Int J Acad Med Pharm, 2020; 2(2); 117-122
International Journal of Academic Medicine and Pharmacy
journal homepage: www.academicmed.org

Cisplatin-Induced Nephrotoxicity in Mouse Kidney Cortical Collection Duct Cells: Protective Role of Curcumin

Bilal ÇİĞ[1*]

[1] Ahi Evran University Medicine Faculty, Department of Physiology, Kirsehir, Turkey

Article info

Received: 10.04.2020
Received in revised form: 05.05.2020
Accepted: 19.05.2020
Available online: 05.06.2020

Keywords

Cisplatin
ROS
Apoptosis
Nephrotoxicity
Curcumin

Abstract

Research Article

The use of Cisplatin in chemotherapeutic treatment is limited to nephrotoxicity. It is stated that cytotoxic DNA platinum inserts, platinum transport mechanisms and pro-apoptotic DNA damage are the main causes of platinum-based renal toxicity. We have discussed the oxidative mitochondrial stress and apoptosis states that occur to understand the basic mechanism of this situation. Various antioxidants have been proposed in Cisplatin (Cisp) induced nephrotoxicities. Here we focused on the protective effects of curcumin (CurC), a powerful antioxidant, against this Cisp-induced nephrotoxicity. Groups were created as Control, Cisplatin, Curcumin and Cisp+CurC. There was a significant increase in Cisp-induced lipid peroxidation (LP), unlike GSH and GSH-Px values. Treatment with curcumin reversed these values. Mitochondrial membrane depolarization, Reactive oxygen species (ROS), caspase activations, and apoptosis levels significantly increased in Cisp groups. Curcumin brought these values back to normal. As a result, these data contributed to the understanding of molecular mechanisms in Cisp-induced nephrotoxicity. Curcumin usage in treatment protocols of cancer patients may be protective for Cisp-induced nephrotoxicity.

INTRODUCTION

Platinum-based cancer drugs provide effective treatment opportunities in malignancies. Cisplatin cis-diamminedichloroplatinum II)[1], a platinum-based primary care antineoplastic agent, discovered approximately 60 years ago, is used in the treatment of cancers of the head, neck, lung, ovaries and testicles.[2,3] In cancer treatment, cisplatin, which shows its antineoplastic effect by binding to the DNA double helix crosswise, turns into its metabolites by rapid enzymatic differentiation. The use of platinum-based chemotherapeutics is limited to many severe negative effects. As these chemotheurapeutics have to be used systemically in the treatment, they potentiate a risk of systemic complications. This is the platinum accumulation in the renal system for cisplatin usage and this dose-limiting side effect appears as nephrotoxicity.[4] The molecular mechanism underlying these side effects remains uncertain. In Cisp-induced nephrotoxicity, the role of reactive oxygen species (ROS), mainly occurring in kidney tubular cells, is an undeniable reality.[5] There are studies suggesting the clearance of ROS in the environment with antioxidative agents to prevent renal toxicity.[6,7]

Glutathione peroxidase (GSH-Px) is one of the leading natural antioxidant enzymes that eliminate oxidative damages in the cells.[8] In this study, GSH, GSH-Px and LP levels were discussed in case of Cisp-induced oxidative stress. Thus, we have evaluated how the strongest mammalian antioxidant enzyme GSH-Px contributes to normalization by eliminating lipid hyperoxides. The second aim of this study is to investigate how much the levels of oxidative stress parameters (ROS, Mitochondrial membrane depolarization, caspase3, caspase-9 and apoptosis) will change with CurC. In addition, the contribution of Curcumin (CurC) to this situation was investigated. Curcumin is an important phenolic compound obtained from Rhizoma curcuma longa which has spread all over the world from Southeast Asia. Curcumin, which reacts to reactive oxygen species by reacting directly to reactive nitrogen species, has been shown to exhibit a very strong anti-oxidant and anti-inflammatory effects.[9,10] In this study, the protective effect of CurC was investigated, on oxidative stress in Cisp-induced nephropathy.

