Ukrainian Journal of Nephrology and Dialysis
Scientific and Practical, Medical Journal

Founder:
- National Kidney Foundation of Ukraine

ISSN 2304-0238;
eISSN 2616-7352
Journal homepage: https://ukrjnd.com.ua

Research paper

Zhenya Yavroyan[1], Anna Grigoryan[2], Nune Hakobyan[1], Agapi Hovhannisyan[1], Tamara Abgaryan[2], Anna Karapetyan[2], Emil Gevorgyan[1]

doi: 10.31450/ukrjnd.4(84).2024.08

Cisplatin and dexamethasone separate and combined effect on nephrotoxic processes in female rats

[1]Interfaculty Research Laboratory of Structural Biophysics, Research Institute of Biology, Yerevan State University, Yerevan, Republic of Armenia
[2]Laboratory of Human and Animal Physiology, Research Institute of Biology, Yerevan State University, Yerevan, Republic of Armenia

Citation: Yavroyan Zh, Grigoryan A, Hakobyan N, Hovhannisyan A, Abgaryan T, Karapetyan A, et al. Cisplatin and dexamethasone separate and combined effect on nephrotoxic processes in female rats. Ukr J Nephrol Dialys. 2024;4(84): 65-74. doi: 10.31450/ukrjnd.4(84).2024.08.

Abstract. *Nephrotoxicity is one of the most severe side effects caused by cisplatin, limiting its use at high, effective concentrations. Dexamethasone, known for its strong anti-inflammatory and immunomodulating properties, is often used to alleviate cisplatin-induced side effects. However, dexamethasone also exhibits pro-oxidant properties and has been associated with morphological impairments and acute kidney injury. Although the mechanisms of cisplatin-induced nephrotoxicity are complex and involve numerous cellular processes, oxidative stress is widely accepted as the primary cause of this pathology. This study aims to investigate how dexamethasone, despite having effects similar to cisplatin, alleviates the side effects caused by this drug.*

Methods. The study measured lipid peroxide product malondialdehyde (MDA) levels using the thiobarbituric acid method and catalase activity using the molybdenum method. For histological examination, 5-6 µm thick tissue sections were prepared from samples processed with formalin and fixed with paraffin. These sections were stained with hematoxylin-eosin and observed under a light microscope.

Results. Cisplatin and dexamethasone independently increased MDA levels to varying degrees. Cisplatin raised MDA by 75% in the homogenate and 38% in the supernatant, while dexamethasone increased these levels by 41% and 25%, respectively. The combined use of cisplatin and dexamethasone produced effects similar to those of dexamethasone alone. Catalase activity decreased following exposure to cisplatin (36% in the supernatant and 14% in the nucleus) and dexamethasone alone (33% in the supernatant and 24% in the nucleus). Combined use of the drugs led to a similar reduction in catalase activity. Histological analysis revealed tissue damage, supporting the pro-oxidant nature of both cisplatin and dexamethasone.

Conclusions. The findings indicate that both cisplatin and dexamethasone exhibit pro-oxidant effects, as demonstrated by increased malondialdehyde levels, reduced catalase activity, and histological evidence of tissue damage. The ability of dexamethasone to mitigate cisplatin-induced side effects is likely attributable to a combination of its anti-inflammatory and immunomodulatory properties, as well as a "preventive or restraining" effect.

Key words: *cisplatin, dexamethasone, malondialdehyde, catalase, nephrotoxicity, histological changes.*

Conflict of interest. The authors declare no conflict of interest.

Article history:
Received October 04, 2024
Received in revised form October 23, 2024
Accepted November 14, 2024

© Zh. Yavroyan, A. Grigoryan, N. Hakobyan, A. Hovhannisyan, T. Abgaryan, A. Karapetyan, E. Gevorgyan, 2024.

Correspondence should be addressed to Nune Hakobyan: nhakobyan@ysu.am

© Явроян Ж., Григорян А., Акопян Н., Оганесян А., Абгарян Т., Карапетян А., Геворгян Е., 2024.

УДК: 616.61-008.6-036.11:599.323.4]-085

Женя Явроян[1], Анна Григорян[2], Нуне Акопян[1], Агапі Оганесян[1], Тамара Абгарян[2], Анна Карапетян[2], Еміль Геворгян[1]

Окремі та комбіновані нефротоксичні ефекти цисплатину і дексаметазону у самок щурів

[1]Міжфакультетська науково-дослідна лабораторія структурної біофізики, Науково-дослідний інститут біології, Єреванський державний університет, Єреван, Республіка Вірменія

[2]Лабораторія фізіології людини і тварин, Науково-дослідний інститут біології, Єреванський державний університет, Єреван, Республіка Вірменія

Резюме. *Нефротоксичність є одним із найважчих побічних ефектів, спричинених цисплатином, що обмежує його використання в ефективних концентраціях. Дексаметазон, відомий своїми сильними протизапальними та імуномодулюючими властивостями, часто використовується для полегшення побічних ефектів, спричинених цисплатином. Проте дексаметазон також проявляє прооксидантні властивості та асоційований з морфологічними порушеннями та гострим ураженням нирок. Хоча механізми індукованої цисплатином нефротоксичності є складними та включають численні клітинні процеси, окислювальний стрес визнаний однією з основних причин цієї патології. Метою цього дослідження було вивчити окремі та комбіновані нефротоксичні ефекти цисплатину і дексаметазону у самок щурів.*

Методи. У дослідженні визначали рівні малонового діальдегіду (MDA) продукту перекису ліпідів за допомогою методу тіобарбітурової кислоти та активності каталази за допомогою молібденового методу. Для гістологічного дослідження із зразків, оброблених формаліном і фіксованих парафіном, готували зрізи тканин товщиною 5-6 мкм. Ці зрізи фарбували гематоксилін-еозином і спостерігали під світловим мікроскопом.

