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Mitochondrial Transplantation Improved Renal Damage - Induced by Uranyl Acetate

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Abstract

Oxidative stress and mitochondrial dysfunction are considered as the main nephrotoxicity mechanisms in uranyl acetate-induced renal damage. The delivery of freshly isolated mitochondria into a damaged cell from healthy tissue is one of the new treatments for mitochondria-related diseases. Transfer of exogenous mitochondria may restore normal cell function.

The effectiveness of inserting newly isolated mitochondria from the rat kidney into uranyl acetate-disrupted rat Renal Proximal Tubular Cells (RPTCs) was the focus of this investigation. By measuring changes in Mitochondrial Membrane Potential (MMP) and mitochondrial Succinate Dehydrogenase Activity (SDH), the proper functioning of isolated mitochondria was initially characterized.

Afterward, the protective effects of mitochondrial transplantation on the impaired RPTCs were assessed through several parameters including MTT, Reactive Oxygen Species (ROS), MMP, lipid peroxidation, GSH and GSSG contents and caspase-3 activity. Our data showed that addition of exogenous mitochondria to the uranyl acetate -treated RPTCs, significantly enhanced cell survival by decreasing of ROS generation, MMP collapse and lipid peroxidation, increasing of GSH contents and reducing of GSSG levels and caspase-3 activity.

In conclusion, our findings demonstrated that mitochondrial transplantation might be a promising option for treating the nephrotoxic effects of uranyl acetate.

Keywords: Uranyl acetate; Mitochondrial transplantation; Nephrotoxicity; Oxidative stress

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Introduction

A byproduct of the Uranium (U) enrichment process, depleted uranium, mostly composed of the uranyl ion U238, has been utilized for decades in industrial and military settings. Inhalation, ingestion, or ingestion can all result in accidental exposure to uranyl acetate dust or spatters, as can wounds sustained during industrial applications, waste disposal, and warfare [1]. The radioactivity of depleted uranium is roughly 60% that of normal uranium, yet it has a similar weighty metal harmfulness as regular uranium [2]. The compound harmfulness of intense and high exposure to depleted uranium explicitly focuses on the kidneys, bringing about serious renal proximal cylindrical putrefaction, renal disappointment, and eventually passing. Kidney hurt is caused when the uranium utilization outperforms 2 mg/kg body-weight or when kidney uranium explanation shows up at 3 µg/g in a short period of time. When humans are exposed to high doses of uranyl salts in a short period of time, they experience a decrease in glomerular filtration rate as well as an increase in serum creatinine, urine protein, and urine catalase [3,4]. Various cell types are toxically affected by uranyl acetate, according to in vitro research. In human dermal fibroblast primary cells, for instance, uranyl acetate causes oxidative stress, mitochondrial membrane potential collapse, glutathione depletion, and apoptosis. Additionally, upon exposure to uranium, Proximal Renal Tubular Cells (RPTCs) release lactate dehydrogenase [2,5].

In uranyl acetate-induced damage, Reactive Oxygen Species (ROS) formation is thought to be a significant toxicological mechanism. The cells' damage to mitochondria and lysosomes is linked to uranium's cytotoxic effect [6].

Thibault et al. reported that uranium induces apoptosis in the rat renal proximal tubular cell line NRK-52E by stimulating ROS generation, caspase-9 and caspase-3. It is abundantly clear that

mitochondria play a significant role in the nephrotoxicity caused by depleted uranium [7]. The mammalian cell's primary source of energy is the mitochondrion, a multifunctional organelle. Mitochondrial dysfunction is linked to more than 100 diseases that affect humans. The goal of mitochondrial therapy (mitotherapy) is to restore cell viability and, by extension, stop the progression of the disease by inserting functional exogenous mitochondria into mitochondria-deficient cells. The need to forestall or mitigate mitochondrial changes, particularly those evoked by oxidative pressure and mitochondrial brokenness, requests new restorative procedures in sub-atomic and cell medication that go further in pharmacotherapy [8,9]. Research has shown that the internalization of isolated mitochondria into cultured mammalian cells is possible with simple co-incubation [10].

Most of mitochondrial harms are irreversible on the grounds that they are brought about by lesions, like the mtDNA transformation, so different techniques, for example, chelators and compounds with antioxidant properties, have been tried to treat uranyl acetate harmfulness recently. However, these strategies can only provide minimal protection [1].