MATERIAL and METHODS

Curcumin, JC-1, Dihydro-rhodamine 123 (DHR 123) and Cell viability dye were obtained from Sigma Aldrich USA. Mouse kidney cortical collection duct cells (mpkCCDcl4) were provided by Prof Dr Franziska Theilig at the University of Friborg in Germany. The mpkCCD$_{cl4}$ cells were preserved as

*Corresponding author: Bilal Çiğ , E-Mail; bilalcig@gmail.com, ORCID ID: 0000-0001-7832-066X ,
http://dx.doi.org/10.29228/jamp.43329

described previously.[11] The cells were passaged in 25 cm^2 culture flasks at 37 °C 5% CO_2, 95% air incubator (HF90, Heal Force Bio-meditech Holdings Limited, Shanghai, China).

The groups were designed as follows

Control: These cells were kept in an incubator for 24 hours without incubation

CurC: These cells were treatment with Curcumin (10 µM) for 24 hours.[12]

Cisp: These cells were treatment for 24 hours with Cisp (80 µM).[13]

Cisp+CurC: These cells were treatment for 24 hours with Cisp (80 µM), CurC (10 µM). Incubator conditions were:(5% CO_2, 95% air, 37 °C,).

Apoptosis and Caspase-3, Caspase-9 analysis

Detection of Cisp-induced apoptotic cells in mouse kidney cortical collection duct cells ($mpkCCD_{cl4}$, $1x10^6$ cell/ml) was performed as described in another study[14] with the Apopercentage kit (Biocolor Ltd., Northern Ireland). Asymmetry loss occurs in the membrane of cells that undergo apoptosis. The membranes of these cells, which have lost their asymmetry, are painted in red by APOPercentage kit and transported into the cell. In this way, quantitative apoptosis can be determined with the help of spectrophotometer. The determination of the activities of these caspases, which are compared to the stair steps on the road to apoptosis, was made on the basis of a previously reported method.[15] Caspase-3 and -9 were analysed with multiple well reader (plate reader, Tecan Austria) at 360, 460 nm excitation and emission wavelengths, respectively. Data were evaluated by calculating fluorescent units/mg protein compared to control.

Mitochondrial membrane depolarization

The cells (mpkCCDcl4, $1x10^6$ cells/ml) were loaded with JC-1 (1µM, 15 minutes at 37 °C) in a shaking water bath as done before in other studies.[16,17] After loading with dye, fresh Na-HEPES was added on the cells and resuspended and centri-fuged. JC-1 are cationic dyes that show mitochondrial potential dependent deposition. Determining the level of mitochondrial membrane depolarization is determined by a shift rate from green (525 nm) to red (590) fluorescent aggregation. The green/red conversion ratio depends on the mitochondrial membrane depolarisation that can affect single-component fluorescent signals.

Intracellular ROS production

Intracellular ROS production was analyzed with multiple well reader (Pleate reader, infinite pro200 brand, Austria). This process was determined by measuring the fluorescence intensity of rhodamine-123 (Rh-123). Briefly, cells ($1x10^6$ cells/ml) were loaded by incubating with 2µM dihydrorodamine-123 (DHR123) in a shaking water bath at 37 °C for 30 minutes. DHR123 marker, treated with these cells for 30 minutes, is converted into rhodamine 123 with fluorescent properties and used to determine the amount of reactive oxygen species. The excitation is 488 nm and the emission wavelengths are determined as 543 nm for this rhodamine.[18]

Cell viability (MTT) analysis

The cell viability activities were evaluated using the MTT assay, as described before.[19] In the cell viability analysis, the diphenyltetrazolium bromide substance, which is yellow in color, is transformed into a purple colored formocant crystal by mitochondrial respiratory tract by living cells. If the cells are not alive, this reaction does not take place. Color formation was also evaluated quantitatively on a colorimetric basis by spectrofluorometric method.[20] MTT dye (0,5 mg/ml), cells ($1x10^6$ cells/ml) were incubated in the dark for 60 minutes in a shaking water bath at 37°C. Optical density was performed at the same absorbance values (490, 650 nm) for each well using a plate reader.