Результати. Цисплатин і дексаметазон незалежно підвищували рівні МДА різним ступенем. Цисплатин підвищував МДА на 75% у гомогенаті та на 38% у супернатанті, тоді як дексаметазон підвищував ці рівні на 41% та 25% відповідно. Комбіноване застосування цисплатину та дексаметазону викликало ефекти, подібні до ефектів окремого застосування дексаметазону. Активність каталази знизилася після впливу цисплатину (36% у супернатанті та 14% у ядрі) та дексаметазону окремо (33% у супернатанті та 24% у ядрі). Спільне застосування лікарських засобів призводило до аналогічного зниження активності каталази. Гістологічний аналіз виявив пошкодження тканин, що підтверджує прооксидантну природу як цисплатину, так і дексаметазону.

Висновки. Отримані результати вказують на прооксидантну дію як цисплатину, так і дексаметазону, про що свідчить підвищення рівня МДА, зниження активності каталази та гістологічні ознаки пошкодження ниркової тканин. Здатність дексаметазону пом'якшувати побічні ефекти, спричинені цисплатином, ймовірно, пояснюється поєднанням його протизапальних та імуномодулюючих властивостей, а також «профілактичного або стримувального» ефекту.

Ключові слова: *цисплатин, дексаметазон, малоновий діальдегід, каталаза, нефротоксичність, гістологічні зміни.*

Introduction. Cisplatin (cis-diamine dichloro platinum (II), CDDP) is a chemotherapeutic drug widely used for the treatment of many solid tumors [1, 2]. However, the clinical application and efficacy of cisplatin are highly limited due to its severe adverse effects, including nephrotoxicity [3, 4]. While cisplatin induces various toxicities, such as neurotoxicity, hepatotoxicity, gastrointestinal toxicity, ototoxicity, and allergic reactions, the most commonly reported and dose-limiting side effect is nephrotoxicity [2, 3, 5]. It is estimated that 20% of patients receiving high-dose cisplatin experience severe renal dysfunction, and approximately one-third of patients develop kidney injury within days of the initial treatment [3, 5].

Nune Hakobyan:
nhakobyan@ysu.am

Cisplatin accumulates in the proximal tubular region of the nephron, where its concentration in tubular cells is higher than in the bloodstream [2, 3]. Additionally, the mitochondria of proximal tubular epithelial cells are major intracellular sites of cisplatin accumulation [5, 6]. Although the mechanisms of cisplatin-induced nephrotoxicity are complex and involve numerous cellular processes, such as oxidative stress, apoptosis, and inflammation, oxidative stress (OS) is generally considered the primary cause of this condition [5, 7]. Substantial evidence suggests that cisplatin-induced nephrotoxicity is due to the stimulation of reactive oxygen species (ROS) production, disruption of antioxidant systems, and accumulation of lipid peroxidation products in the kidney [3, 2, 5, 6]. The generation of ROS by cisplatin is directly linked to its cytotoxicity [7,

8]. Moreover, cisplatin negatively affects antioxidant enzymes such as superoxide dismutase (SOD), glutathione peroxidase, and catalase [5, 9]. Stimulation of OS is considered an alternative mechanism of cisplatin action [1, 2].

To alleviate the undesirable side effects caused by cisplatin, dexamethasone is frequently administered in high doses at various times before, during, and after cisplatin treatment [10, 11]. In addition to its role in reducing acute toxicity, glucocorticoids (GCs) have been shown to prevent the late effects of cytotoxic therapy on normal tissues [10, 11]. Specifically, dexamethasone is used as a concomitant agent for its anti-inflammatory and immunoregulatory properties [10, 11]. However, it has been established that dexamethasone exhibits pro-oxidant properties, stimulates the formation of reactive oxygen species, and increases the degree of lipid peroxidation [10, 12, 13]. Both cisplatin and dexamethasone have also been shown to induce morphological impairments in rat kidney cells [3–5, 13].

Thus, both cisplatin and dexamethasone stimulate the formation of reactive oxygen species, increase the degree of lipid peroxidation, and induce morphological changes in kidney cells, causing nephrotoxicity [7, 8, 10, 12, 13]. In light of the above, it is of interest to investigate the lipid peroxidation levels, antioxidant enzyme (catalase) activity, and histological changes in kidney tissue resulting from the separate and combined use of cisplatin and dexamethasone.

The aim of this study is to determine how dexamethasone, which exhibits effects similar to those of cisplatin, alleviates the side effects caused by this drug.

Materials and methods. ***Animal care and design of experiment.*** The study was performed on adult Wistar albino female rats (120-150 g weight, 20 rats). Experiments were conducted according to the "International Recommendations on Carrying out of Biomedical Researches with Use of Animals" (CIOMS, 1985; 2016), to the "Human Rights and Biomedicine the Oviedo Convention" (CE, 1997), to the European Convention for the Protection of Vertebral Animals Used for Experimental and Other Scientific Purposes (CE, 2005), and were approved by the National Center of Bioethics (Armenia). Animals were placed in a controlled environment at a temperature of 25±2°C, 12-hour day and night cycle, fed commercial rat feed ad-libitum, and given free access to water in the animal house of the faculty of biology at Yerevan State University.

For a single injection of cisplatin and dexamethasone usually chosen doses near the intermediate doses of these drugs. The doses we used (8 mg/kg for cisplatin and 4 mg/kg for dexamethasone) are consistent with this principle. The dose of cisplatin was chosen according to [15], and the dose of dexamethasone according [16]. Cisplatin was purchased from Sigma Aldrich, USA. Dexamethasone (dexamethasone phosphate) 4 mg/ml, solution for injection was purchased from Krka Sl-Slovenia. 2-Thiobarbituric Acid (TBA) (75241) (4,6-Dihydroxy-2-Mercaptopyrimidine, 4,6-Dihydroxypyrimidine-2-Thiol) SRL India.

The animals were divided into 4 groups, 5 rats in each group. Group 1 (n=5) served as a control group of animals without treatment. Animals in group 2 (n=5) and group 4 (n=4) received a single dose of cisplatin (8 mg/kg) by peritoneal injection and were decapitated 24 hours after administration. Group 3 (n=5) was treated with dexamethasone (4 mg/kg, peritoneal injection) and decapitated 4 hours after administration. Animals in group 4 (n=5) received the same single dose of dexamethasone within 20 hours after the cisplatin injection (4 hours before decapitation). All animals were euthanized by decapitation at an appropriate time following inhalation ether anesthesia. Then, animals were sacrificed, and the kidneys were extracted from each group of animals and used for the isolation of nuclei and histopathological examination. Nuclear fraction from the kidney was isolated by the method of Blobel and Potter [17].

