experimental design

All animals were weighed prior to the experiments. Wistar albino rats were divided into four groups of ten animals each and treated for seven consecutive days. The agomelatine treatment was performed 30 minutes before the cisplatin injection in each group. The first group was used as the control and seven-day oral saline treatment (1 ml/kg) was given. The second group was administered with a single injection of cisplatin (7 mg/kg, i.p). The third and fourth groups were administered with single dose of cisplatin (7 mg/kg, i.p) and agomelatine at the doses of 20 and 40 mg/kg, respectively, for seven days, by oral gavage. At the end of the treatments, animals were anesthetized and decapitated (Bilgic *et al.,* 2018). Blood samples were immediately collected and serum samples isolated (4°C, 300 g for 10 min), then stored at -20°C. Kidney and liver samples were carefully removed and kept for histopathological and biochemicals analysis.

Biochemical analysis

Serum samples were thawed on the day of the analysis and left to reach room temperature. Serum levels of the creatinine (Cr), blood urea nitrogen (BUN), aspartate transaminase (AST) and alanine transaminase (ALT) were then measured using a Cobas Mira Plus CC Chemistry Analyzer (Switzerland). In addition, paraoxonase-1 (PON-1) levels were established using a commercially available ELISA kit. Kidney and liver samples were homogenized with a glass homogenizer in phosphate buffered saline (PBS). Tissue lysates were centrifuged at 4 °C, 300 g for 10 min, then Lowry's method was used to determine the total protein content (Lowry *et al.,* 1951). Superoxide dismutase (SOD), catalase (CAT), glutathione-S-hydroxylase (GSH) for evaluating the antioxidant status and advanced oxidation protein products (AOPP) for evaluating protein peroxidation, were determined with commercially available ELISA kits, while strictly following the manufacturer's instructions.

Histopathological analysis

Kidney and liver samples were fixed with a 10% neutral formalin solution for 48 hours. Tissue samples were washed with tap water overnight. Samples were then passed through ethanol series for dehydration before xylene treatment and paraffinization. Paraffin blocks were sectioned with a rotary microtome (RM2125RTS, Leica, Germany) to 4-5 µm thickness. For the histopathological evaluation, all samples were stained using hematoxylin and eosin staining. Kidney sections were evaluated for tubular epithelial changes (dilatation, desquamation, vacuolization, atrophy), glomerular changes (sclerosis, necrosis) and interstitial changes (inflammatory cell invasion, edema, fibrosis), and liver sections were evaluated for hepatocellular degeneration, portal area fibrosis, inflammatory cell invasion, vascular congestion, sinusoidal dilatation. Individual pathological features, if present, were graded according to the changes that were observed (score 0 with no changes, 1 with <20%, 2 with 20–40%, 3 with 40–60%, 4 with 60–80%, and 5 with >80% changes). The sum of all numerical scores in each group was recorded as the total histopathological score. Photographs of the sections were then obtained via a camera attached to a binocular light microscope (ECLIPSE Ni-U, Nikon, Tokyo, Japan).

Statistical analysis

All data from the experiments was expressed as mean±standard deviation. The SPSS v21.0 software (Illinois, US) was used for data analysis. After the determination of data distribution, one-way ANOVA and Kruskal-Wallis tests were performed. The Tukey and Tamhane's tests were performed for post hoc analysis. P values less than 0.05 were considered statistically significant.

RESULTS

Agomelatine (20 mg/kg) reversed the impairment in liver and kidney function caused by cisplatin and increased the PON-1 level

Cr and BUN for kidney function, AST, and ALT for liver function are commonly used biomarkers. In addition, PON-1, one of the main enzymes that hydrolyzes the toxic metabolites, platinum-based compounds and organophosphorus insecticides, was also evaluated. Our serum results demonstrated that cisplatin significantly increased Cr (Figure 1a), BUN (Figure 1b), AST (Figure 1d), ALT (Figure 1e) levels and decreased PON-1 (Figure 1c) levels, as expected (Figure 1, Table I). At the dose of 20 mg/kg, agomelatine significantly decreased Cr, BUN, AST and ALT levels. Additionally, agomelatine (20 and 40 mg/kg) significantly inhibited the decrease in PON-1 levels due to the cisplatin insult (Figure 1), although the 40 mg/kg agomelatine did not significantly affect Cr, BUN, and it caused a significant increase in AST and ALT levels (Figure 1d, e).