Analysis of lipid peroxidation, GSH and GSH-Px levels

The determination of lipid peroxidation (LP) as the release of malondialdehyde (MDA) in Cisp-induced nephrotoxicity in kidney collection duct cells was evaluated by measuring the thiobarbituric acid (TBA) reaction as described in a previous study.[21] While GSH levels were determined with the help of Ellman reagent (412 nm wavelength), GSH-Px activity were analysed in the light of the literatüre.[22]

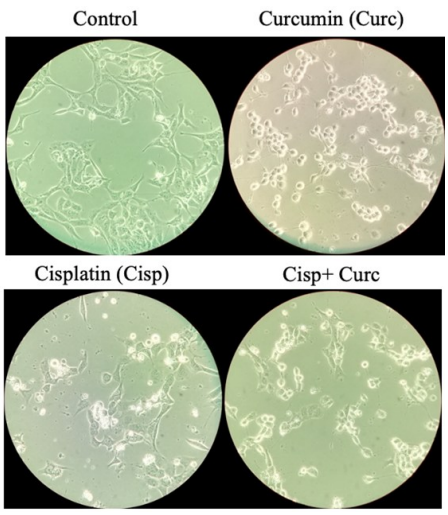
Statistical analysis

SPSS statistics program (version 17.0, software, SPSS, Chicago, IL) was used to analyze the data. Expressing the results was mean ± standard deviation (SD). Unpaired Mann-Whitney U-test and Variance analysis (ANOVA) were used and p values were accepted as less than 0.05 and equal. Values with statistical significance were evaluated with the lowest significance difference test.

RESULTS

Cisplatin-induced oxidative stress-related apoptosis and caspase findings in mpkCCD$_{cl4}$

The increase in oxidative stress parameters in the environment causes disruptions in cellular energy production, disruption of mitochondrial membrane integrity and mitochondrial dysfunction leading to apoptosis[23]. Here the researchers wanted to examine the stages that started from this mitochondrial dysfunction and dragged the cell to apoptosis. In this study, where the protective role of CurC in Cisp-induced nephrotoxicity was investigated (Pict.1), although there was a significant increase in reactive oxygen species production levels (ROS) and apoptosis values, these values approached to normal by CurC treatment (Fig.1 a, b, ($a_p \leq 0.05$, $b_p \leq 0.001$ vs control, $c_p \leq 0.01$ vs Cisp)). The caspase-3, -9 levels, also known as pro-apoptotic cascades, also increased in the Cisp group. CurC treatment significantly reduced this toxic effect (Fig. 2 a, b, ($p \leq 0.01$ vs control, $p \leq 0.05$ vs Cisp)). The most important effect of apoptotic pathways is the decrease of mitochondrial membrane potential, namely membrane depolarization. Therefore, in this study, also mitochondrial membrane depolarization was evaluated. Cisp significantly increased mitochondrial membrane depolarization and CurC improved these values (Fig.3e, ($a_p \leq 0.001$, $b_p \leq 0.01$ vs control; $c_p \leq 0.01$ vs Cisp)). Also, Cisp treatment caused a decrease in cell viability values and CurC reversed this situation (Fig.3 f, ($a_p \leq 0.05$, $b_p \leq 0.01$ vs control, $c_p \leq 0.01$ vs Cisp)). Our data show that the use of CurC to eliminate oxidative damage in Cisp-induced nephrotoxicity will be beneficial, and the use of CurC can be beneficial in renal failure problems in cancer patients undergoing Cisp treatment.



Pict.1 Images of cells belonging to all groups under Sterio microscope

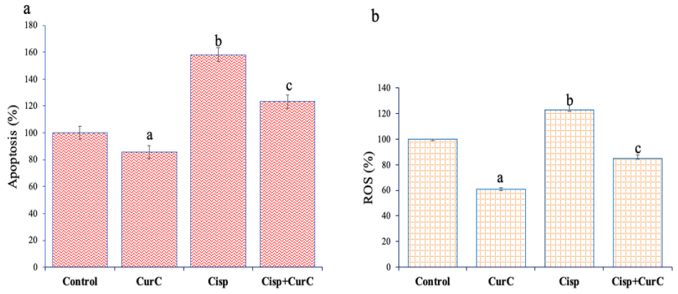


Fig.1 Effects of Cisp (80 µM) and CurC (10 µM) treatment **(a)** on apoptosis levels **(b)** on ROS production levels in mouse kidney cortical collection duct cells (mpkCCD$_{cl4}$) ($^a p \leq 0.05$, $^b p \leq 0.001$ vs control, $^c p \leq 0.01$ vs Cisp) (mean ± SD and n = 6). Values are expressed as percent compared to control.

