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Protective effect of melatonin on cisplatin induced acute kidney injury: Role of regulation of heat shock proteins expressions

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Chemotherapy is one of the major treatment approaches for cancer, and these agents are known to cause severe side effects, including damaging vital organs. Cisplatin (CP) is a commonly used chemotherapeutic agent in the treatment of many cancer types, particularly lung, breast, ovarian, testicular and head-neck cancers. CP is reported to cause damage to damage on brain, kidney, liver and gonads. In this study, is we investigated the protective effects of melatonin (MEL) in acute kidney injury (AKI) induced by CP via assessment of heat-shock protein (HSP) induction in rats. For this purpose, total 40 Wistar albino rats were divided into four groups: Control (n=10), MEL (n=10, 10 mg/kg/i.p. melatonin for 8 days), CP (n=10, 7 mg/kg/i.p. cisplatin at the 5th day), and CP+MEL (n=10, 10 mg/kg/i.p. melatonin for 8 days and 7 mg/kg/i.p. cisplatin at the 5th day). After kidney tissues were extracted, histopathological changes were evaluated and immunoreactivities of HSP47, HSP60, HSP70 and HSP90 in renal cortex were detected via immunohistochemistry. Moreover, blood serum BUN (blood urea nitrogen), creatinine and uric acid levels were measured to assess kidney function. CP group showed histopathological deterioration, and MEL treatment attenuated this damage in CP+MEL group. An increase in HSPs immunoreactivities were detected in renal cortex of CP group when compared with the control and MEL groups, showing increased HSP response because of CP-induced AKI. However, CP-induced HSP induction was significantly lower in the CP+MEL group. Similarly, blood serum BUN, creatinine and uric acid levels were higher in CP group while CP+MEL group showed decreased levels of these parameters. Our results suggest that MEL could exert a significant protective effect against CP-induced AKI via reducing HSP response.

Keywords: Cancer chemotherapy

As the success rate of chemotherapy increases, the long-term survival rates and life quality of cancer patients in cancer treatment have also increased. However, chemotherapeutics can cause serious damage to healthy organs such as kidney[1]. The kidney is one of the organs most negatively affected by chemotherapeutics, and chemotherapy-induced kidney injury is one of the most common and investigated side effects[2,3]. Cisplatin (CP), CP-diaminedichloroplatin-II, is widely used in the several cancer treatments such as lung, breast, ovarian, testicular, head-neck cancers, because of its antiproliferative effects on cancer cells[4,5]. However, it causes a serious damage on brain, kidney, liver, and gonads[6-10].

Although CP can bind a variety of biological components, such as proteins, RNA, membrane phospholipids, microfilaments, and peptides containing thiols, DNA is thought to be one of its primary targets. Therefore, CP causes a serious DNA damage in many healthy tissues. When CP enters cells, it goes via aquation, and the platinum atom forms a covalent bond with the N7 position of purines. This results in roughly 65% GpG, 25% ApG 1,2 intra-strand crosslinks, 5-10% GpNpG 1,3 intra-strand crosslinks, and a lesser percentage of interstrand crosslinks. The principal mechanism for detecting and repairing CP-induced DNA adducts is the nucleotide excision repair route, which is one of several repair mechanisms that cells activate in response to CP[11].

Application of CP in cancer therapy causes acute kidney injury (AKI) in patients, and CP-induced AKI is a complex pathophysiological process that is associated with many intracellular events such as DNA damage[12], apoptosis, vascular damage, oxidative and endoplasmic reticulum stress, and inflammation[13]. Several studies have shown that renal function is impaired in CP-induced AKI and, accordingly, various renal parameters of the kidney are severely altered. Blood urea nitrogen (BUN), creatinine, and uric acid, whose serum levels are elevated due to impaired kidney function in CP-induced AKI, are commonly used markers of kidney damage[14].

Exposure to stress conditions such as thermal stress induces overexpression of various stress-related proteins like heat shock proteins (HSPs) in many cells. HSPs are overexpressed in the situation of various processes (oxidative stress, inflammation, ischemia etc.) as protective markers to keep tissues or organs safe against injury. One of the most effective strategies for protecting kidney tissue is the action of HSPs (chaperones) in renal injury. HSPs are divided into many subfamilies according to molecular weights. HSPs are constitutively expressed in healthy kidney, but expression patters of these chaperons vary under stress conditions.