The malondialdehyde amount estimation. The malondialdehyde (MDA) amount was estimated in 10% homogenate of rat kidney tissue and in supernatant that formed after the first centrifugation of 10 min at 1000g of this homogenate (centrifuge Sigma 3-18K, Germany) [18]. The assay was based on a condensation reaction of two molecules of thiobarbituric acid with one molecule of MDA, in which the reaction rate depends on temperature, pH value, and concentration of thiobarbituric acid. The reaction mixture contains 30% trichloroacetic acid, 5 N HCl, 0,8% solution of thiobarbituric acid, and an appropriately diluted biological sample of 1 ml each. After heating for 20 min in a boiling water bath and after cooling, the solution was centrifuged at 3000 rpm/min for 10 min and the precipitate obtained was removed [18]. The absorbance of the pink supernatant was determined at 532nm against a blank that contained all reagents without the biological sample. The measurements were carried out with the Chinese-made BK-UV 1800 Spectrophotometer (Biobase China). The MDA concentration (in nmol/mg protein) was calculated using the molar extinction coefficient (ε)-of MDA -$1.56 \cdot 10^5 M^{-1} \cdot cm^{-1}$ at 532 nm; and the appropriate formula [19].

Catalase enzyme activity assay. Catalase (EC 1.11.1.6) activity in supernatant that formed after the first centrifugation of 10% homogenate and in the nuclear fraction of rat kidney tissue was estimated by method [20]. The method is based on the registration of the rate of H_2O_2 degradation by the action of catalase. The activity of catalase was determined by measuring a decrease in the hydrogen peroxide (H_2O_2) concentration at 410 nm. The method of defining catalase activity was based on the development of a stable blue-colored complex as a result of ammonium molybdate reaction with H_2O_2 and subsequent photometric measurement of the recovered complex [20]. The enzyme activity was expressed in μmol H_2O_2 /mg

protein per min by the formula as described in [20]. The protein amount was determined by the spectrophotometric method [21].

$$A_{Cat} = \frac{(D_0 - D_T) V_1}{C V_2 \varepsilon l t}$$

Where:

A_{Cat} is the activity of catalase (µmol/mg protein, minute);

D_o – is the optical density of the blank sample;

D_T – is the optical density of the Test sample;

V_1 – is the volume of the reaction mixture (3.1 ml);

V_2 – is the volume of the homogenate (0.1 ml),

C – is the amount of protein in 0.1 ml (mg);

ε – is the extinction coefficient of the substrate H_2O_2, (0.02 $mol.cm^{-1}$),

l – is the thickness of the cuvette (1 cm),

t – is the time (10 minutes).

Histopathological examination. For histological examination, the kidneys were removed and fixed with a 10% formalin solution according to the protocol [22]. The fixed material was subjected to standard histological processing. The tissue dehydration in increasing concentrations of alcohol to remove water was performed By Spin Tissue Processor STP-120(MYR, Italy). For embedding tissue samples with paraffin wax we use Tissue Embedding Center EC-500(MYR, Italy). Further, about 5-6µm thick paraffin sections were cut with the semi-automatic precision microtome CUT 5062 (SLEE Medical, Germany). The slides were stained with hematoxylin-eosin [22]. Hematoxylin-eosin (Hematoxylin, Sigma-Aldrich, Eosin, Sigma-Aldrich, USA) stained samples were used to examine the morphological alterations in rat kidneys. The stained specimens were mounted with DPX and underwent light microscopy using trinocular microscope B-293, OptikamB5 Digital Camera M- 114(Italy). All captured images were recorded via OptikaLiteview software with magnifications x100 and x400.

Hematoxylin and Eosin Staining Procedure. *Deparaffinization and rehydration:*

For deparaffinization and rehydration, it is necessary to incubate the slides for 5 minutes in xylene (2 times). Then transfer to 100% ethanol for 2 minutes (2 times), after which you should move to 95% ethanol for 2 minutes (2 times) and rinse in distilled water. Slides are ready for Hematoxylin Staining.

Hematoxylin Staining: For Hematoxylin Staining it is necessary to immerse slides in Hansen's Hematoxylin for 5-10 minutes. Then Rinse in running tap water until the blue color appears.

Rinsing and Differentiation: The washed slides should be immersed in 1% hydrochloric acid in ethanol for 10-30 seconds and rinse immediately in running water until the blue color returns. Next, for Bluing, soak in an alkaline solution (such as Scott's Tap Water Substitute) for 1 minute, then rinse in running tap water.

Dehydration: For dehydration, the slide should be kept in 95% ethanol for 1 minute, then transferred to 100% ethanol and kept for 1 minute.

Eosin Staining: For Eosin Staining, it is necessary to dip the slides into the eosin solution and leave it for 1-2 minutes. After that, rinse in 95% ethanol for 30 seconds, then in Eosin Y Solution for 1-2 minutes. Then you have to implement dehydration and clearing.

Dehydration and Clearing: For this purpose, the slides should be immersed in 100% ethanol for 1 minute and then immersed in xylene, leaving for 5 minutes (2 times).

Mounting: Apply a few drops of mounting medium (e.g., DPX) to the slide. Place a coverslip over the tissue section.

Statistical analysis. All results were expressed as Mean±SE from 5 independent experiments. Statistical analysis was performed using paired Student's t-test for grouped data, where P<0.05 was considered statistically significant (P<0.05 indicates significant differences compared with the control group). Statistical comparisons between experimental groups were performed using Statgraphics Centurion 19 Software (Stagraphics Technologies, Inc., USA). Statistical comparisons between all experimental groups were tested by analysis of variance (ANOVA), and #P< 0.05 considered significant differences in the case of intergroup comparison.

Results. The data presented in Table 1 show significant changes in the concentration of MDA after the treatment of rats with cisplatin and dexamethasone alone, as well as after co-treatment with these drugs, compared to baseline (Table 1). The results indicate that the MDA level significantly increased in the kidney homogenate of rats treated with cisplatin by approximately 75% (P<0.01) compared to the control group [19].