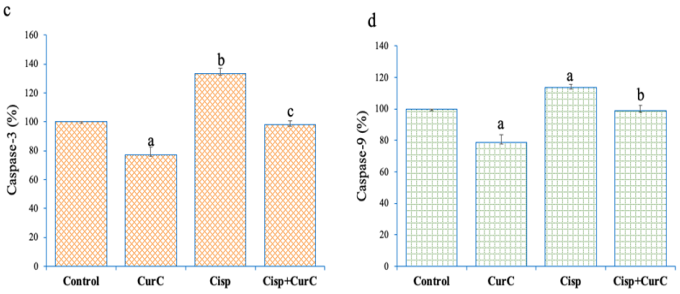


Fig.2 Effects of Cisp (80 µM) and CurC (10 µM) treatment **(c)** on caspase 3 levels in mouse kidney cortical collection duct cells (mpkCCD$_{cl4}$) ($^a p \leq 0.01$, $^b p \leq 0.01$ vs control, $^c p \leq 0.01$ vs Cisp) **(d)** on caspase 9 levels ($^a p \leq 0.01$ vs control, $^b p \leq 0.05$ vs Cisp), (mean ± SD and n = 6). Values are expressed as percent compared to control.

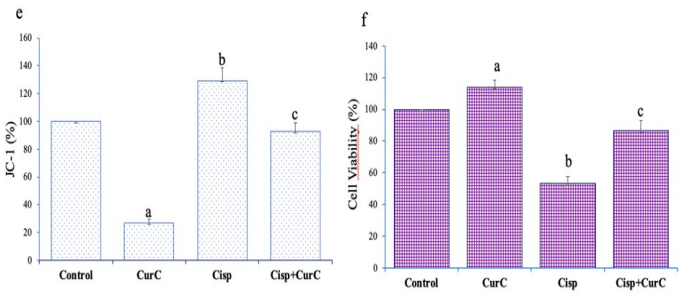


Fig.3 Effect of Cisp (80 µM) and CurC (10 µM) treatment **(e)** on mitochondrial membrane depolarization (JC-1) levels in mouse kidney cortical collection duct cells (mpkCCD$_{cl4}$) ($^a p \leq 0.001$, $^b p \leq 0.01$ vs control; $^c p \leq 0.01$ vs Cisp) **(f)** Effects on cell viability (MTT) levels ($^a p \leq 0.05$, $^b p \leq 0.01$ vs control, $^c p \leq 0.01$ vs Cisp) (mean ± SD and n = 6). Values are expressed as percent compared to control.

Effect of Curcumin on GSH, GSH-Px and LP values in mpkCCD$_{cl4}$ cells treated with Cisplatin.

Lipid peroxidation levels were higher in Cisplatin-treated mpkCCD$_{cl4}$ mouse kidney cortical collection duct cells compared to the control group ($p < 0.001$), with a decrease in GSH and GSH-Px values ($ap < 0.01$ and $cp < 0.001$). Although in the curcumin treated mpkCCD$_{cl4}$ cells, GSH-px levels significantly increased when compared to the control group, LP levels decreased significantly ($p < 0.01$). The LP levels of cells in Cisp+CurC groups showed a significant decrease compared

to Cisplatin ($p < 0.001$), but there was a significant increase in GSH ($p < 0.01$) and GSH-px levels ($p < 0.001$) (Table.1).

Table.1 Glutathione peroxidase (GSH-Px), reduced glutathione (GSH) and Lipid Peroxidation (LP) levels in mpkCCDcl4 mouse kidney cortical collection channel cells (Control, Curcumin, Cisplatin and Cisp + CurC) (mean ± SD) ([a]$p < 0.01$ and [c]$p < 0.001$ copared to control; [b]$p < 0.01$ and [d]$p < 0.001$ compared to Cisplatin).