Heat shock protein (HSP) 47 is one of the chaperones that regulates the conformational structure of procollagen[15]. Overexpression of the HSP47 in the glomerular region is thought to lead to glomerular sclerosis as a result of excessive collagen production[16]. Therefore, it is suggested that HSP47 has an important function in the regulation of the synthesis and degradation of extracellular matrix in tubulointerstitial fibrosis in the kidney[17]. Chaperones in the HSP60 family include molecular chaperones that are involved in crosslinking monomeric proteins and assembling them into oligomeric complexes. It has been reported that tubular damage can be delayed by preventing Bax-mediated apoptosis through early stimulation of HSP60 in acute kidney injury caused by CP[18]. Among HSP families, HSP70 has several functions like all chaperone proteins. These tasks include acquiring the conformational shape of newly generated peptides, recovering partially damaged proteins and degrading permanently denaturized proteins, and transporting proteins from intracellular membranes to organelles via cytoskeleton contact[19]. HSP70 serves as a phosphorylation blocker of stress kinases such as JNK and p38 MAPK leading to suppression of apoptotic pathways and overexpression of proinflammatory cytokines, thereby suppressing inflammation and apoptosis[20]. Constitutively expressed in healthy cells, the HSP90 complex helps to gain the conformational structure of various proteins such as tyrosine kinases (Akt and MEK), transcription factors (androgen receptor, estrogen receptor and p53), structural proteins (tubulin, actin), and hypoxia-inducible factor-1alpha (HIF-1α)[21]. Several studies showed that HSP90 expressions are increased in tubular cells in AKI induced by ischemia or toxic agents. Therefore, HSP90 is thought to be an important component of the preservative system that plays an active role in repairing damaged tubules and regeneration through differentiation of new tubular cells[22].

Melatonin (MEL), as a hormone, plays a role in regulating and maintaining the circadian rhythm. It regulates reduction/oxidation mechanisms under various stress conditions such as heat stress and toxicity and has many biological properties such as anti-inflammation, antioxidant and anti-apoptosis[23-25]. Many studies have showed that MEL protects kidney tissue from the side effects of CP by inhibiting oxidative stress, inflammation, and apoptosis in CP-induced AKI[26].

It is known that overexpression of heat shock proteins is triggered when cell function gets corrupt and is disrupted by many reasons such as ischemia and toxicity. Therefore, it can be expected that oxidative stress, inflammation, and apoptosis induced by CP administrations in the kidney will not activate HSPs[27]. Induction of HSPs by tissue damage indicates that a serious damage has been induced in the tissue. The suppression of heat shock response by various therapeutics is important in terms of determining their therapeutic efficacy. Therefore, this study focuses on the protective effect of melatonin, which has many biological activities, against cisplatin-induced acute kidney injury on the regulation of heat shock proteins. After literature review, it was determined that there are in sufficient studies investigated the protective effects of MEL in the CP-induced AKI via regulation of HSPs. Therefore, we studied the effects of MEL on damage induced HSP induction in AKI caused by CP chemotherapy. For this purpose, we evaluated kidney tissue damage histologically and compared the expression patterns of HSP47, HSP60, HSP70 and HSP90 among experimental groups. In addition, we demonstrated the effect of MEL on the increased serum BUN, creatinine, and uric acid levels by CP applications via biochemical methods. In this study, we have made an attempt to demonstrate the ameliorative potential of Melatonin (MEL) on heat shock proteins (HSP) induction against cisplatin (CP)-induced acute kidney injury (AKI).

Materials and Methods

Chemicals

Cisplatin was bought from Koçak Farma Pharmaceuticals and Chemical Industry Inc., Turkey with the name of Sisplatin. Cisplatin, 50 mg iv/1 flk + 1 amp, containing concentrated solution for direct injection, was used. Melatonin (73-31-4, Purity ≥98%) was purchased from Sigma-Aldrich Company Ltd., USA. All kits used for biochemical analysis of BUN, creatinine and uric acid were purchased from Sigma-Aldrich Company Ltd., USA, and they were suitable for rat studies.