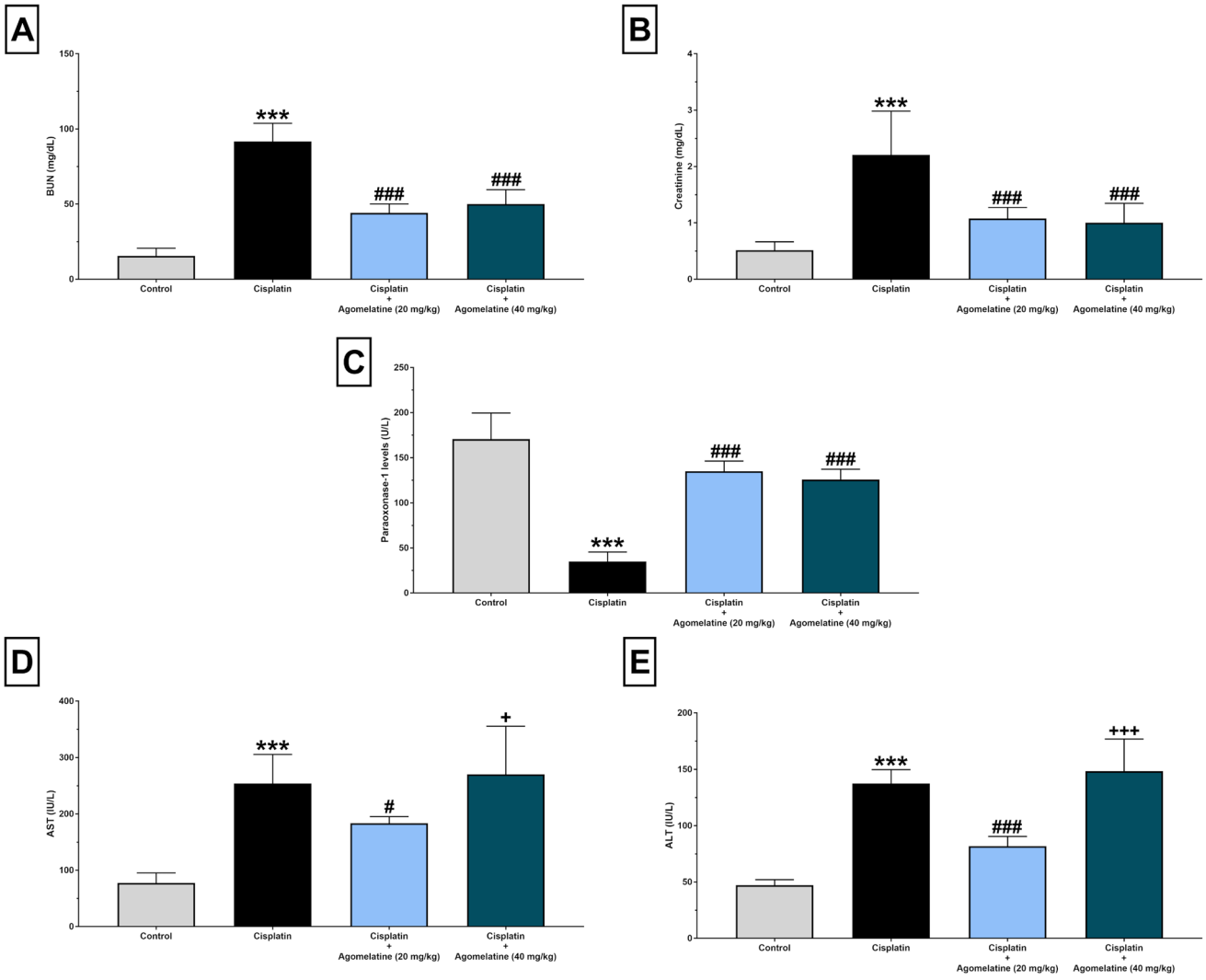

FIGURE 1 - Serum levels of selected markers. A: Blood urea nitrogen (BUN) B: Creatinine (Cr) C: Paraoxonase 1 (PON-1), D: Aspartate transaminase (AST), E: Alanine transferase (ALT). At the doses of 20 and 40 mg/kg, agomelatine prevented BUN and Cr increase and PON-1 decrease in serum levels. However, agomelatine (20 mg/kg) diminished cisplatin-induced AST and ALT increase, whereas agomelatine (40 mg/kg) failed to prevent the cisplatin-induced increase in AST and ALT levels. All data represented as mean±SD and stand for eight animals per group. $^{***}p<0.001$ versus control group and $^{###}p<0.001$, $^{#}p<0.05$ versus cisplatin group and $^{+++}p<0.001$, $^{+}p<0.05$ versus cisplatin+agomelatine (20 mg/kg) group.