Parameters	Control	Curcumin (CurC)	Cisplatin (Cisp)	Cisp+Cur C
GSH (µg/g protein)	11,67 ± 0,61	12,63 ± 0,45	7,14 ± 0,20[a]	10,50 ± 0,76[b]
GSH-Px (IU/g protein)	24,11 ± 2,94	28,55 ± 1,25[a]	14,10 ± 2,50[c]	20,77 ± 1,41[d]
LP (µmol/g protein)	16,98 ± 0,71	13,34 ± 1,76[a]	24,24 ± 1,29[c]	17,58 ± 0,94[d]

DISCUSSION

In this study, we investigated the caspase cascades and apoptosis responses by using molecular techniques to investigate the Cisp-induced nephrotoxicity model in-vitro using mouse kidney collection channel cells ($mpkCCD_{cl4}$). Previous studies on cisp-induced nephrotoxicity have been targeted to form DNA lesions as a source of apoptosis caused by this toxicity.[4] According to studies, platinum damage was found in tubular and glomerular kidney cells in Cisp-mediated nephrotoxicity. This resulted in the division of procaspase-3 and procaspase-7, and ultimately with apoptosis.[24] Here, CurC prevented Cisp-induced apoptosis, which forms DNA-platinum adducts by entering the cell nucleus (Fig.1a, $bp \leq 0.001$ vs control, $cp \leq 0.01$). Our findings are consistent with the histopathological findings obtained from previous Cisp-induced nephrotoxicities.[25-28] In addition, Curcumin significantly suppressed the ROS, Mitochondrial membrane depolarization levels and caspase-3, caspase-9 activities, which increased with the Cisp effect. Curcumin's contribution to cell vitality is by maintaining cell proliferation with DNA damage prevention by fighting against DNA-platinum additions. As seen in Fig.3f, CurC contributed significantly to cell viability when given to the cell with Cisp ($p \leq 0.01$). The protective role of curcumin against Cisp-induced nephrotoxicity has not been previously investigated in renal cortical collection duct cells. We have shown here that Curcumin's protection against this toxicity develops by removing reactive oxidative species from the cellular environment.

Cisplatin-induced nephrotoxicity is the source of ROS, and this reactive oxidative stress in cells is expected to depolarize the mitochondria. Here we wanted to investigate the events that will occur after this mitochondria membrane depolarization after Cisp treatment. It is released into cytosol due to cytochrome C membrane depolarization, which is normally located between the two membranes of the mitochondria and has a very important role in the electron transport chain. The release of cytochrome C into cytosol triggers caspase cascades. Protecting the membrane integrity of mitochondria, BCL-2, BCL-X proteins act anti-apoptotic by preventing the release of any protein from mitochondria to cytosol.[29] When damage occurs in a cell DNA, P53 triggers protein production. This protein leads to the transformation of many proteins. Bim, Bid and bad proteins, which are triggered for reasons such as toxic effects occurring in the cells, prevent the activities of BCL-2 and Bcl-x proteins, and help the membrane integrity of the mitochondria. In this case, Bax and bak proteins form channels in the mitochondria membrane and accelerate this situation. In this way, the cytochrome C[30] flowing into the cytosol combines with Apaf-1 to form the apoptosome. Apoptosome catalyzes the conversion of caspase-9 from procaspasase-9. Active caspase-9, on the other hand, triggers the active caspase-3 transformation from procaspase-3 and eventually takes the cell to apoptosis. From these stages, we performed mitochondrial membrane depolarization, caspase-3, caspase-9, apoptosis and cell viability analysis, especially ROS.

To summarize, we observed that in the in vitro Cisp-induced nephrotoxicity model in kidney collection duct cells, the increase in ROS amount, depolarized the mitochondrial membranes, increased caspase-3, caspase-9 and apoptosis. CurC played an important role in the inhibition of apoptotic pathways by reversing these effects. While Cisp increased LP levels, it decreased GSH and GSH-Px levels. Curcumin reversed this situation and exhibited a complete antioxidant property and exhibited a nephroprotective property. Consequently, to prevent kidney complications, researchers should consider curcumin supplementation in cancer treatment protocols. More research on the benefits of using CurC in cancer treatment will be valuable for the topic under discussion.

Conflicts of Interest

The authors declare that they have no conflict of interests.