Hence, this study plans to assess the chance of utilizing another treatment in light of the transplantation of sound mitochondria isolated from the kidney into Renal Proximal Tubular Cells (RPTCs) with mitochondria compromised by uranyl acetate exposure.

Materials and Methods

Chemicals

Uranyl acetate dehydrate was purchased from BDH, UK. rhodamine 123, collagenase type II, N-(2-Hydroxyethyl)Piperazine-N'-(2-Ethanesulfonic acid) (HEPES), 3-(4,5-dimethylthiazol2-yl)-2,5-diphenyltetrazolium bromide, (MTT), 2',7'-Dichlorofluorescein Diacetate (DCFH), Coomassie Blue, trypan blue, cytochalasin D, EIPA, methyl-β-cyclodextrin, Bovine Serum Albumin (BSA), Dimethyl Sulfoxide (DMSO) and trichloroacetic acid were acquired by the Sigma-Aldrich Chemie GmbH, Tutschkrichen in Germany. The most excellent commercially accessible review was all the other chemicals.

Animals

In this study, we used male rats that weighed between 280 and 300 grams. The animals came from the Faculty of Medicine Animal House at Shahid Beheshti University of Medical Sciences, Tehran, Iran. Each animal had their own space and were taken care of every day. They could eat and drink whenever they wanted. The light and dark changed every 12 h. The temperature was kept between 20 and 22 degrees Celsius, and the humidity was between 50% and 60%. The Shahid Beheshti University of Medical Sciences Animal Ethics Committee allowed working with animals in this study with the approval code: IR.SBMU.PHARMACY.REC.1401.132.

Isolation of proximal tubular cells from rat kidney

RPTCs were isolated as described before by Boom et al., Schafer et al. [11,12]. Animals were anesthetized with 40 mg/kg of ketamine and 10 mg/kg of xylazine. The kidneys were subjected to a 5-min wash with a specialized salt solution known as HBSS lacking Ca and Mg and supplemented with 0.5 mM EGTA and 50 mM Hepes (pH 7.4) at 37 ~ for 5 min. The temperature was 37 degrees Celsius. The kidneys were removed immediately after perfusion then the renal capsule was removed and the cortex was trimmed from the medulla in Petridish and minced into very small pieces (about 1 mm^3). Separation of RPTCs was effectively created employing a 0.05% collagenase sort II HBSS that contained 4 mM of $CaCl_2$ and 1% penicillin/streptomycin. After assimilation, the arrangement was passed through a 120-mesh sifter to evacuate cell flotsam and jetsam and glomeruli. Once more, the suspension was passed through a 60-mesh estimate strainer to evacuate distal tubules. The suspension was centrifuged for 10 min at 1000 rpm at 4°. The cell pellet was resuspended in Earl's arrangement (pH=7.4).

Cell viability assay

The cell viability of the confined RPTCs was surveyed from the intactness of the plasma layer as measured by the MTT (3-(4,5-dimethylthiazol-2-yl)) test. RPTCs were plated onto 96 well plate (1×10^6 cells/ml) and brooded with diverse concentration of uranyl acetate (250, 500, 750 and 1000 μM) for 6 h. After the brooding time, the viability of cells was measured with MTT method [13].