Treatment with dexamethasone increased the MDA level by about 41% (P<0.01). Co-injection of cisplatin and dexamethasone also led to a statistically significant increase in MDA levels by approximately 50.5% (P<0.02) compared to baseline (see Table 1).

As evidenced by the data, the MDA level in the kidney homogenates of animals in all experimental groups was, on average, about four times higher than in the supernatants from the first centrifugation of these homogenates (Table 1). Statistically significant changes in MDA levels compared to the control group were also observed in the supernatants obtained after the first centrifugation of kidney homogenates from all experimental groups. Specifically, cisplatin exposure led to a 38% increase in MDA levels (P<0.01) [19]. Similarly, a separate injection of dexamethasone resulted in a 25% increase in MDA levels in the supernatant compared to baseline (P<0.01) (see Table 1). The combined use of cisplatin and dexamethasone resulted in a 27% increase in MDA levels (P<0.02) (see Table 1).

Statistically significant changes were also observed in intergroup comparisons of the data. An increase in the amount of MDA (by 16%, #P<0.05) was recorded in the

kidney homogenates of rats treated with cisplatin alone compared to the experimental group of animals receiving a combined injection of cisplatin and dexamethasone (see Table 1). Conversely, no statistically significant difference was found when comparing the data from the dexamethasone-alone group with the results from the co-injected experimental group. Cisplatin increased the amount of MDA by 24% (#P<0.05) compared to dexamethasone, while dexamethasone decreased the amount of MDA by 19% (#P<0.05) compared to cisplatin alone (see Table 1). No statistically significant changes in MDA levels were observed in intergroup comparisons of results obtained from the supernatants of all experimental groups.

Table 1

The amount of MDA in a 10% homogenate of kidney tissue and the supernatant from the first centrifugation of this homogenate in all experimental groups [19]

Experimental groups		Homogenate		Supernatant	
		MDA quantity (in nmol /mg protein)	P-value	MDA quantity (in nmol /mg protein)	P-value
Group 1 (n=5)	Baseline without treatment	$^{+}$4.36 ±0.10		$^{+}$1.26±0.071	
Group 2 (n=5)	Cisplatin alone treatment	$^{+}$7.62 ±0.33	<0.01	$^{+}$1.74±0.070	<0.01
Group 3 (n=5)	Dexamethasone alone treatment	6.15 ±0.28	<0.01	1.58±0.065	<0.01
Group 4 (n=5)	Cisplatin+Dexamethasone	6.56 ±0.15	<0.02	1.60±0.057	<0.02

The next part of the investigation focuses on the measurement of antioxidant enzyme catalase activity in the supernatants from the first centrifugation of these homogenates and in the nuclear fractions from all experimental groups. The data are presented in Table 2.

Table 2

Catalase activity in supernatants of first centrifugation of kidney tissue homogenates and nuclear fractions of rats from all experimental groups [23]

Experimental groups		Supernatant		Nuclear fraction	
		μM/min, mg protein	P-value	μM/min, mg protein	P-value
Group 1 n=5)	Baseline without treatment	$^{++}$553.27 ± 9.77		$^{++}$213.00±8.70	
Group 2 (n=5)	Cisplatin alone treatment	$^{++}$354.15 ± 9.66	<0.05	$^{++}$182.34± 6.35	<0.01
Group 3 (n=5)	Dexamethasone alone treatment	368.00 ±11.16	<0.05	162.11±10.24	<0.02
Group 4 (n=5)	Cisplatin+Dexamethasone	467.00 ± 18.60	<0.01	187.00± 5.78	<0.02

The obtained results showed that cisplatin treatment caused a significant decrease in catalase activity in the supernatant fraction of kidney tissue by approximately 36% (P<0.05) compared to the baseline level. In contrast, the decrease in the nuclear fraction was less pronounced (approximately 14%; P<0.01) [23] (see Table 2). In the case of dexamethasone alone, a reduction in catalase enzyme activity by 33.5% (P<0.05) in the supernatant fraction of kidney tissue was observed (Table 2). In the nuclear fraction, dexamethasone alone caused a 24% reduction in catalase activity (P<0.02) compared to the baseline level (see Table 2).

The combined injection of cisplatin and dexamethasone resulted in a 16% decrease in catalase enzyme activity (P<0.01) in the supernatant fraction of kidney tissue compared to the baseline (see Table 2). In the nuclear fraction, the combined treatment caused a reduction in catalase activity by approximately 12% (P<0.02) compared to the baseline.

Statistically significant changes in catalase enzyme activity in the supernatant fraction were also observed in intergroup comparisons. Cisplatin treatment alone led to a 24% reduction in catalase activity (#P<0.05) compared to the group receiving combined cisplatin and dexamethasone treatment (see Table 2). A statistically significant reduction in catalase enzyme activity (approximately 21%; #P<0.05) was also observed in the dexamethasone-alone treatment group compared to the combined treatment group (see Table 2). However, no statistically significant differences were recorded when comparing the cisplatin-alone and dexamethasone-alone treatment groups.

Intergroup comparisons of the nuclear fraction data also revealed statistically significant changes in catalase activity (see Table 2). Dexamethasone alone caused a 13% decrease in catalase activity (#P<0.05) compared to the group receiving combined cisplatin and dexamethasone treatment (see Table 2). At

the same time, the data for cisplatin treatment alone showed no statistically significant differences compared to the combined treatment group.

A statistically significant increase in catalase enzyme activity (by 12%; #P<0.05) was observed when comparing the cisplatin-alone treatment group to the dexamethasone-alone group. Conversely, dexamethasone treatment alone decreased catalase activity by 11% (#P<0.05) compared to cisplatin-alone treatment (see Table 2).

The histological observation of the kidney tissue of animals from the control group revealed normal glomerular and tubular structures (Fig. 1-A; 1-4). The kidneys of the normal group showed normal glomerular podocytes (Fig. 1-A; 1-4). Histopathology examination of renal slices from rats treated with cisplatin alone showed different alterations. (Fig. 1-B; 1-4).