REFERENCES

1. Rosenberg B, VanCamp L, Trosko JE, Mansour VH. Platinum compounds: a new class of potent antitumour agents. *Nature*. 1969 Apr 26;222(5191):385-6.
2. Ali I, Wani WA, Saleem K, Haque A. Platinum compounds: a hope for future cancer chemotherapy. *Anticancer Agents Med Chem*. 2013 Feb;13(2):296-306.
3. Weijl NI, Cleton FJ, Osanto S. Free radicals and antioxidants in chemotherapy-induced toxicity. *Cancer Treat Rev*. 1997 Jul; 23(4):209-40.
4. Yao X, Panichpisal K, Kurtzman N, Nugent K. Cisplatin nephrotoxicity: a review. *Am J Med Sci*. 2007 Aug;334(2):115-24.
5. Pabla N, Huang S, Mi QS, Daniel R, Dong Z. ATR-Chk2 signaling in p53 activation and DNA damage response during cisplatin-induced apoptosis. *J Biol Chem*. 2008 Mar 7;283 (10):6572-83.
6. Materna V, Liedert B, Thomale J, Lage H. Protection of platinum-DNA adduct formation and reversal of cisplatin resistance by anti-MRP2 hammerhead ribozymes in human cancer cells. *Int J Cancer*. 2005 Jun 20;115(3):393-402.
7. Yoshida M, Iizuka K, Terada A, Hara M, Nishijima H, Akinori, Shimada, Nakada K, Satoh Y, Akama Y. Prevention of nephrotoxicity of cisplatin by repeated oral administration of ebselen in rats. *J Exp Med*. 2000 Aug;191(4):209-20.
8. Legallicier B, Leclere C, Monteil C, Elkaz V, Morin JP, Fillastre JP. The cellular toxicity of two antitumoural agents derived from platinum, cisplatinum versus oxaliplatinum, on cultures of tubular proximal cells. *Drugs Exp Clin Res*. 1996;22(2):41-50.
9. Osawa T. Nephroprotective and hepatoprotective effects of curcuminoids. *Adv Exp Med Biol*. 2007;595:407-23.
10. Ueki M, Ueno M, Morishita J, Maekawa N. Curcumin ameliorates cisplatin-induced nephrotoxicity by inhibiting renal inflammation in mice. *J Biosci Bioeng*. 2013 May;115(5):547-51.
11. Kuo KT, Yang CW, Yu MJ. Dexamethasone enhances vasopressin-induced aquaporin-2 gene expression in the mpkCCD cells. *J Physiol Renal Physiol*. 2018 Feb 1;314(2):F219-F229.
12. Hu A, Huang JJ, Jin XJ, Li JP, Tang YJ, Huang XF, Cui HJ, Xu WH, Sun GB. Curcumin suppresses invasiveness and vasculogenic mimicry of squamous cell carcinoma of the larynx through the inhibition of JAK-2/STAT-3 signaling pathway. *J Cancer Res*. 2014 Dec 15;5(1):278-88.