TABLE I - Serum levels of investigated parameters in all experimental groups

Group	BUN	Creatine	PON-1	AST	ALT
Control	15.6±5.08	0.518±0.148	171±28.8	77.3±18	47.3±4.82
Cisplatin	$91.7±12^{***}$	$2.21±0.777^{***}$	$34.8±10.6^{***}$	$254±51.7^{***}$	$137±12.3^{***}$
Cisplatin + Agomelatine 20 mg/kg	$44.2±5.9^{###}$	$1.08±0.196^{###}$	$135±11.3^{###}$	$184±11.6^{#}$	$81.8±8.58^{###}$
Cisplatin + Agomelatine 40 mg/kg	$50±9.58^{###}$	$1±0.342^{###}$	$126±11.3^{###}$	$270±85.3^{+}$	$148±28.5^{+++}$

All data expressed as mean±SD. $^{***}p<0.001$ versus control group and $^{###}p<0.001$, $^{#}p<0.05$ versus cisplatin group and $^{+++}p<0.001$, $^{+}p<0.05$ versus cisplatin+agomelatine (20 mg/kg) group

Agomelatine (20 and 40 mg/kg) conferred protection against cisplatin-induced oxidative stress in the kidney tissue

By determining CAT, SOD, GSH and AOPP levels, oxidative damage caused by cisplatin in kidney tissue was assessed (Figure 2, Table II). Cisplatin significantly decreased CAT, SOD and GSH levels (Figure 2a-c). Additionally, cisplatin caused a significant increase in the AOPP levels (Figure 2d). Nevertheless, agomelatine at 20 and 40 mg/kg doses significantly inhibited the cisplatin-induced decrease in CAT, SOD, and GSH levels (Figure 2a-c). Additionally, agomelatine (20 and 40 mg/kg) significantly blocked cisplatin-induced protein peroxidation, as seen in AOPP levels (Figure 2d). However, there was no significant difference seen between 20 and 40 mg/kg agomelatine treatments in SOD, CAT, GSH and AOPP levels.

TABLE II - Levels of investigated parameters in kidney tissue for all experimental groups

Group	SOD	CAT	GSH	AOPP
Control	325±38.4	99.7±13.9	36.9±4.32	4.77±1.38
Cisplatin	112±25.9***	19±2.24***	14.1±2.11***	40±6.73***
Cisplatin + Agomelatine 20 mg/kg	204±18$^{\#\#\#}$	45.1±4.73$^{\#\#\#}$	22.7±1.77$^{\#\#\#}$	21±3.73$^{\#\#\#}$
Cisplatin + Agomelatine 40 mg/kg	205±15.1$^{\#\#\#}$	43±5.62$^{\#\#\#}$	23.7±2.64$^{\#\#\#}$	38.3±9.21$^{\#\#\#}$

All data expressed as mean±SD. All data expressed as mean±SD. ***$p<0.001$ versus control group and $^{\#\#\#}p<0.001$ versus cisplatin group