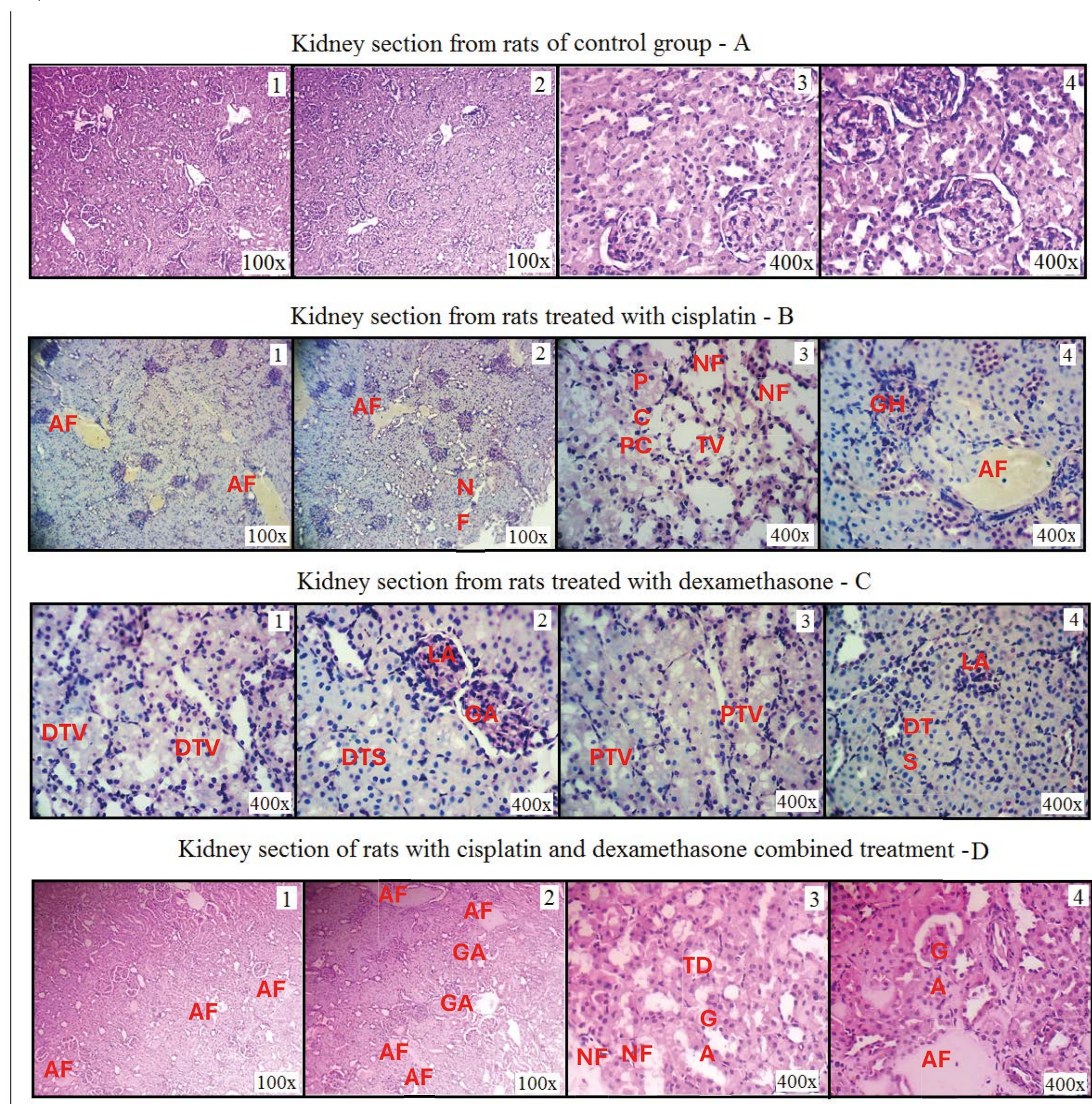

Fig. 1. Photomicrograph of rat kidney section after the staining with hematoxylin-eosin. Photomicrograph of kidney section of rats from the control group (A; 1-4), animals of the experimental group with cisplatin alone treatment (B; 1-4), after dexamethasone injection (C; 1-4), after combined use of cisplatin and dexamethasone (D; 1-4).
100x - magnification:100x; 400x - magnification:400x.

Abbreviations: *AF-amyloidal foci; NF-necrotic foci; PC-picnic cells; TV-tubular vacuolization; GH- hemorrhagance; DTV-distall tubular vacuolization; LA- lymphocytes accumulation; GA- glomerular atrophy; PTV- proximal tubular vacuolization; DTS- distal tubular swelling; TD-tubular dilatation.*

In the kidneys of rats treated with cisplatin alone, distinct regressive phenomena are found. Specifically, in the epithelium of the proximal and distal tubules of the nephron, there is cytoplasmic vacuolization of cells (Fig. 1-B; 1). Additionally, in certain areas, the integrity of the tubule epithelium is compromised (Fig. 1-B; 1). The nuclei of most cells exhibit abnormal morphology, with occasional pyknotic

nuclei also present (Fig. 1-B; 2-4). Furthermore, necrotic foci and amyloidosis are evident in the kidney (Fig. 1-B; 2-4). In some segments of the Malpighian coils of the nephron, hemorrhages occur, and the cells show pyknosis, along with acute tubular necrosis. (Fig. 1-B; 2-4).

Dexamethasone alone injection also caused histomorphological alterations of kidney cells (Figure 1-C; 1-4). These changes are characterized by a narrowing of the lumen in the distal tubules, likely attributed to cellular swelling (Fig. 1-C; 1-4). This phenomenon may be due to the effect of dexamethasone on water-salt metabolism. Histological alterations are evident in the epithelial cells of both the distal and proximal tubules (Fig. 1-C; 3 and 4). Besides cytoplasmic vacuolization (Fig. 1-C; 3 and 4) the presence of lymphocyte accumulations in specific regions was also registered (Fig. 1-C; 1, 2, and 4). Cytoplasmic vacuolization and the presence of lymphocyte accumulations in specific regions are likely associated with inflammatory foci (Fig. 1-C; 2, and 4).

In the kidney of rats with dexamethasone and cisplatin combined treatment simultaneously hematoxylin and eosin (HE) staining showed severe pathological kidney lesions including glomerular atrophy (Fig. 1-D; 1-4), degeneration and necrosis of renal tubular epithelial cells (Fig. 1-D; 2,3,), amyloidosis is evident [Fig. 1-D; 3].