Isolation, normalization, and evaluating function of Mitochondria

Mitochondria were arranged from the Wistar rat's kidney utilizing differential centrifugation. Kidneys were rapidly expelled and set in ice-cold mannitol buffer (D-mannitol (0.225 M), EDTA (0.2 mM), as well as sucrose (75 mM)). Kidneys were flushed, minced and washed with cold mannitol buffer and homogenized with a glass homogenizer. Cores and cell flotsam and jetsam were evacuated by implies of centrifugation at 1000 g for 10 minutes at 4°C, after that, the supernatant was treated with 250 μL of BSA arrangement, which was at that point ceaselessly sifted through 40- and 5- μm networks, individually. Mitochondria were disconnected on centrifugation of the supernatant at 10,000 g for 10 min. The dull pressed foot layer was re-suspended within the mannitol arrangement and re-centrifuged twice at 10,000 × g for 10 min. the sedimentation of mitochondrial was carried out in a Tris arrangement containing 0.05 M Tris–HCl buffer (pH 7.4), 0.25 M sucrose, 20 mM KCl, 2.0 mM $MgCl_2$, and 1.0 mM Na_2HPO_4 at 4°C and at long last weakened to contain around 0.5 mg/ml mitochondrial. investigation of mitochondrial Succinate Dehydrogenase (SDH) activity as a mitochondrial viability marker was carried out through the estimation of the MTT diminishment at the absorbance at 570 nm utilizing an ELISA Peruser (Tecan, Rainbow Thermo, Mannedorf, Switzerland). Too, Rhodamine123 (Rh123) mitochondrial take-up usage was with the reason of MMP recognizable proof. The brooding of 0.5 mg protein/mL of the mitochondrial test with Rh123 (10 μM) was at first carried out within the MMP measure arrangement. Moment, fluorescence observing methods at 490 nm λ excitation and 535 nm λ emission wavelengths were conducted employing a spectrofluorometer (Shimadzu RF5000U, Kyoto, Japan) [14,15].

Study design

RPTCs were taken out of a rat kidney (10^6 cells/ml). They were put in a solution called Earle's buffer and kept at 37°C temperature for 2 h. Then they were exposed to a chemical called uranyl acetate at a specific concentration. The kidney cells were separated and prepared for transplant by cooling them to 4°C. Then dilution to the optimal doses were performed. The mixture in the container was changed from one containing RPTC to one containing mitochondria, and then placed in a container circulated on a 37°C water bath for four hours.

Quantification of ROS level

ROS generation in the isolated rat kidney following mitochondrial

transplantation was determined by use of the fluorescent probe DCFH-DA (1.6 µM). In the presence of ROS, DCFH penetrates the RPTCs and is hydrolyzed to highly fluorescent Dichlorofluorescein (DCF). Measurement of fluorescence of DCF was accomplished by a spectrofluorometer (Shimadzu RF5000U) with Excitation 500 and Emission 520 nm wavelengths. Data were demonstrated as fluorescence intensity in cellular media [16].

Mitochondrial membrane potential (MMP) assay

Rhodamine123 (Rh123) as a cationic dye which can store in mitochondria of live cells, was used for measurement of mitochondrial membrane potential.

RPTCs suspension (0.5 mL) were centrifuged at 1,000 g for 1 min. Following removal of the supernatant solution, RPTCs were incubated with 2 mL of the 1.5 µM Rh123 for 10 min. Finally, the amount Rh123 remaining in the RPT cell suspensions was assessed by a spectrophotometer (Shimadzu RF5000U) with Excitation 490 and Emission 520 nm wavelengths [17].

Lipid peroxidation measurement

Malondialdehyde (MDA) as an indicator for oxidative stress was assayed according to the Thiobarbituric Acid (TBA) method. The MDA-TBA complex were observed at 535 nm with a microplate reader using a colorimetric method [18].

Measurement of GSH and GSSG content

After putting TCA 10% (0.5 mL) into the RPTCs, they were spun around for two minutes at 11,000 g. Next, a liquid called EDTA buffer was added to a small amount of another liquid called supernatant. The amount added was 4.5 mL of EDTA buffer and 0.5 mL of supernatant. Then, a small amount of OPA and a slightly larger amount of the diluted supernatant were mixed with a special buffer solution. The brightness of something was measured using a special machine called a spectrofluorometer from a company in Japan called Shimadzu, by fluorescence light at 350 nm excitation and 420 nm emission wavelengths and seeing how much light was given off after letting it sit for 15 min at a 25°C [19].

Caspase 3 activity assay

We checked how much caspase 3 was active using a special kit. We did this by seeing how well it broke down a substance called a peptide substrate (AC- DEVE- pNA). We used a machine that measures how much pNA absorbed light. It measured at a specific wavelength 405 nm [20].

Statistical analysis

The data were presented as mean ± SD. One-way Analysis of Variance (ANOVA) followed by appropriate post hoc tests were used for statistical analysis of each result.

The normality test was the Turkey's post hoc test. the minimal level of significance was set at P<0.05. This study used GraphPad Prism 8 (GraphPad, La Jolla, CA, USA).