Experimental protocol

The sample size of this experimental study was calculated by power analysis using the G*Power v3.1 software. There was a total of 40 rats in four groups, and 90.94% power expectation was found with 10 rats in each group. The number of experimental animals per group was determined according to these results. Moreover, the experimental protocol was approved by the Erciyes University's Experimental Animal and Local Ethics' Committee with number 21/136/2021. Experimental animals used in this experimental study received necessary care according to the standard guidelines. Forty Wistar albino rats (male, 8 wk old, weighing 200-250 g) were purchased from Hakan Cetinsaya Experimental and Clinic Research Center, Erciyes University, Kayseri, Turkey. Animals were host at room temperature (20-24°C) for 12 h light/dark cycle and optimum humidity. Standard chow and tap water were given to animals *ad libitum*. Total 40 Wistar albino rats were randomly separated into four groups; The control group (n=10) was not treated with any drugs, the MEL group (n=10) was administered 10 mg/kg/i.p. melatonin for 8 days[28], and the CP group (n=10) was given 7 mg/kg/i.p. cisplatin on the 5th day of the experiment[29], and the CP+MEL group (n=10) was injected with 10 mg/kg/i.p. melatonin for 8 days and were given 7 mg/kg/i.p. cisplatin on the 5th day of the experimental period. Treatments continued for 8 days. After experimental procedure, 30 mg/kg ketamine and 4 mg/kg xylazine were used for anesthesia and they were sacrificed. Kidney tissues were collected for histopathological and immunohistochemical examinations and blood samples were taken for biochemical examinations.

Histopathological evaluation

Kidney tissues were fixed in 4% formaldehyde solution for histopathological investigations. After dehydration and clearing, kidney samples were embedded in paraffin blocks. Sections were stained and photographs were taken with a light microscope for histopathological evaluation (Olympus BX51, Center Valley, PA).

Hematoxylin and eosin staining (H&E)

For deparaffinization, sections were heated to 58°C for 2 h. Sections were deparaffinized, then rehydrated with an alcohol series and washed with tap water. Sections were stained with Hematoxylin and Eosin at room temperature. After dehydration and cleaning, histopathological changes in the kidney tissues of the experimental groups were determined by a light microscope to detect histological alterations.

Kidney injury score

For histopathological scoring of kidney sections, following criteria were used; degeneration of glomerulus, tubular epithelial degeneration, tubular dilatation, hemorrhage and mononuclear cell filtration. Scoring was performed as follows: 0 = not at all, 1 = 0-25%, 2 = 26-45%, 3 = 46-75%, and 4 = 76-100%[30]. Quantitative data obtained were analyzed and compared between experimental groups.

Calculation of Bowman's space percentage

Bowman's capsule diameter and glomerular capillary tuft diameter were measured by Image J program (1.45s, National Institute of Health, USA, RRID: SCR_003070) on the photographs. After measuring, Bowman's space percentage was calculated via a formula given below. Changes in the % Bowman's space area were statistically analyzed and compared among the experimental groups[31].

$$\%\ Bowman'space = \frac{(\text{BC diameter} - \text{GCT diameter})\ x\ 100}{\text{BC diameter}}$$

where BC, Bowman's capsule and GCT, Glomerular capillary tuft.

Immunohistochemical staining

Immunohistochemistry method was applied to determine the changes in the HSP47, HSP60, HSP70, and HSP90 immunoreactivities in the kidney. Kidney tissues embedded in paraffin blocks were cut into 5 µm pieces. The parts were kept in the oven for at least 2 h at 60°C for melting of paraffin. Xylene and alcohol series were used to deparaffinize and rehydrate the tissues. Antigen retrieval was achieved by immersing sections in 0.01 M citrate buffer and heating them in a microwave oven at 350 W. The portions were then immersed in phosphate buffered saline (PBS) three times for five minutes each time. To suppress endogenous peroxidase activity, the sections were immersed in 3% (w/v) H_2O_2 for 10 min. The tissues were then immersed in ultra v block solution for 5 min after being washed three times in PBS. After then, sections were incubated with HSP47 (Cat. No: BS-1538R, Bioss, USA), HSP60 (Cat. No: BS-0191R, Bioss, USA), HSP70 (Cat. No: BS-0126R, Bioss, USA) and HSP90 (Cat. No: BS-0135R, Bioss, USA) antibodies overnight at 4°C. Sections were washed three times with PBS the next morning before being instilled with the secondary antibody (TA-125-HDX, Thermo Fisher Scientific, Waltham, MA, USA) for 10 min at the room temperature. The immunoreaction was amplified with the streptavidin-avidin-peroxidase complex after washing with PBS, and the sections were visualized with 3,30-P-diaminobenzidine tetrahydrochloride (TA-060-HDX, Thermo Fisher Scientific, Waltham, MA, USA) mildly counter-stained with Gill hematoxylin. Finally, increasing alcohol series was used for dehydration, xylene for clearing, and finally they were covered with entellan[2].