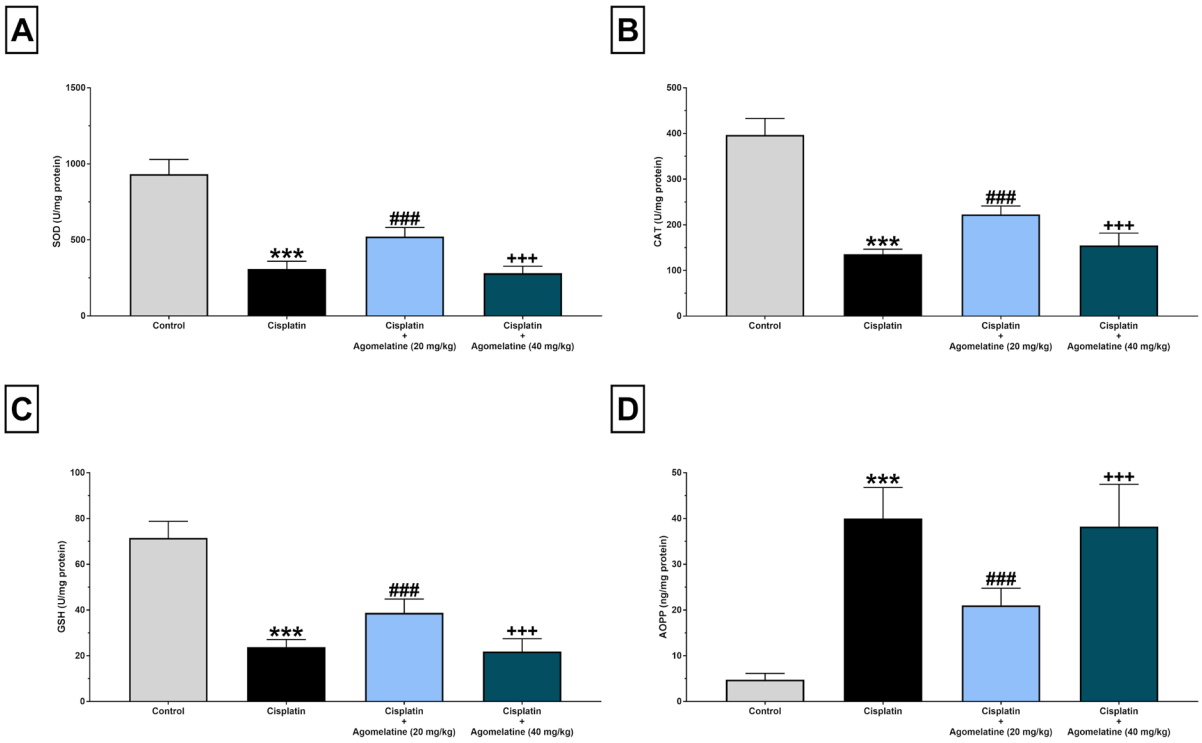

FIGURE 2 - Levels of antioxidant and oxidant markers in kidney tissue. A: Superoxide dismutase (SOD), B: Catalase (CAT), C: Glutathione-S-hydroxylase (GSH), D: Advanced oxidation protein products (AOPP). Agomelatine 20 and 40 mg/kg significantly attenuated cisplatin-induced oxidative stress and resulted in a decrease in antioxidant enzymes. All data represented as mean±SD and stand for five animals per group. $^{***}p<0.001$ versus control group and $^{###}p<0.001$ versus cisplatin group.

Agomelatine (40 mg/kg) does not attenuate cisplatin-induced oxidative stress in liver tissue

It was important to delineate whether agomelatine administration had deleterious effects on liver tissue, therefore CAT, SOD, GSH and AOPP levels were also evaluated. In parallel with kidney tissue, cisplatin significantly decreased CAT, SOD and GSH levels and increased AOPP levels (Figure 3, Table III). However, whereas agomelatine at the dose of 20 mg/kg significantly diminished CAT, SOD and GSH decrease, and AOPP increased due to the cisplatin insult, agomelatine at the dose of 40 mg/kg for its part failed to show this preventive action (Figure 3).

TABLE III - Levels of investigated parameters in liver tissue for all experimental groups

Group	SOD	CAT	GSH	AOPP
Control	933±96.1	397±35.9	71.5±7.2	17.9±4
Cisplatin	$309±51.1^{***}$	$136±10.6^{***}$	$23.8±3.32^{***}$	$83±9.92^{***}$
Cisplatin + Agomelatine 20 mg/kg	$522±59.2^{###}$	$223±18.4^{###}$	$38.7±6.05^{###}$	$44.3±9.54^{###}$
Cisplatin + Agomelatine 40 mg/kg	$282±45.2^{+++}$	$155±26.7^{+++}$	$21.9±5.59^{+++}$	$40.8±8.5^{+++}$

All data expressed as mean±SD. All data expressed as mean±SD. $^{***}p<0.001$ versus control group and $^{###}p<0.001$ versus cisplatin group and $^{+++}p<0.001$ versus cisplatin+agomelatine (20 mg/kg) group