Results

Appropriate function and efficacy of fresh isolated mitochondria

Figure 1A demonstrates that the succinate dehydrogenase activity of freshly isolated mitochondria did not show a significant change after 1, 2, 3 and 4 h of incubation (p<0.05).

Figure 1B shows that there was no significant decrease in MMP after 1 h to 4 h of incubation (p<0.05). MMP measurement for mitochondria was performed by Rhodamine 123 fluorescent probe assay.

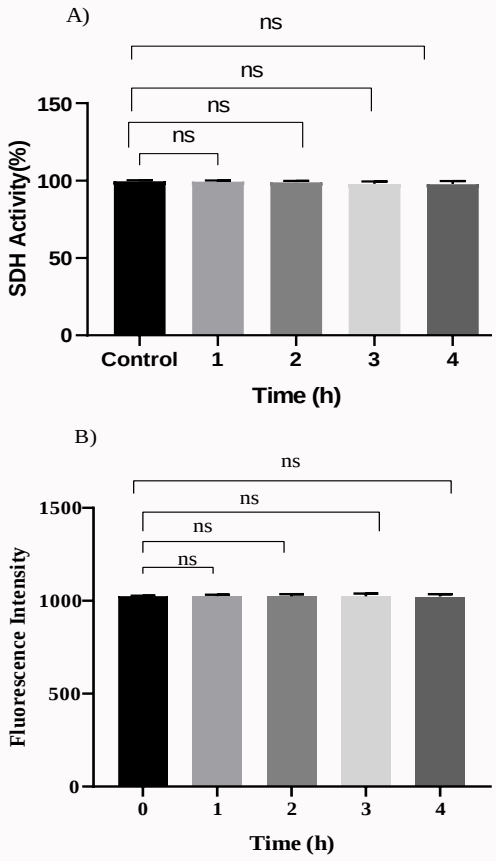

Figure 1A, 1B): Functional assurance exams of isolated mitochondria. Evaluation of succinate dehydrogenase A, and potential of mitochondrial membrane; B, of isolated mitochondria.
One way ANOVA used to determine significant differences. Data were presented as Mean ± SD (n=3); ns = not significant

Effect of transplanting exogenous mitochondria on viability analysis

RPTCS survival were obtained by SDH activity assay with MTT tetrazolium reagent). Our finding showed that uranyl acetate at concentrations of 250, 500, 750 and 1000 µM caused a significant decrease of SDH activity in RPT cells after 2 h of incubation (P<0.05). Exogenous isolated mitochondria were exposed to uranyl acetate -treated RPTCs for 4 h in an incubator. It was observed that the concentrations of 200, 400 and 800 µg protein/mL mitochondria which were determined based on our previous Studies, significantly inhibited the reduction of cell viability (SDH activity) (Figure 2, 3).

Effect of transplanting exogenous mitochondria on ROS level

The results showed that ROS formation in the RPTCs exposed to uranium acetate for a 2 h (750 µM) increased significantly compared to the control group (P<0.0001;). After 4 h of incubation of cells treated with urinal acetate and exogenous mitochondria in contact with each other, the amount of ROS production decreased significantly in uranyl acetate treated cells. These results indicate that mitotherapy reduces the oxidative stress caused by urinal acetate in RPTCS (Figure 4).

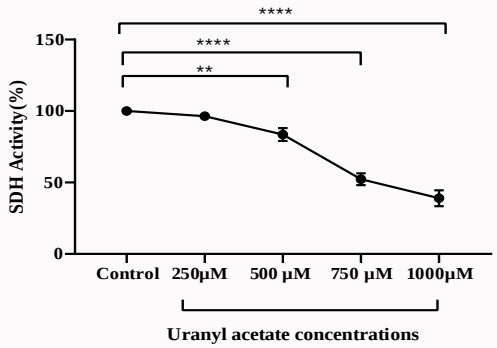

Figure 2: Viability of RPTCs following treatment with Uranyl acetate by Trypan Blue assay with various dosages of uranyl acetate. One way ANOVA used to determine significant differences. Data were presented as Mean ± SD (n=3); **p<0.01 and ****p<0.0001 show a significant difference between indicated group and control group.