Semi-quantitative immunohistochemistry

All stained sections from each experimental group were evaluated for the immunoreactivity of HSP47, HSP60, HSP70 and HSP90, and photographs were taken in 32 microscopic fields for each group. The intensity of immunoreactivity in both the cortical tubules and the glomerulus on these 32 photographs was measured with the Image J program (1.45s, National Institute of Health, USA, RRID: SCR_003070). In this measurement system, immunostained areas were detected via colour deconvolution section of this software on each photograph and the density of immunoreactivity was measured on these stained areas. For each experimental group, a total of 100 measurements were taken for HSP47 and HSP60, and a total of 160 measurements were taken for HSP70 and HSP90 expressions. The quantitative measurements data were statistically analyzed and compared among the experimental groups[32].

Biochemical analyses

Blood samples from experimental animals were centrifuged and serum samples were obtained for further biochemical analysis. Before the examinations of the serum samples, they were kept at −80°C. To assess the kidney function, serum blood urea nitrogen (BUN) (Cat. No: 04460715190, Biolab, Italy), creatinine (Cat. No: 04810716190, Biolab, Italy) and uric acid (Cat No: 03183807190, Biolab, Italy) levels were measured by Roche™ Cobas 8000 Modular Analyzer device at the Erciyes University Central Biochemistry Laboratory. All kits were suitable for rat studies. Obtained measurements were statistically analyzed and compared among the experimental groups.

Statistical analysis

GraphPad Prism version 9.00 for Mac, GraphPad Software, La Jolla, California, USA, was used for all statistical analyses. The normal distribution of the data was determined using the D'Agostino Pearson omnibus test and the Shapiro-Wilk test. One-way analysis of variance (ANOVA) and Tukey's post-hoc test were used to compare quantitative variables. The data were reported as the standard deviation of the mean of normalized data. It was determined that $P<0.05$ was statistically significant.

Results

Histopathological findings

Melatonin reduced cisplatin induced kidney injury

The histological alterations in the kidney tissue were investigated using H&E staining and light microscopy. Normal tissue morphology was determined in the Control and MEL groups. However, in the CP group glomerular degeneration, tubular epithelial degeneration, tubular dilatation, hemorrhage, and mononuclear cell filtration were observed and kidney injury score increased based on these criteria compared to the control and MEL groups ($P<0.0001$) (Fig. 1A). Unlikely, the kidney tissue morphology in the CP+ MEL group was similar to the Control and MEL groups. Glomerular degeneration, tubular epithelial degeneration and mononuclear cell filtration were negligible. However, tubular dilatation and hemorrhagic areas were detected in only a few sites (Fig. 1A). In terms of these histopathological criteria, a significant decrease was determined in the kidney injury score in CP+MEL group compared to CP group ($P<0.0001$) (Fig. 1B).

Improvement of degenerated glomerular morphometry by melatonin:

To determine the differences in glomerular morphometry among the experimental groups, the %Bowman’s space was calculated using the formula

Table 1 — Glomerular morphometry measurements of experimental groups

Groups	Control	MEL	CP	CP +MEL	*P*
Bowman's capsule diameter (μ)	76.6±1.02	76.61±0.74	75.33±0.96	74.2±1.05	0.219
Glomerular capillary tuft diameter (μ)	68.6±1.02	67.9±0.72	55.5±0.61*	65.7±1.05	0.001
% Bowman's space area	10.7±0.23	11.5±0.16	26.6±0.68*	11.4±0.15	0.001

[All data are expressed as the mean±standard error of mean (n=10). *P*<0.05 was considered as significant. * shows significant difference in comparison of CP group and the other experimental groups. CP, cisplatin; MEL, melatonin]