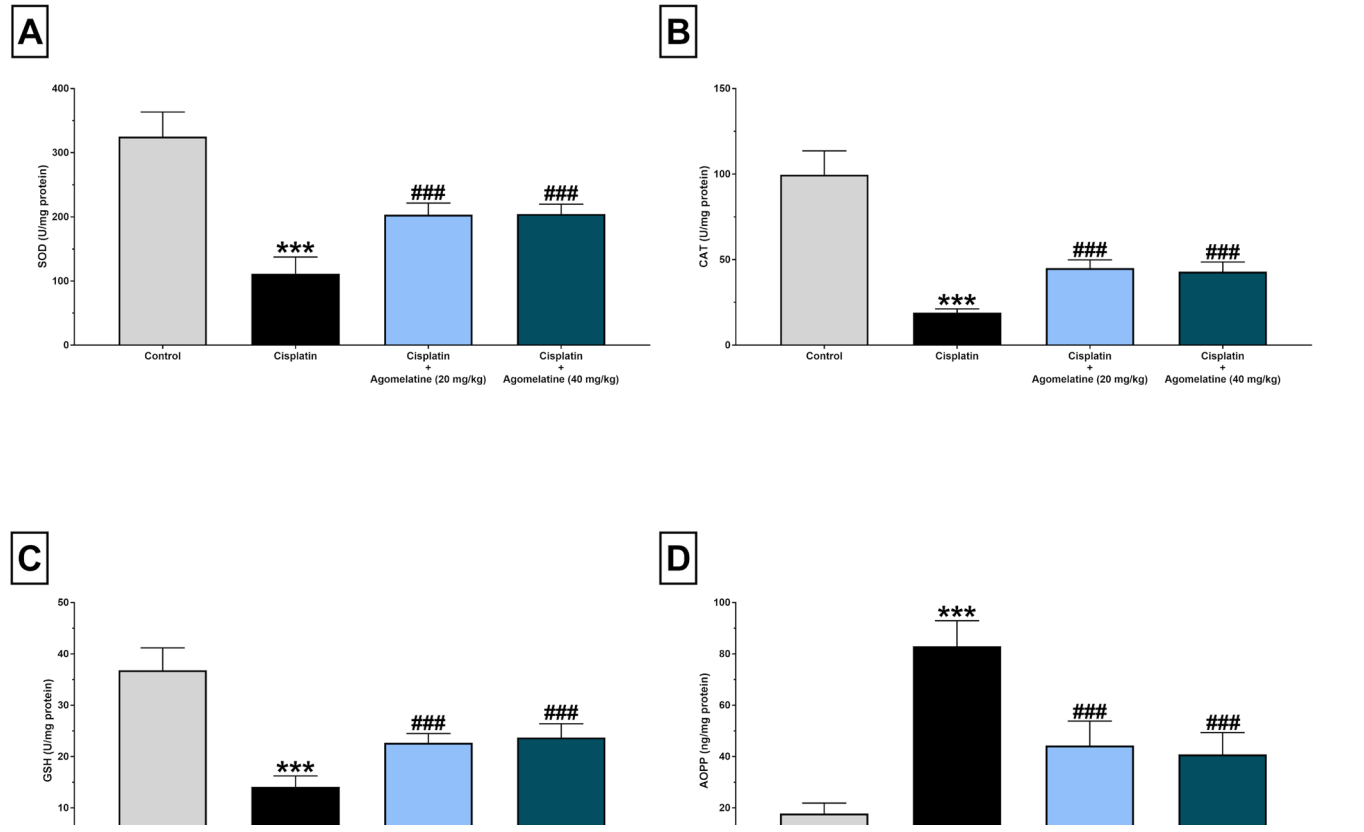

FIGURE 3 - Levels of antioxidant and oxidant markers in liver tissue. A: Superoxide dismutase (SOD), B: Catalase (CAT), C: Glutathione-S-hydroxylase (GSH), D: Advanced oxidation protein products (AOPP). Agomelatine 20 mg/kg significantly attenuated cisplatin-induced oxidative stress and resulted in a decrease in antioxidant enzymes. But, agomelatine at the dose of 40 mg/kg failed to protect liver tissue against cisplatin insult. All data represented as mean±SD and stand for five animals per group. $^{***}p<0.001$ versus control group and $^{###}p<0.001$ versus cisplatin group and $^{+++}p<0.001$ versus cisplatin+agomelatine (20 mg/kg) group.

Effects of agomelatine on kidney and liver tissue structures

Histopathological analysis was performed to observe and evaluate the effects of agomelatine on kidney and liver tissue. No significant pathological mark was observed in the control group (Figure 4a). Significant dilatation proximal and distal tubules, desquamation in tubular epithelia with vacuolization and atrophy was noted in the kidney sections in the cisplatin group (Figure 4b). Furthermore, sinusoidal dilatation, vascular congestion, pyknotic nucleus, hepatocellular degeneration and fibrosis, was seen in the liver sections in the cisplatin group (Figure 5e). Cisplatin therefore significantly increased the histopathological score (Figure 4e, 5e). At the dose of 20 mg/kg, agomelatine significantly ameliorated the cisplatin-induced pathology score in both tissues and caused a decrease in histopathological signs (Figure 4c, 5c, 4e, 5e, Table IV). Although agomelatine 40 mg/kg also inhibited cisplatin-induced kidney damage, albeit without showing the significant difference with a dose of 20 mg/kg (Figure 4e), it failed to inhibit cisplatin-induced damage liver (Figure 4d, 5d).