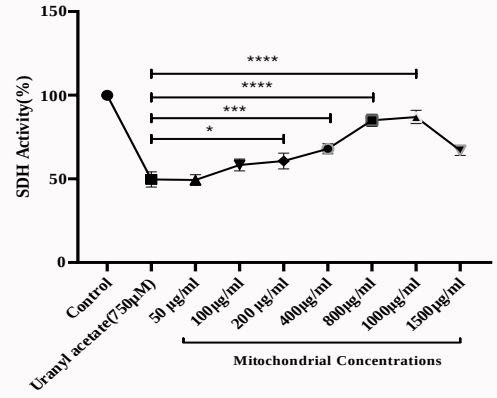

Figure 3: Mitochondrial transplantation effect on Succinate Dehydrogenase (SDH) activity in Renal Proximal Tubular Cells (RPTCs) One way ANOVA used to determine significant differences. Data were presented as Mean ± SD (n=3); *p<0.05, ***p<0.001, ****p<0.0001 show a significant difference between indicated group and uranyl acetate group.

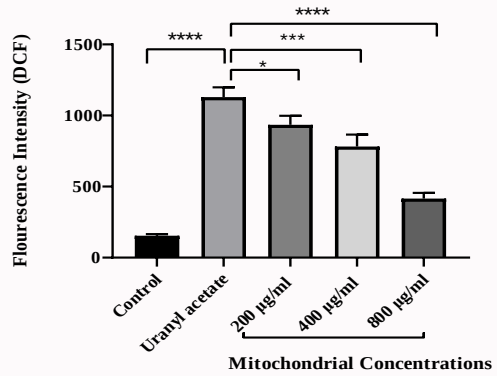

Figure 4: Mitochondrial transplantation effect on uranyl acetate-induced ROS formation in Renal Proximal Tubular Cells (RPTCs). One way ANOVA used to determine significant differences. Data were presented as Mean ± SD (n=3); *p<0.05, ***p<0.001, ****p<0.0001 show a significant difference between indicated group and uranyl acetate group.

Effect of transplanting exogenous mitochondria on MMP analysis

Figure 5 indicated that uranium acetate-treatment (750 µM) caused significant collapse of mmp in RPTCS. The results showed that fresh mitochondria inhibited the reduction of MMP after 2 h of

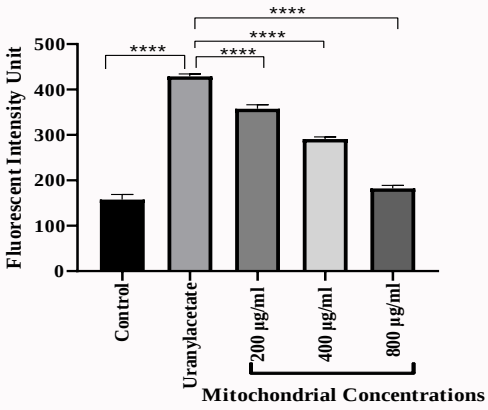

Figure 5: Mitochondrial transplantation effect on collapse of mitochondrial membrane potential in Renal Proximal Tubular Cells (RPTCs). One way ANOVA used to determine significant differences. Data were presented as Mean ± SD (n=3); ****p<0.0001 show a significant difference between indicated group and uranyl acetate group.

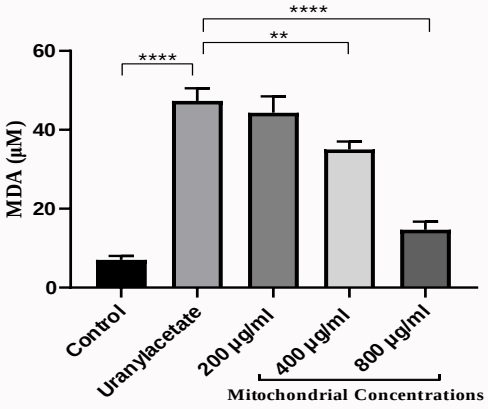

Figure 6: Mitochondrial transplantation effect on Lipid Peroxidation (LPO) in Renal Proximal Tubular Cells (RPTCs). One way ANOVA used to determine significant differences. Data were presented as Mean ± SD (n=3); ** p<0.01 and ****p<0.0001 show a significant difference between indicated group and uranyl acetate group.

exposure to uranium-treated RPTCS.