All the observed phenomena undeniably point to the nephrotoxic effects of dexamethasone and cisplatin. Furthermore, the retrograde changes are especially pronounced during the alone injection of cisplatin.

Discussion. It is well known, that the primary targets of ROS are lipids, which undergo oxidation with lipoperoxyl radicals and lipid hydroperoxide formation upon interaction with oxidants [24]. In normal physiological conditions, the presence of ROS is vital for the normal functioning of cells [24]. Moreover, a certain physiological amount of ROS is maintained by balancing the processes of their generation and destruction, that is, due to the oxidant/antioxidant balance. This imbalance arises from either increased production of oxidants, decreased levels of antioxidants, or both [9, 24].

Cisplatin disrupts the oxidant/antioxidant balance by inducing ROS formation, oxidative stress, and reducing antioxidant levels [1, 2, 9]. Markers of oxidative stress are usually classified as molecules that become modified by interactions with ROS and as molecules of the antioxidant system that become changed in response to increased redox stress [25]. Currently, more than 20 different markers of oxidative stress have been identified, but still MDA is accepted as a marker of OS and lipid peroxide oxidation (LPO) levels [25, 26]. The amount of malondialdehyde expresses the intensity and level of oxidative stress and the resulting lipid peroxidation [24, 26]. Malondialdehyde, in turn, disrupts the functions of various biomacromolecules by directly binding to them or cross-linking them to each other through Schiff bases [24, 26].

The amount of MDA in 10% homogenates of rat kidney tissue after separate and combined exposure to cisplatin and dexamethasone was determined. The results confirm that compared to the baseline statistically significant changes in MDA quantity were revealed in all experimental groups (Table 1). In fact, both cisplatin and dexamethasone when injected separately increase the amount of MDA, thus stimulating both LPO and OS. This is also evidenced by the data obtained by different authors [7, 9, 12, 13]. However, it should be noted that the pro-oxidant properties of cisplatin are stronger compared to dexamethasone. This is evidenced not only by the changes in the amount of MDA registered compared to the control (75% and 41% in the homogenate, and 38% and 25% in the supernatant), but also by the results of the intergroup comparison of the data. The latter indicates a stronger effect of cisplatin compared to dexamethasone, both in the homogenate and in the supernatant.

The result of the joint action of these drugs is not the arithmetic sum of the effects caused by their separate use. Admittedly, co-injection also increased the amount of MDA, but these data are close to the effect shown by dexamethasone. It allows us to speculate about a certain antagonism between the cisplatin and dexamethasone action. In case of combined use, dexamethasone acts as a buffering factor for cisplatin. This is probably due to the predominance of anti-inflammatory and immunomodulatory properties of dexamethasone, which contributes to the mitigation of unwanted side effects of cisplatin by this hormone.

The pro-oxidant properties of cisplatin and dexamethasone are also evidenced by the changes in the activity of the antioxidant enzyme catalase as a result of the separate and combined effects of these drugs. In the supernatant, cisplatin and dexamethasone reduced catalase activity to an equal extent when applied separately, while the effect of co-injection is smaller than the data obtained when these drugs are used separately. The same trend is observed in the nuclear fraction of kidney cells (Table 2).

A reduction in catalase enzyme activity as a result of both cisplatin and dexamethasone exposure has also been reported by other researchers [9, 27]. The recorded results once again indicate activation of oxidative stress and LPO processes in rat kidney cells by cisplatin and dexamethasone. These processes are known to be the main companions of kidney toxicity. Moreover, it has been evidenced that OS and LPO products are the main cause of nephrotoxicity. Although nephrotoxicity is the result of a complex interaction of various factors, it has been evidenced that OS and LPO products are the main cause of nephrotoxicity [2, 28].

Nephrotoxicity is the most common and most serious of the unwanted side effects caused by cisplatin [5, 29]. This is due not only to the fact that any drug, including cisplatin, is removed from the body through the kidneys but also to the fact that cisplatin accumulates the most in the kidneys [5, 6, 29]. The level of cisplatin

in the proximal tubules of the kidney is almost five times higher than in the serum. Due to this high cisplatin accumulation, the proximal tubular cells suffer significant toxicity [5, 29]. In addition, cisplatin can also cause harm to renal arteries and decrease the glomerular filtration rate [5].

In order to visualize the nephrotoxic effect of applied drugs, histopathological studies were carried out. The obtained results show that cisplatin and dexamethasone cause different histological changes in kidney tissue (Figure 1-B-C). Cisplatin caused tubular vacuolization, amyloid foci, necrotic foci, hemorrhaging, and other degenerative changes. As a result of exposure to cisplatin, similar changes were recorded also by other authors, who noted that this drug injection partial shedding of renal tubular epithelial cells, and caused vacuolar degeneration, dilation of proximal tubules, desquamation of tubular epithelium, and acute tubular necrosis, atrophy of glomerulus [7, 30, 31]. These changes lead to cisplatin-induced renal dysfunction including alterations in glomerular function [7, 30, 31].

Separate injection of dexamethasone also caused histological changes, including atrophy of glomerulus, lymphocyte accumulations, amyloid and necrotic foci, etc. (Figure 1-B-C) [27, 32].

In fact, both cisplatin and dexamethasone compared to the control group, where normal glomeruli and tubular structures were observed, cause different changes in kidney cells, which result in acute kidney injury (AKI).

In the case of combined use these drugs caused the histological injuries that manifested when they were used separately. However, it should be noted that these changes do not have the same strength and depth as in the case of separate use of these drugs. Literature data on the combined use of cisplatin and dexamethasone were not found.

Changes in oxidative stress markers recorded as a result of separate and combined exposure to cisplatin and dexamethasone indicate the activation of free radical formation and lipid peroxidation processes. These processes underlie cisplatin-induced side effects, especially nephrotoxicity [4, 6, 7, 14, 33]. Numerous studies have demonstrated that cisplatin induces damage to an array of renal components, encompassing the vasculature, glomerular apparatus, and, most prevalently, the renal tubules [29-33]. Therefore, it can be argued that the morphological changes in the kidney tissue were recorded due to the OS effect induced by the pro-oxidants we used. However, to understand the concept of oxidative damage, several factors must be investigated in order to have a clearer understanding of these processes.