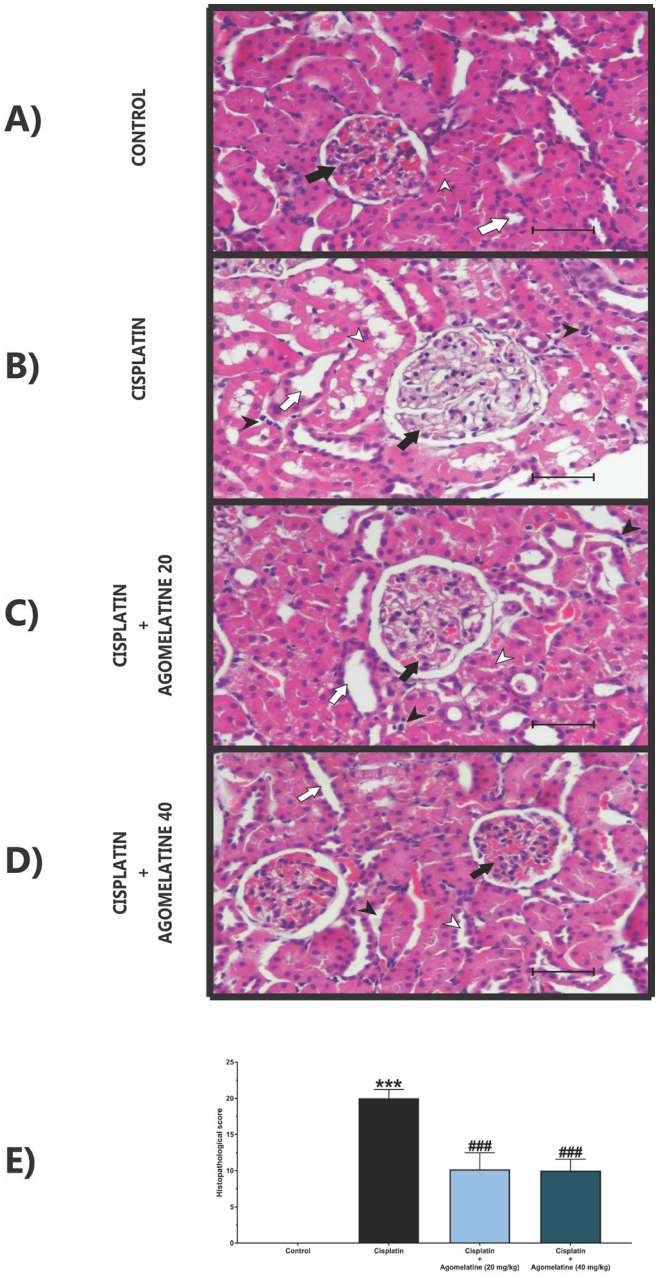

FIGURE 4 - Histopathological presentation of kidney tissue with H&E staining. Scale bar=50 µm with 40x magnification. Standard kidney histology in the control group. Representation of healthy glomerular (black arrow), distal tubule (white arrow) and proximal tubule (arrowhead) (a). Glomerular sclerosis and necrosis (black arrow), dilatation in distal (white arrow) and proximal tubules (white arrowhead) and edema in cisplatin group (b). Compared with the cisplatin group, there were significantly fewer pathological findings in agomelatine 20 and 40 mg/kg groups (c, d). Total histopathological scores of all groups (e). All data represented as mean±SD and stand for three animals per group. $^{***}p<0.001$ versus control group and $^{###}p<0.001$ versus cisplatin group.