    Kim JH, Jeong SJ, Kim B, Yun SM, Choi DY, Kim SH. Melatonin synergistically enhances cisplatin-induced apoptosis via the dephosphorylation of ERK/p90 ribosomal S6 kinase/heat shock protein 27 in SK-OV-3 cells. *J Pineal Res*. 2012 Mar;52 (2):244-52.
14. Omoyeni OA, Hussein A, Meyer M, Green I, Iwuoha E. Pleiocarpa pycnantha leaves and its triterpenes induce apoptotic cell death in Caco-2 cells in vitro. *BMC Complement Altern Med*. 2015 Jul 14;15:224.
15. Sagar S, Esau L, Moosa B, Khashab NM, Bajic VB, Kaur M. Cytotoxicity and apoptosis induced by a plumbagin derivative in estrogen positive MCF-7 breast cancer cells. *Anticancer Agents Med Chem*. 2014 Jan;14(1):170-80.
16. Granados MP, Salido GM, Pariente JA, Gonza´lez A Effect of H2O2 on CCK-8-evoked changes in mitochondrial activity in isolated mouse pancreatic acinar cells. *Biol Cell.* 2005;97:847–856
17. González D, Espino J, Bejarano I, López JJ, Rodríguez AB, Pariente JA. Caspase-3 and -9 are activated in human myeloid HL-60 cells by calcium signal. *Mol Cell Biochem*. 2010 Jan;333 (1-2):151-7.
18. Hastuti SD, Quach A, Costabile M, Barton MD, Pyecroft SB, Ferrante A. Measuring the Asian seabass (Lates calcarifer) neutrophil respiratory burst activity by the dihydrorhodamine-123 reduction flow cytometry assay in whole blood. *Fish Shellfish Immunol*. 2019 Sep;92:871-880.
19. Espino J, Bejarano I, Paredes SD, González D, Barriga C, Reiter RJ, Pariente JA, Rodríguez AB. Melatonin counteracts alterations in oxidative metabolism and cell viability induced by intracellular calcium overload in human leucocytes:changes with age. *Basic Clin Pharmacol Toxicol*. 2010 Jul;107(1):590-7.
20. Bejarano I, Espino J, Barriga C, Reiter RJ, Pariente JA, Rodríguez AB. Pro-oxidant effect of melatonin in tumour leucocytes: relation with its cytotoxic and pro-apoptotic effects. *Basic Clin Pharmacol Toxicol*. 2011 Jan;108(1):14-20.
21. Placer, Z.A., Cushman, L., Johnson, B.C. (1966) Estimation of products of lipid peroxidation (malonyl dialdehyde) in biological fluids. *Anal Biochem*, 16, 359–364.
22. Lawrence, R.A., Burk, R.F. (1976) Glutathione peroxidase activity in selenium-deficient rat liver. *Biochem Biophys Res Commun*, 71, 952–958.
23. He J, Zhang X. miR-668 inhibitor attenuates mitochondrial membrane potential and protects against neuronal apoptosis in cerebral ischemic stroke. *Folia Neuropathol*. 2020;58(1):22-29.
24. Krüger K, Thomale J, Stojanović N, Osmak M, Henninger C, Bormann S, Fritz G. Platinum-induced kidney damage: Unraveling the DNA damage response (DDR) of renal tubular epithelial and glomerular endothelial cells following platinum injury. *Biochim Biophys Acta*. 2015 Mar;1853(3):685-98.
25. Trujillo J, Molina-Jijón E, Medina-Campos ON, Rodríguez-Muñoz R, Reyes JL, Loredo ML, Barrera-Oviedo D, Pinzón E, Rodríguez-Rangel DS, Pedraza-Chaverri J. Curcumin prevents cisplatin-induced decrease in the tight and adherens junctions: relation to oxidative stress. *Food Funct*. 2016 Jan;7(1):279-93.

26. Okur ME, Ayla Ş, Karadağ AE, Çiçek Polat D, Demirci S, Seçkin İ. Opuntia ficus indica Fruits Ameliorate Cisplatin-Induced Nephrotoxicity in *Mice. Biol Pharm Bull.* 2020;43(5):831-838.
27. Liu YH, Li K, Tian HQ. Renoprotective Effects of a New Free Radical Scavenger, XH-003, against Cisplatin-Induced Nephrotoxicity. *Oxid Med Cell Longev*. 2020 Apr 18;2020:9820168.
28. Khalifa EA, Nabil Ahmed A, Hashem KS, Allah AG. Therapeutic Effects of the Combination of Alpha-Lipoic Acid (ALA) and Coenzyme Q10 (CoQ10) on Cisplatin-Induced Nephrotoxicity. *Int J Inflam*. 2020 Apr 9;2020:5369797.
29. Luo Y, Fu X, Han B, Zhang F, Yuan L, Men H, Zhang S, Tian S, Dong B, Meng M. The Apoptosis Mechanism of Epirubicin Combined with BCG on Human Bladder Cancer Cells. *Anticancer Agents Med Chem*. 2020 May 1.
30. Mercado-Uribe H, Andrade-Medina M, Espinoza-Rodríguez JH, Carrillo-Tripp M, Scheckhuber CQ. Analyzing structural alterations of mitochondrial intermembrane space superoxide scavengers cytochrome-c and SOD1 after methylglyoxal treatment. *PLoS One*. 2020 Apr 30;15(4):e0232408.