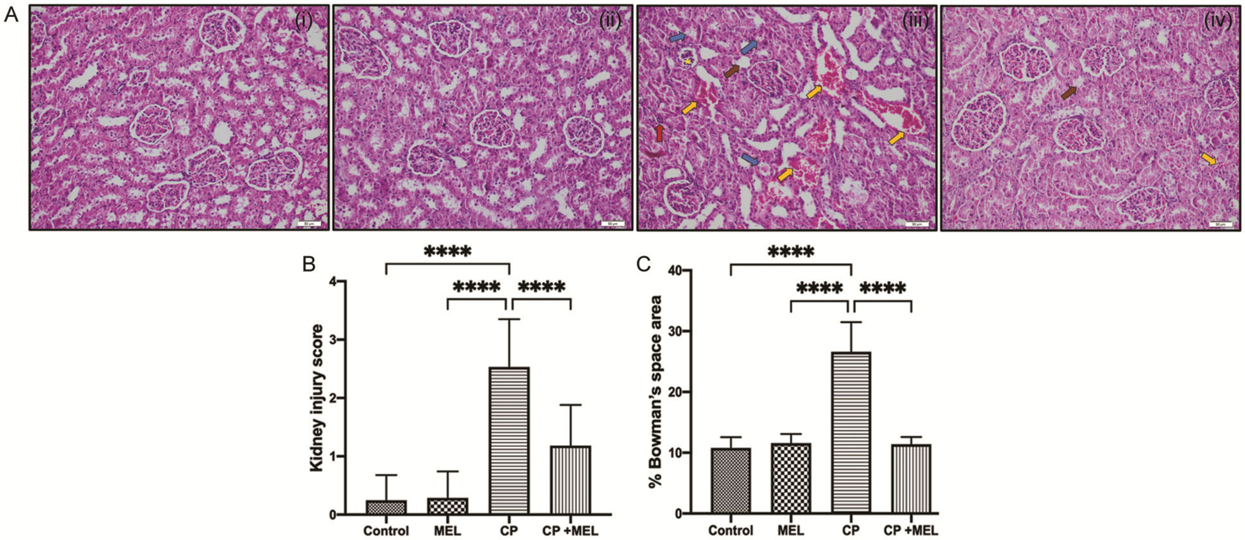

Fig. 1 — (A) Light microscopy of kidney tissues stained with H&E; (B and C) statistical analysis of kidney injury score and % Bowman's space area among experimental groups. Yellow asterisk (*) shows glomerular degeneration, blue arrows indicate tubular epithelial degeneration, brown arrows representtubular dilatation, yellow arrows point out hemorrhage, and red arrow shows mononuclear cell filtration. [Scale bar = 50 µm. *= *P* <0.05, **= *P* <0.01, ***= *P* <0.001, ****= *P* <0.0001. H&E, hematoxylin eosin; MEL, melatonin; CP, cisplatin]

given in the materials and methods section (Table 1). As a result of the statistical evaluation of the %Bowman's spaces, it was determined that the %Bowman's space was significantly increased in the CP group compared to the control and MEL groups, indicating that CP significantly reduced the glomerular capillary tufts by causing glomerular degeneration in the kidney tissue. However, the %Bowman's space in the CP+MEL group was similar to that of the control and MEL groups. This indicates that melatonin has an ameliorative effect on glomerular degeneration (Fig. 1C).

Melatonin reduces the increased heat-shock protein expressions

The expressions of HSP47, HSP60, HSP70 and HSP90 in kidney tissue were determined by immunohistochemistry utilizing the avidin-biotin method. Immunohistochemical stainings and statistical analysis of the immunoreactivity intensity measurements demonstrated the presence of HSP47, HSP60, HSP70 and HSP90 immunoreactivity in the kidney sections of all experimental animals. The HSP47, HSP60, HSP70 and HSP90 expressions in the MEL group were similar to those in the control group. However, immunoreactivity of these factors significantly increased in kidney tissue of the experimental animals in the CP group. In addition, HSP47, HSP60, HSP70 and HSP90 expressions in the CP+MEL group were substantially less compared to those in the CP group. Figure 2 shows immunohistochemical staining of these factors and Fig. 3 shows the statistical analysis of the density of immunoreactivity in the experimental groups.

Impaired kidney function was reduced by melatonin pre-administrations

The levels and statistical analyzes of kidney function parameters measured by biochemical assays in blood serum are shown in Fig. 4. Serum BUN, creatinine and uric acid levels in the control and MEL groups were quite similar and there was no statistically significant difference between these groups (*P* >0.05). However, the levels of these parameters in the CP group showed a significant increase compared to the control and MEL groups (*P* <0.05). In addition, serum levels of these parameters were lower in the CP+MEL group than