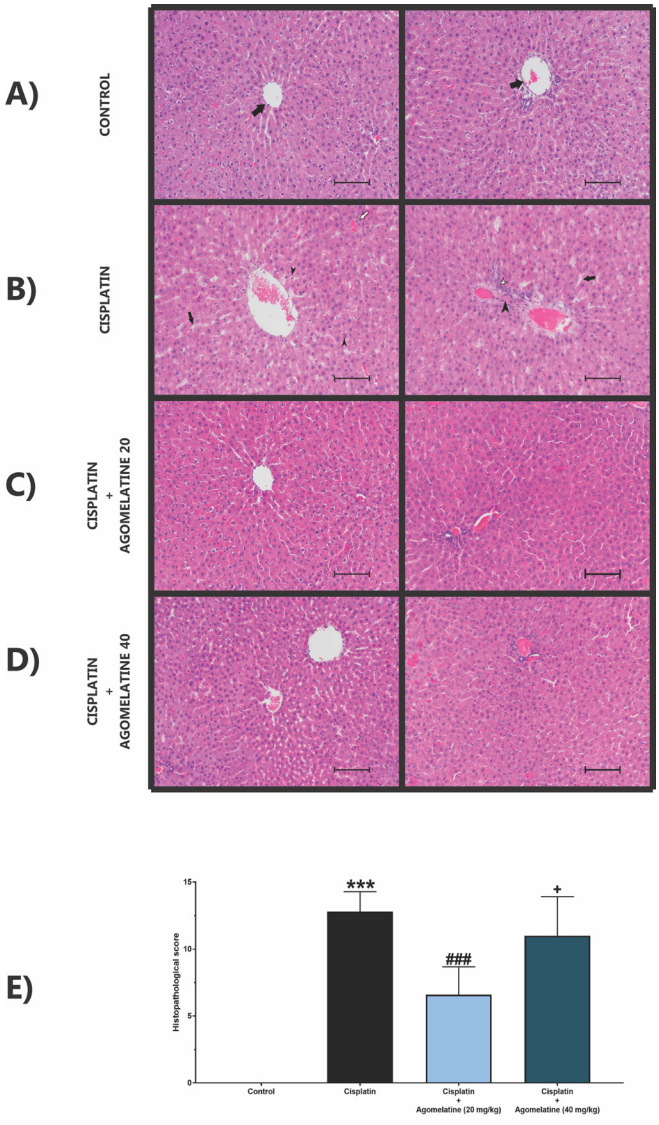

FIGURE 5 - Histopathological presentation of liver tissue with H&E staining. Scale bar=50 µm with 40x magnification. Standard liver histology in the control group. Representation of terminal hepatic venule (black arrow) and hepatic cords circumventing area with the normal sinusoidal look (a). Dilatation in sinusoids (black arrow), vascular congestion (white arrow) and hepatocellular degeneration (white arrowhead) in the cisplatin group (b). Compared with cisplatin group, there were significantly fewer pathological findings in the agomelatine 20 mg/kg group (c). But at the dose of 40 mg/kg agomelatine, similar findings with the cisplatin group were present (d). Total histopathological scores of all groups (e). All data represented as mean±SD and stand for three animals per group. $^{***}p<0.001$ versus control group and $^{###}p<0.001$ and $^{+}p<0.05$ versus cisplatin+agomelatine (20 mg/kg) group.

TABLE IV - Results of histopathological scores in all experimental groups in kidney and liver tissues

Group	Kidney	Liver
Control	0±0	0±0
Cisplatin	20±1.22***	12.8±1.48***
Cisplatin + Agomelatine 20 mg/kg	10.2±2.28$^{\#\#\#}$	6.6±2.07$^{\#\#\#}$
Cisplatin + Agomelatine 40 mg/kg	10±1.58$^{\#\#\#}$	11±2.92^{+}

All data expressed as mean±SD. $^{***}p<0.001$ versus control group and $^{\#\#\#}p<0.001$ and $^{+}p<0.05$ versus cisplatin+agomelatine (20 mg/kg) group

DISCUSSION

In the current study, we demonstrated whether agomelatine could exacerbate or protect cisplatin-induced nephro/hepatotoxicity. Because of the dose-limiting effects of cisplatin, reliable therapeutic interventions for preventing or treating cisplatin-induced kidney and liver damage during aggressive tumor therapy, are urgently needed. Although most of the studies focused on cisplatin-induced kidney injuries, only a few investigated cisplatin-induced hepatotoxicity, or both. Therefore, our study aimed to demonstrate the effects of agomelatine on both tissues. Our results suggested that agomelatine (20 and 40mg/kg) prevented cisplatin-induced kidney injury, possibly increasing antioxidant enzymes. However, agomelatine (20 mg/kg) protected against cisplatin-induced hepatoxicity but at the dose of 40 mg/kg, it failed to protect liver tissue's structural integrity. PON-1 has been a widely studied esterase, which mainly synthesized in the liver (Camps, Marsillach, Joven, 2009). It is known to have a role in protection against poisoning against organophosphate or deactivation of reactive molecules, such as platinum-based compounds (Litvinov, Mahini, Garelnabi, 2012). Additionally, the protective role of PON-1 in oxidative stress and protein oxidation is described in previous studies (Koyuncu *et al.,* 2017). Thus, in our study, we also investigated possible changes in plasma PON-1 levels after cisplatin insult. Our results demonstrated that agomelatine prevented the cisplatin-induced PON-1 decrease in plasma. Our results are also in line with other studies which showed that agomelatine treatments alleviated cisplatin-induced total oxidative stress (Demirdaş, Nazıroğlu, Ünal, 2016; Yigitturk *et al.,* 2017).

Cisplatin-induced nephrotoxicity has several different mechanisms, but oxidative stress is regarded as a major contributor (Sun *et al.,* 2019). Cisplatin is known to accumulate in the mitochondria and disturb ATP production of all mitochondrial complexes (I-V) (Galgamuwa *et al.,* 2016). Therefore, this detrimental metabolic shift causes an enormous amount of reactive oxygen species (ROS) and superoxide anion production. Following impaired ATP production and increased ROS levels, cellular structures rapidly oxidized, unable to maintain cell integrity after that point. However, antioxidant enzymes and systems in the cells, attempting to cope with that increased stress, then start the regenerative process. In particular, proximal tubular cells in the kidney have regeneration capacity after tissue damage in pro-oxidant conditions (Galgamuwa *et al.,* 2016). In cisplatin-induced oxidative damage, accumulated cisplatin overcomes this regenerative and cellular antioxidant system, and causes progressive kidney damage. Compounds with high antioxidant capacity were shown to be protective against cisplatin insult in kidney tissue (Hajian, Rafieian-Kopaei, Nasri, 2014). One of these compounds, melatonin, was demonstrated as being nephroprotective in cisplatin-induced toxicity with increased cellular antioxidant enzymes, by several research groups (Kilic *et al.,* 2013; Şener *et al.,* 2000). Şener *et al*. (2000), in particular showed that antioxidant action of melatonin in cisplatin-induced kidney damage. Moreover, the nephroprotective action of melatonin has been recently demonstrated