This study has several limitations. First, it was conducted on a small sample size of female Wistar albino rats, which may limit the generalizability of the findings to other populations or species, including humans. Second, the study focused on specific biochemical and histological markers, such as MDA levels and catalase activity, without exploring other oxidative stress markers or pathways that might contribute to nephrotoxicity. Additionally, while the chosen doses of cisplatin and dexamethasone were consistent with existing literature, dose-response relationships and long-term effects were not investigated. Finally, the study did not include mechanistic evaluations at the molecular level to elucidate the observed antagonistic interactions between cisplatin and dexamethasone. Future research could address these gaps by incorporating larger sample sizes, additional biomarkers, and molecular analyses.

Conclusions. The observed alterations in MDA levels, combined with the decreased catalase enzyme activity, reaffirm the pro-oxidant nature of both cisplatin and dexamethasone. Notably, the oxidative effect of cisplatin is significantly greater than that of dexamethasone when these drugs are administered separately. However, during combined treatment, dexamethasone appears to act as a buffering factor, partially mitigating the oxidative effects of cisplatin.

Histological studies further demonstrate that while both cisplatin and dexamethasone exhibit pro-oxidant properties, they induce distinct histological changes in kidney tissue when used independently. The nephrotoxic injuries caused by each drug alone differ in nature, yet in combined use, these injuries are noticeably milder, likely due to the mitigating presence of dexamethasone.

These findings unequivocally highlight the nephrotoxic effects of both cisplatin and dexamethasone. However, it is hypothesized that dexamethasone's ability to alleviate the side effects of cisplatin may stem from a combination of its anti-inflammatory and immunomodulatory properties, along with a «preventive or restraining» effect on cisplatin-induced damage.

Ethics statement. Experiments were conducted according to the "International Recommendations on Carrying out of Biomedical Researches with Use of Animals" (CIOMS, 1985; 2016), to the "Human Rights and Biomedicine the Oviedo Convention" (CE, 1997), to the European Convention for the Protection of Vertebral Animals Used for Experimental and Other Scientific Purposes (CE, 2005), and were approved by the National Center of Bioethics (Armenia).

Conflict of interest. The authors declare that there are no conflicts of interest related to this research.

Funding source. No financial support was sought for the completion of this study.

Author contribution.

Zhenya Yavroyan: Methodology, software, validation, formal analysis, investigation, data curation, writing-original draft;

Anna Grigoryan: Histological sample preparation, interpretation of the results;

Nune Hakobyan: Investigation, data curation;

Agapi Hovhannisyan: Resources, investigation;

Tamara Abgaryan: Histological sample preparation;

Anna Karapetyan: Interpretation of the results

Emil Gevorgyan: Conceptualization, writing- review and editing, supervision.

Data availability statement. The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

References:

1. *Aldossary SA.* Review on pharmacology of cisplatin: clinical use, toxicity and mechanism of resistance of cisplatin. Biomed Pharmacol J. 2019;12(1):7-15. doi: 10.13005/bpj/1608.
2. *Ghosh S.* Cisplatin: The first metal based anticancer drug. Bioorg Chem. 2019;88: 102925 doi: 10.1016/j.bioorg.2019.102925.
3. *Jadon AS, Bhadauriya P, Sharma M.* An integrative review of cisplatin: the first metal anti-tumor drug. Journal of Drug Delivery Ther. 2019;9(3):673-7. doi: 10.22270/jddt.v9i3.2679.
4. *Fawzy MH, Moustafab YM, Khodeerb DM, Saeeda NM, El-Sayed NM.* Molecular mechanisms of cisplatin induced nephrotoxicity. Rec Pharm Biomed Sci. 2022;6(3):128-35. doi: 10.21608/rpbs.2022.145126.1152.
5. *Oh GS, Kim HJ, Shen AH, Lee SB, Khadka D, Pandit A, et al.* Cisplatin-induced kidney dysfunction and perspectives on improving treatment strategies. Electrolyte Blood Press. 2014;12:55-65. doi: 10.5049/Ebp.2014.12.2.55.
6. *Koulouridis I, Koulouridis E.* Cisplatin nephrotoxicity: New insights in an old problem. Medical Research Archives. 2022;10(3):1-17. doi: 10.18103/mra.v10i3.2725.
7. *Soni H, Kaminski D, Gangaraju R, Adebiyi A.* Cisplatin-induced oxidative stress stimulates renal Fas ligand shedding. Ren Fail.2018;40(1):314–22. doi: 10.1080/0886022X.2018.1456938.
8. *Wang L, Zhao X, Fu J, Xu W, Yuan J.* The role of tumour metabolism in cisplatin resistance. Front Mol Biosci. 2021;8:1-13. 691795. doi: 10.3389/fmolb.2021.691795.
9. *Ognjanović BI, Djordjević NZ, Matić MM, Obradović JM, Mladenović JM, Štajn AŠ, et al.* Lipid peroxidative damage on cisplatin exposure and alterations in antioxidant defense system in rat kidneys: A possible protective effect of selenium. Int J Mol Sci. 2012;13:1790-803. doi: 10.3390/ijms13021790.
10. *Cook AM, McDonnella AM, Lakea RA, Nowaka AK.* Dexamethasone co-medication in cancer patients undergoing chemotherapy causes substantial immunomodulatory effects with implications for chemo-immunotherapy strategies. Oncoimmunology. 2016;5(3):e1066062. doi: 10.1080/2162402X.2015.1066062.
11. *Chow R, Warr DG, Navari RM, Tsao M, Milakovic M, Popovic M, et al.* Efficacy and safety of 1-day versus 3-day dexamethasone for the prophylaxis of chemotherapy-induced nausea and vomiting: a systematic review and meta-analysis of randomized controlled trials. J Hosp Manag Health Polic. 2018;25(2):1-13. doi:10.21037/jhmhp.2018.04.05.
12. *Liu W, Zhao Zh., Na Y, Meng Ch, Wang JR.* Dexamethasone-induced production of reactive oxygen species promotes apoptosis via endoplasmic reticulum stress and autophagy in MC3T3-E1 cells. Int J Mol Med. 2018;41(4):2028-36. doi: 10.3892/ijmm.2018.3412 .
13. *Owu DU, Okon IA, Ufot UF, Beshel JA.* Cardiac and renal protective effect of vitamin E in dexamethasone-induced oxidative stressed wistar rats. Niger J Physiol Sci. [Internet].2020;35(1):52-60. Available from: https://pubmed.ncbi.nlm.nih.gov/33084616/.
14. *Jasim NH, Kareem AD, Majeed MF, Abbas BA.* Effect of long-term treatment with dexamethasone on the liver and kidney histopathology, as well as blood biochemistry in male rabbits (Lepus Cuniculus). Arch Razi Inst. 2022;77(1):333-43. doi: 10.22092/ARI.2021.356468.1847.
15. *Sahu AK, Verma VK, Mutneja E, Malik S, Nag TC, Dinda A, et al.* Mangiferin attenuates cisplatin-induced acute kidney injury in rats mediating modulation of MAPK pathway. Mol Cell Biochem.2019;452(1-2):141-152. doi: 10.1007/s11010-018-3420-y.
16. *Nagendranayak IM, Chinta R, Nagaraju KB, Jetti R.* Short-term administration of high dose dexamethasone can induce maximum insulin resistance in Wistar albino rats. Rom J Diabetes Nutr Metab Dis. [Internet].2021;28(4):352–362. Available from: https://www.rjdnmd.org/index.php/RJDNMD/article/view/945.
17. *Blobel G, Potter VR.* Nuclei from rat liver: isolation method that combines purity with high yield. Science. 1966;154(3757):1662–65. doi: 10.1126/science.154.3757.1662.
18. *Uchiyama M, Mihara M.* Determination of malonaldehyde precursor in tissues by thiobarbituric acid test. Anal Biochem. 1978;86(1):271-78. doi: 10.1016/0003-2697(78)90342-1.
19. *Yavroyan ZhV, Hovhannisyan AG, Hakobyan NR, Gevorgyan ES.* Changes in malondialdehyde level in female rats brain, kidney and liver cells after the separate and joint action of cisplatin and steroids. Biolog Journal of Armenia. 2021;1(73):17-25.
20. *Koroliuk M, Ivanova L, Maĭorova I, Tokarev V.* A method of determining catalase activity. Lab Delo. [Internet].1988;1:16-19. (Article in Russian). Available from: https://pubmed.ncbi.nlm.nih.gov/2451064/.