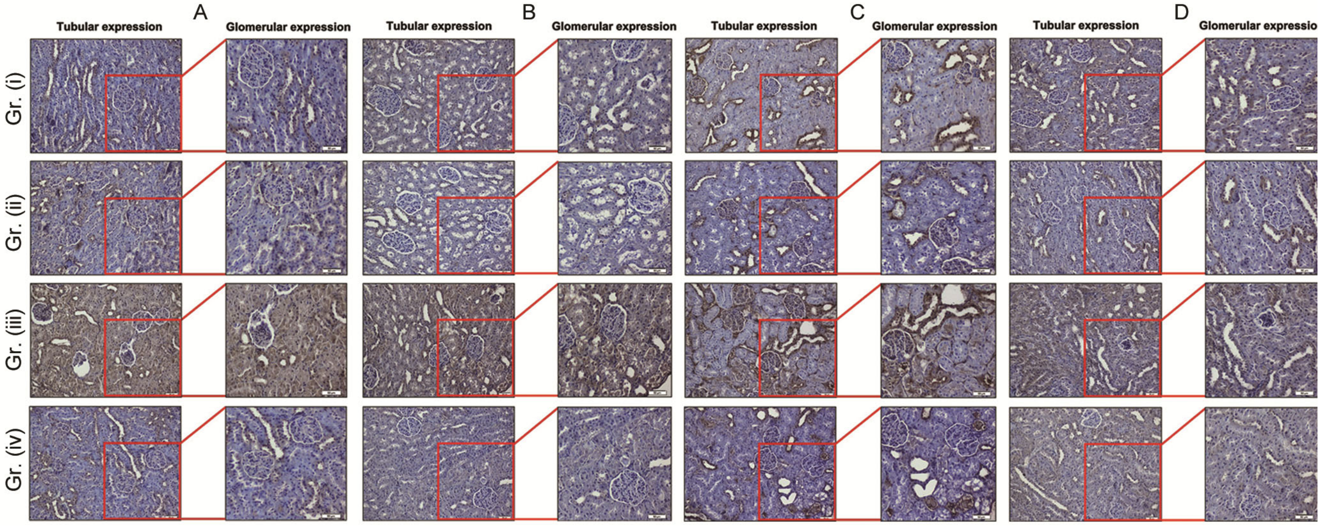

Fig. 2 — Immunohistochemical staining of (A) HSP47; (B) HSP60; (C) HSP70; and (D) HSP90 in kidney tissues. [Scale bar: 50 µm. MEL, melatonin; CP, cisplatin; HSP, heat-shock protein]

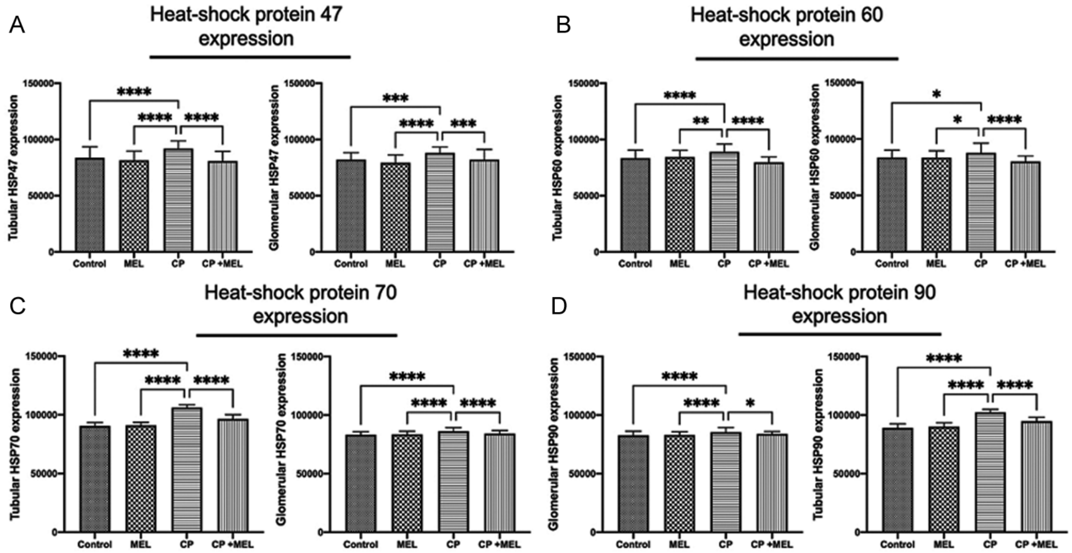

Fig. 3 — Immunoreactivity measurements of (A) HSP47; (B) HSP60; (C) HSP70; and (D) HSP90 in the glomerular and tubular region of renal cortex of kidney tissue sections of experimental groups. [*P <0.05, **P <0.01, ***P <0.001 and ****P <0.0001. CP, cisplatin; MEL, melatonin; HSP, heat-shock protein]

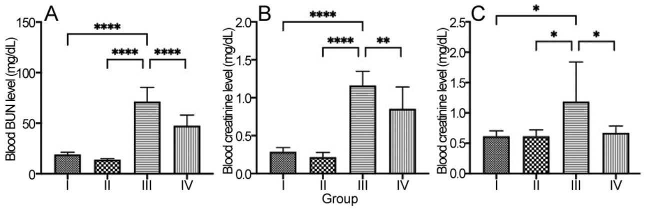

Fig. 4 — Measurements of (A) serum BUN; (B) creatinine; and (C) uric acid levels obtained by biochemical assay and statistical analysis of them among experimental groups. [*P <0.05; **P <0.01; ***P <0.001; and ****P <0.0001. CP, cisplatin; MEL, melatonin; BUN, blood urea nitrogen; HSP, heat-shock protein]

in the CP group, showing the ameliorative effects of melatonin on impaired renal function (P <0.05).