in cisplatin-induced kidney injury in humans. In contrast to melatonin, insofar as we have seen, this is the first study that showed nephroprotective action of agomelatine against cisplatin-induced kidney injury, although antioxidant and protective effects of agomelatine have been reported by several experimental models (Aguiar *et al.,* 2013). Demirdas *et al.,* demonstrated that antioxidant effects of agomelatine in chronic mild stress-induced depression model in the brain, kidney and liver (Demirdaş, Nazıroğlu, Ünal, 2016). Although their group chronically administered agomelatine, it is rational to think the difference between our liver tissue results is the result of the differences between the experimental models and that chronic, unpredictable, mild stress does not contain potent stressor such as cisplatin. In our study, agomelatine inhibited cisplatin-induced protein peroxidation and increased cellular antioxidant enzymes in kidney tissue. In addition, agomelatine inhibited an increase of markers that indicates kidney damage, which also underpinned a protective effect against cisplatin injury. Several contradictory reports have also suggested that agomelatine treatment could exacerbate oxidative stress and damage in living animals. Gunaydin *et al*. (2019) showed that agomelatine caused aggravated brain pathology in rotenone-induced Parkinson's disease model. The detrimental effect of agomelatine has also been suggested in chemically induced seizures (Aguiar *et al*., 2013). These reports suggested that strong antioxidant therapies might result in different toxicities in investigated tissues, when concomitantly administered. It is therefore important to clarify the possible relation between agomelatine and cisplatin in different tissues.

Although most studies have investigated cisplatin-induced nephrotoxicity, hepatotoxicity is another crucial problem limiting its usefulness (Lu, Cederbaum, 2006). In this study, we also investigated hepatic tissue and possible hepatic damage after cisplatin treatment. As is often used in the clinics, the serum levels of AST and ALT are generally considered hepatic damage markers (Sun *et al.,* 2019). In our study, cisplatin caused a marked increase in these enzymes' levels and agomelatine at the dose of 20 mg/kg inhibited this increase, as seen in the kidney tissue. Production of ROS and decreased antioxidant enzymes after the cisplatin treatment were seen in the liver tissue. Increased protein oxidation and decreased antioxidant capacity after the cisplatin treatment has already been demonstrated, which we also showed in our study (Ozkok, Edelstein, 2014; Soni *et al.,* 2018). Otherwise, agomelatine 20 mg/kg attenuated cisplatin-induced protein oxidation and prevented the decrease of antioxidant enzymes and although this agomelatine 20 mg/kg inhibited these cisplatin-induced oxidative stress, agomelatine 40 mg/kg for its part failed to show the same protection. Furthermore, the effects of cisplatin and 20 and 40 mg/kg doses of agomelatine were consistent with histopathological analysis, however the agomelatine 20 mg/kg inhibited cisplatin-induced structural alterations in hepatic tissue, and these effects were absent in the agomelatine 40 mg/kg group. In contrast to the current knowledge about agomelatine effects on liver tissue, we failed to observe any antioxidant action of agomelatine at the dose of 40 mg/kg. However, hepatotoxicity and liver damage seen in patients who have chronically used agomelatine was reported (Freiesleben, Furczyk, 2015; Pladevall-Vila *et al.,* 2019). As a result, we strongly hypothesize that increased dose of agomelatine resulted with deleterious effects on liver tissue. Acute liver injury after agomelatine treatment has also been recently reported (Montastruc *et al.,* 2014). Although this toxic effect was not fully clarified, studies demonstrated that agomelatine causes an accumulation in the hepatocytes (Freiesleben, Furczyk, 2015). With current knowledge and our experimental results, we conclude that deleterious effects might be mediated by two different mechanisms. First, in line with these studies, even though the agomelatine showed antioxidant and protective action in 20 mg/kg, this protective action possibly waned after agomelatine accumulation in the hepatocytes, resulting in aggravated pathology. Second, a possible slightly declined hepatic activity of CYP1A2, which is the enzyme for agomelatine metabolism, could cause a decrease in agomelatine detoxification while under cisplatin insult, and result in liver damage (Masubuchi, Kawasaki, Horie, 2006). These hypotheses should be further investigated for validation and clarifying the mechanism responsible for the liver toxicity we observed and which has been elsewhere reported.

Our results provide evidence for the possible protective action of agomelatine in cisplatin treatment. However, attention should be paid for agomelatine treatment during cisplatin chemotherapy because of the effects of agomelatine on hepatic tissue. These results need to be investigated in more detail, in order to identify the underlying mechanisms responsible for this discrepancy.

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Conflicts of interest

The authors declare no conflict of interest present in this study

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