21. *Kalb V, Bernlohr R.* A new spectrophotometric assay for protein in cell extracts. Anal Biochem.1977;82(2):362-71. doi: 10.1016/0003-2697(77)90173-7.
22. *Korjevsky DE, Gilyarov AB.* Basics of histological technique. Spec. Lit. Sankt Peterburg. 2010. https://www.books-up.ru/ru/book/osnovy-gistologicheskoj-tehniki-4424130.
23. *Yavroyan ZhV, Hovhannisyan AG, Hakobyan NR, Gevorgyan ES.* Cisplatin and estradiol separate and joint action on catalase activity in liver and kidney tissues of rats. Eurasian Union of Scientists (ESU). 2019;68(11):12-16. doi: 10.31618/ESU.2413-9335.2019.2.68.439.
24. *Ayala A, Muñoz M, Argüelles S.* Lipid peroxidation: production, metabolism, and signaling mechanisms of malondialdehyde and 4-Hydroxy-2-Nonenal. Oxid Med and Cell Longev. 2014;2014:360438. doi: 10.1155/2014/360438.
25. *Fontana J, Zima M, Vetvicka V.* Biological markers of oxidative stress in cardiovascular diseases: after so many studies, what do we know? Immunological investigations. J Mol Cell Immunol. 2018;47(8):823-43. doi: 10.1080/08820139.2018.1523925.
26. *Jadoon S, Malik A.* A review article on the formation, mechanism and biochemistry of MDA and MDA as a biomarker of oxidative stress. Int J Adv. Res. 2017;5(12):811-18. doi: 10.21474/IJAR01/6024.
27. *Hasona NA, Alrashidi AA, Aldugieman TZ, Alshdokhi AM, Ahmed MQ.* Vitis vinifera extract ameliorate hepatic and renal dysfunction induced by dexamethasone in albino rats. Toxics. 2017;5(2):1-9. doi: 10.3390/toxics5020011.
28. *Fang CY, Lou DY, Zhou LQ, Wang JC, Yang B, He QJ, et al.* Natural products: potential treatments for cisplatin-induced nephrotoxicity. Acta Pharmacol Sin. 2021;42(12):1951- 69. doi: 10.1038/s41401-021-00620-9.
29. *McSweeney KR, Gadanec LK, Qaradakhi T, Ali BA, Zulli A, Apostolopoulos V.* Mechanisms of cisplatin-induced acute kidney injury: Pathological mechanisms, pharmacological interventions, and genetic mitigations. Cancers. 2021;13(7):1572. doi: 10.3390/cancers13071572.
30. *Ravindra P, Bhiwgade DA, Kulkarni S, Rataboli PV, Dhume CY.* Cisplatin induced histological changes in renal tissue of rat. J Cell Anim Biol. 2010;4(7):108-111. Article Number - 8ACF04113170. doi: 10.5897/JCAB.9000003.
31. *Gong Q, Yin J, Wang M, He L, Lei F, Luo Y, et al.* Comprehensive study of dexamethasone on albumin biogenesis during normal and pathological renal conditions. Pharm Biol. 2020;58(1):1261-71. doi: 10.1080/13880209.2020.1855214.
32. *Danaiyan S, Abbasi MM, Raeisi S, Argani H, Ghorbanihaghjo A, Shanehbandi D, et al.* The efects of remdesivir and dexamethasone on renal sirtuin1 expression and renal function in male rats. Appl Biochem Biotechnol. 2024;196(2):632–642. doi: 10.1007/s12010-023-04529-3.
33. *Katanić Stanković JS, Selaković D, Rosić G.* Oxidative Damage as a Fundament of Systemic Toxicities Induced by Cisplatin-The Crucial Limitation or Potential Therapeutic Target? Int J Mol Sci. 2023; 24(19):14574. doi: 10.3390/ijms241914574.