Discussion

Acute kidney injury (AKI) induced by drugs or toxic agents is one of the most common health problems in the world. Therefore, researchers are constantly looking for new combinations of treatments to alleviate AKI resulting from treatments such as cancer treatment. For safer chemotherapy, finding non-nephrotoxic drugs strategies and therapeutics is crucial for effective protection of kidney against AKI caused by nephrotoxic antineoplastic chemotherapeutics such as CP. Many studies have demostrated that CP chemotherapy causes AKI in the experimental animal models by triggering tubular epithelial injury, changes in glomerular space and infiltration of the inflammatory cells[33]. This study aimed to reveal the potential therapeutic effects of MEL on CP-induced AKI via regulation of HSP expressions. Our histopathological examinations showed that CP induced a serious kidney damage by causing glomerular degeneration, tubular epithelial degeneration, tubular dilatation, hemorrhage, and mononuclear cell filtration. However, in the CP+MEL group, a significant improvement was determined in the kidney sections. Therefore, we determined that MEL exerted an inspiring ameliorative effect against the detrimental effects of CP in kidney tissue at a dose of 10 mg/kg. This ameliorative effects of MEL in kidney tissue could be attributed to its well-known inhibitory effects on oxidative stress, inflammation and apoptosis.

HSP47 plays a substantial role in the conformation of the functional structure of procollagen and inhibiting the production of procollagen with abnormal conformation under various stress conditions such as heat stress. It has been shown in many studies that the expression of HSP47 in the kidney is increased under various toxicity stress conditions[34]. In our study, we observed that HSP47 expressions increased in both glomeruli and tubules in the renal cortex of experimental animals in the CP group. This increase in HSP47 expressions in the renal cortex could be due to glomerular sclerosis and tubulointerstitial fibrosis induced by CP applications. Therefore, we propose that increased HSP47 expression in the renal cortex is crucial for the amelioration of glomerular sclerosis and tubulointerstitial fibrosis because of the mechanism of its substantial role in the conformation of procollagen. On the other hand, we showed that MEL, which is used as a protective agent against CP-induced AKI, exerted a serious inhibitory effect against HSP47 induction. This suggests that MEL has a strong protective effect at 10 mg/kg in the renal cortex in the CP+MEL administered group which showed relatively less glomerular sclerosis and tubulointerstitial fibrosis.

HSP60 is a molecular chaperone that plays an active role in the proper folding of newly synthesized peptides, refolding of misfolded peptides, prevention of Bax-mediated apoptosis, induction of translocation of proteins to mitochondria, and regulation of anti-inflammatory response. Many studies have reported that HSP60 expressions are significantly increased in CP-induced AKI[35]. In our study, we suggest that a significant increase in the HSP60 expressions in both the glomerulus and tubules in the renal cortex of experimental animals in the CP group when compared with the Control and MEL groups are caused by the defects in protein conformations because of the oxidative damage caused by CP in the cells. Hence, the cells increase their HSP60 expression as a mechanism of protection against oxidative stress induced by CP. In addition, since CP induces oxidative stress induced inflammation and apoptosis in kidney tissue, we can say that the effects of HSP60 on Bax-dependent apoptosis and suppression of inflammation leading to increased HSP60 expression of glomerular and tubular cells in the kidney. Unlikely, HSP60 expressions were significantly less in the 10 mg/kg MEL administered group compared to the CP group. It suggests that MEL protects kidney tissue from the harmful effects of CP by causing inhibition of oxidative stress due to its antioxidant properties.