Effect of transplanting exogenous mitochondria on lipid peroxidation

Figure 6 shows that the amount of MDA in rat RPTCs increased as a result of uranium toxicity in the concentration used (750 µM). The study found that when mitochondria were transferred to kidney cells, the LPO caused by uranium was reduced.

Effect of transplanting exogenous mitochondria on GSH Level

As shown in Figure 7A, 7B, concentrations of reduced glutathione (GSH) in the RPTCS was decreased significantly following uranyl acetate (2 h exposure with 750 µM uranyl acetate) exposure and oxidized glutathione (GSSG) was increased. Four hours of incubation of uranyl-affected RPTCS with exogenous kidney mitochondria significantly ($p<0.05$) inhibited these GSH and GSSG alterations in RPTCs.

Effect of transplanting exogenous mitochondria on Caspase-3 activity

The results showed that activity of caspase 3 (critical executioner

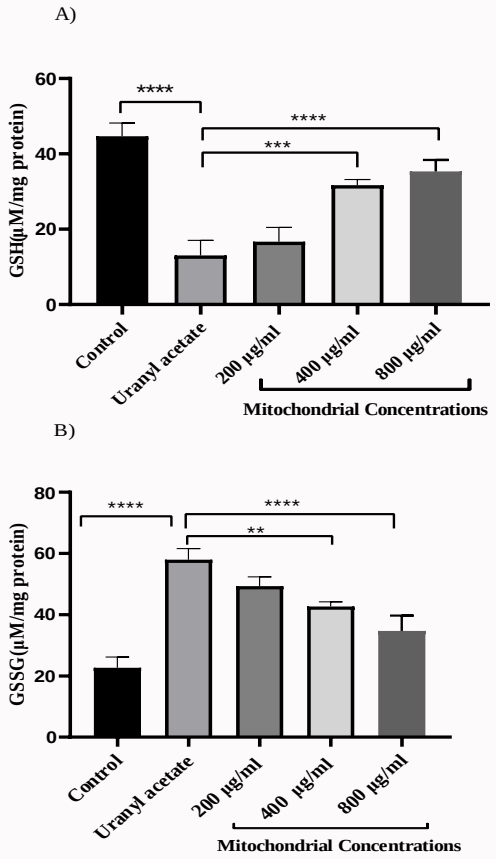

Figure 7A, 7B: Mitochondrial transplantation effect on GSH and GSSG content in Renal Proximal Tubular Cells (RPTCs). One way ANOVA used to determine significant differences. Data were presented as Mean ± SD (n=3); ** p<0.01 and ****p<0.0001 show a significant difference between indicated group and uranyl acetate group.

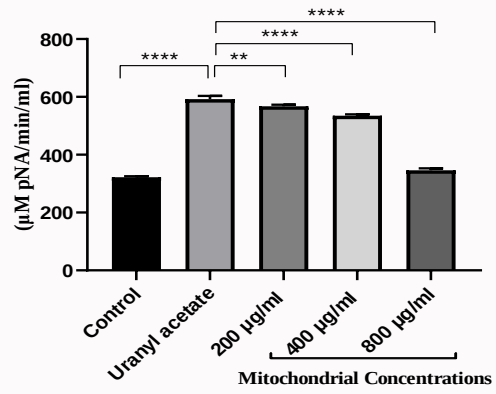

Figure 8: Mitochondrial transplantation effect on caspase-3 activity in Renal Proximal Tubular Cells (RPTCs). One way ANOVA used to determine significant differences. Data were presented as Mean ± SD (n=3); ** p<0.01 and ****p<0.0001 show a significant difference between indicated group and uranyl acetate group.

of apoptosis) was significantly increased in the RPTCs treated with 750 µM uranyl acetate (during 2 h exposure). Again, mitochondrial transplantation inhibited this increased caspase 3 activity in uranyl treated RPTCs (Figure 8).

Discussion

Due to the fact that there is no study related to the efficacy of healthy and fresh exogenous mitochondria transfer into rat RPTCs toxicity affected by uranyl acetate so far, we decided to evaluate the success of mitochondrial transplantation as a new promising tool against depleted uranium (uranyl salt) induced nephrotoxicity.

Mitochondria is the major place for Reactive Oxygen Species (ROS) generation in the cell, which includes 60% to 80% of ROS formation in the cell [21]. Mitochondria are also the main powerhouse of energy supply and provide more than 90% of the cell's ATP content [22].