HSP70 is one of a family of chaperone proteins and has a variety of intracellular functions such as controlling the correct folding of newly synthesized natural proteins in kidney tissue, repairing misfolded proteins, and removing damaged proteins to a complete loss of function[36]. It also contributes to the suppression of inflammation and apoptosis by inhibiting the phosphorylation of stress kinases such as JNK and p38 MAPK[37]. Several studies have showed that HSP70 is upregulated in CP-induced AKI and is related to cell protection and survival[38]. In this study, the expression levels of HSP70 in glomerulus and tubules in the renal cortex were investigated in CP-induced AKI. In line with the data obtained from immunohistochemical staining, the significantly increased HSP70 expressions in the kidney tissues of experimental animals administered CP indicate a serious response to CP in the renal cortex. Thus, we propose that the HSP70 expressions increased in the renal cortex in the CP treated group to eliminate misfolded proteins because of oxidative damage caused by CP and to ensure the proper conformation of newly synthesized proteins. In addition, since the inhibitory effect of HSP70 on inflammation and apoptosis is known, we can say that the cells in the kidney tissue try to protect themselves by suppressing inflammation and apoptosis caused by oxidative stress induced by CP. Contrarily, MEL, which was used as a protective agent in this study, showed a serious protective effect on kidney tissue at a dose of 10 mg/kg, causing HSP70 levels in the glomerulus and tubules in the renal cortex to be like those in the control and MEL groups. We think that this situation occurred because of the inhibitor effects of MEL on protein misfolding and oxidative stress-induced inflammation and apoptosis due to its protective feature in kidney tissue.

It is known that HSP90, which is one of the heat shock proteins that is structurally synthesized in the body and plays an important role in the functional conformation of proteins, is also expressed in healthy kidney tissue. Recent studies have reported that HSP90 expression is increased in toxic conditions associated with inflammation, oxidative stress, and apoptosis, such as CP-induced AKI[39]. In the presented study, we support the idea that the increase in the expression of HSP90 in the glomerulus and tubules in the renal cortex of experimental animals administered with CP is due to protein misfolding because of CP-induced oxidative stress. We also suggest that because of these protein misfoldings due to oxidative stress, cells increase their HSP90 expression in the kidney tissue to ensure translation accuracy. Furthermore, we determined that MEL has a protective effect against CP applications in kidney tissue, and considering the decrease in HSP90 expressions, this therapeutic agent also has effects on the elimination of protein misfoldings resulting from CP-induced oxidative stress. These results indicate that MEL has positive effects on translation accuracy against the detrimental effects of CP-induced AKI.

Among the kidney function parameters, BUN, creatinine and uric acid are commonly used markers of kidney damage. In many studies, it has been reported that the serum levels of these parameters are significantly increased in AKI induced by CP[40]. In our study, significantly increased serum levels of BUN, creatinine and uric acid in the CP group when compared with the control and MEL groups indicates that CP causes serious damage to the kidney tissue and impairs kidney functions. Moreover, serum BUN, creatinine and uric acid levels in the CP+MEL group were similar to those in the control and MEL groups. It could be attributed to MEL's protective effect on CP-induced AKI at 10 mg/kg. Our histological and immunohistochemical data also support the above observation.

The present study has shown that cisplatin (CP) causes histopathological changes in the kidney tissues of the experimental animals. The significant increase in the expression of HSP47, HSP60, HSP70 and HSP90 in the glomeruli and tubules in the renal cortex indicates the cellular response to CP. We think that this increase in HSPs expression is due to the increase in the amount of misfolded protein in the renal cortex because of CP-induced glomerular sclerosis, tubular fibrosis, and oxidative stress. In addition, inflammation and apoptosis are induced in kidney tissue with the deterioration of cellular homeostasis possibly due to the misfolded protein accumulation. Further, the significant increase in the expression of HSP60, HSP70 and HSP90 in the renal cortex, which play an important role in the suppression of inflammation and apoptosis in the CP administered group, supports this idea. In this study, it has been proven that 10 mg/kg MEL administration is a chemical agent that affects HSPs and kidney function markers. Our immunohistochemical results showed that MEL caused lower expression of HSPs. Overexpression of HSPs induced by drugs or various chemicals are known to play a crucial role in eliminating glomerular sclerosis and tubular fibrosis in renal tissue, as well as repairing or eliminating misfolded or damaged proteins accumulating as a result of oxidative stress. Therefore, the findings obtained in this study showed that MEL showed a strong protective effect against CP-induced damage in kidney tissue, resulting in lower HSP induction.

Conclusion

Patients treated with chemotherapy are recommended by clinicians to receive supportive treatment of therapeutics with properties viz. antioxidant, anti-inflammatory, etc. Our results in the present study have demonstrated that melatonin (MEL) @10 mg/kg has the potential to protect patients receiving cisplatin (CP) chemotherapy from the harmful effects of acute kidney injury (AKI). Hence, MEL may be applied as a supportive treatment during the chemotherapy process.

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Conflict of Interest

Authors declare no competing interests.

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