Mitochondrial dysfunction often occurs in the initial stages of toxicological processes, and they are importantly involved in the occurrence of various morbidities, including cancer, cardiovascular and neurodegenerative diseases [23].

Kidneys are the most important way to remove uranium from the body and the proximal tubule is the main site that is damaged by uranium toxicity [24].

Previous studies have shown that uranium causes renal toxicity in experimental animals and increases the level of blood urea nitrogen and creatinine [25].

The results of studies show that depleted uranium increases N-Acetylglucosamine (NAG), Lactate Dehydrogenase (LDH) and ROS and decreases Catalase (CAT) and Glutathione (GSH) in proximal tubule cells (HK-2 cell) [3].

Therapeutic effects of mitochondrial transplantation have been proven as a potential method in the treatment of cardiovascular diseases, aging, type 2 diabetes, cancer, and neurodegenerative diseases in which mitochondrial dysfunction occurs [26]. Due to the fact that uranium toxicity in the proximal tubular cells of the kidney causes a disruption in the mitochondrial function, mitotherapy can be one of the new strategies for its treatment.

Hernandez-Cruz et al. showed that mitochondrial transplantation can be used as a new method in the treatment of cadmium toxicity in the kidney, which reduces the amount of reactive oxygen radicals [27].

In another study, it was shown that the transfer of healthy mitochondria to the kidney proximal tubular cells in rats can reduce mitochondrial dysfunction, oxidative stress and toxicity parameters caused by cisplatin [28].

The important mechanism of uranyl acetate toxicity is considered to be oxidative stress and increased ROS production in cells. The uranium cytotoxicity is related to the mitochondrial/lysosomal disruption inside the cells [6]. Thibault et al. showed that rat renal proximal tubular cells exposed to UA leads to the production of ROS and the reduction of MMP and mitochondrial apoptotic pathway [7].

Our data showed that the exposure of RPT cells to uranyl acetate enhanced ROS formation and declined the mitochondrial membrane potential 4 h after incubation. While mitotherapy inhibited uranyl acetate induced ROS production in RPT cells and decreased cytotoxicity of uranyl acetate. Also, reduction of MMP collapse was observed after delivery of 200, 400 and 800 µg protein/mL of fresh exogenous mitochondria to uranyl acetate treated RPTCs.

Lipid peroxidation is one of the expected complications of the ROS formation biological environment [21]. Our findings demonstrated that the concentration of Malondialdehyde (MDA) in uranyl acetate affected RPTCs was significantly increased compared

to the control group, which again confirms the role of stress oxidative in uranyl acetate toxicity. The lipid peroxidation induced by uranyl acetate on different cell types has been previously published in several papers [5]. In this study, uranyl acetate induced lipid peroxidation was significantly reduced by mitotherapy.

Glutathione is a low molecular weight main component in the cytoplasm that scavenges oxygen free radicals. One of the important roles of glutathione is neutralizing the reactive oxygen species by converting GSH to oxidized form of Glutathione (GSSG). The export of excess GSSG to the outside of the cell is usually called glutathione depletion [29].

The results of our study confirm the previous study as shown that oxidation of glutathione occurred due to oxidative stress caused by uranyl acetate. After incubation with freshly isolated mitochondria, an increase in GSH and decrease in GSSG was observed in RPT cells.

According to previously published data, uranyl acetate can lead to the opening of MPT pore by targeting mitochondria. As a result of MPT pore openings, apoptogenic factors are released into the cytosol. Subsequently, caspases cascade is activated, especially caspase 3, which is the most important apoptosis executioner [29]. Our data showed that the caspase 3 toxic activity was inhibited in uranyl acetate treated RPTCs after incubation with fresh mitochondria.

Conclusion

Considering that oxidative stress and mitochondrial damage are reported to be the most important mechanisms of uranyl acetate toxicity, the present study aims to investigate the cytoprotective role of healthy mitochondrial transplantation to the uranyl acetate treated RPTCs. Our results showed that mitotherapy can improve cytotoxicity, ROS production level, GSH and GSSG content and inhibit cellular caspase 3 activity. In conclusion, transplantation of mitochondria can be suggested as a promising therapeutic tool for the treatment of diseases related to mitochondria.

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