Antioxidative Effects of Nano-curcumin on Liver Mitochondria Function in Paraquat-induced Oxidative Stress

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Abstract

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Introduction

Poisoning with Paraquat (PQ) is one of the most dangerous poisonings physicians face in the emergency wards. However, PQ is widely used in agriculture due to its herbicidal effect and low cost. PQ poisoning damages the cell membrane by generating free radicals and exerts adverse effects on body organs [1]. PQ can be absorbed through mouth or skin, and with massive exposure, it can kill humans within 3.5 hours.
The PQ toxicity in humans and animals appears as acute and chronic. Respiratory failure is the leading cause of death and causes disability and damage to the liver, kidney, heart, adrenal glands, spleen, and central nerves. It can also affect systems and organs like the central nervous system, liver, kidney, eye, and immune system. Accordingly, this chemical can endanger the health of humans, animals, and even aquatic organisms [2]. PQ toxicity is associated with the oxidation system and mitochondrial restoration. It seems that PQ-induced lung injury involves the production of PQ cation radicals from redox cycling. Furthermore, in PQ toxicity, the Release of Reactive Oxygen Species (ROS) such as superoxide anion, considerably decrease the levels of vital reducing equivalents such as NADPH (Nicotinamide adenine dinucleotide phosphate) and reduced glutathione [3].

None of the antidotes used in PQ poisoning have been clinically fully effective [4]. Treatments sometimes include clinically and, in some cases, empirically tested chemicals, including corticosteroids, immunosuppressive agents, fibrinolytic agents, colchicine and radiotherapy, iron-chelating by chelating agents like deferoxamine, selenium, exogenous glutathione and N-acetyl cysteine [2, 5].

Antioxidants are chemicals that prevent the absorption of ROS [6]. Curcumin or diferuloylmethane is a biologically active ingredient of Curcuma longa from the ginger family. It is extracted from the rhizome of this plant, which is traditionally and commonly cultivated in Iran [7]. Different studies have documented the potential therapeutic effects of curcumin in a wide range of diseases like cancer, lung disease, neurodegenerative diseases, kidney disease, metabolites, heart disease, and other inflammatory diseases.

Curcumin has anti-oxidative, anti-inflammatory, and anti-cancer effects [8]. Moreover, curcumin shows a high ability to scavenge free radicals, exerts anti-inflammatory effects, and inhibits tumor growth. In cancerous cells, curcumin inhibits growth factors related to signaling pathways such as extracellular kinases and protein kinase C [9]. Curcumin can exert antioxidant activity by intensifying glutathione synthesis in poisoning [10].

Many approaches have been examined to increase the bioavailability of curcumin. These techniques include using adjuvants like piperine, liposomal curcumin, structural curcumin analogs such as 3,5-bis[(2-fluorophenyl)methylene]-4-piperidinone, curcumin phospholipid complex, and curcumin nanoparticles [11]. Some studies have shown that nanotechnology and encapsulation of curcumin in nano-emulsions (nano-curcumin) can enhance the medical properties of this material [7, 12]. The current study aimed to evaluate the effects of nano-curcumin compared with curcumin against the PQ-induced liver mitochondrial dysfunction.

Materials and Methods

Experimental Protocols

In this study, 36 adult Wistar rats weighing 250-280 g were randomly divided into 6 following groups: normal saline, 5 mg/kg PQ [7], 100 mg/kg curcumin, 100 mg/kg nano-curcumin, curcumin + 5 mg/kg PQ, and nano-curcumin + 5 mg/kg PQ.

Preparation of liver mitochondria

The liver was cut up with a scalpel in a cold mannitol solution containing 0.225 M D-mannitol, 75 mM sucrose, and 0.2 mM Ethylenediaminetetraacetic acid (EDTA). The pieces of livers (30 g) were gently homogenized in a glass homogenizer with a Teflon pestle and then centrifuged at 700×g for 10 min at 4°C to remove its nuclei, intact cells, and other non-subcellular components. The supernatants were collected in another falcon and were again centrifuged at 7000×g for 20 min. The supernatant contained crude microosomal fraction, and the pale, loose upper layer was rich in swollen or broken mitochondria, lysosomes, and some microsomes that were washed away from sediments [13].

Total protein measurement

Total protein concentration was measured using Bradford reagent and BSA standard. To prepare Bradford reagent, 100 mg of Coomassie Brilliant Blue G-250 was dissolved in 50 mL of 95% ethanol and added to 100 mL of 85% phosphoric acid. Protein contents of the samples were read at 595 nm by adding 10 µL of sample to 5 mL of reagent against the standard [14].

Total Antioxidant Capacity (TAC) level measurement

Total Antioxidant Capacity (TAC) was measured according to the FRAP method [15]. Briefly, in this method, the antioxidant compounds can be measured at low pH by reduction of Fe III complex ([3, 5-bis[(2-fluorophenyl)methylene]-4-piperidinone] TPTZ) to ferrous (Fe II) (with bluish color) by measuring the absorption changes at 593 nm wavelength [15].
Measuring Lipid Peroxidation (LPO) level

Lipid peroxidation was assayed with the Yagi method. This method is based on the reaction between Malondialdehyde (MDA) — as the final product of lipid peroxidation — and Thiobarbituric Acid (TBA), which produces a red complex with optimum absorbance at 532 nm. The standard curve was prepared by tetraethoxypropane solution [16].

Measuring catalase (CAT) activity

The activity of Catalase (CAT) was measured with Ai et al. approach followed by hydrogen peroxide (H₂O₂) decomposition at 240 nm. The reaction started by adding 30 mM H₂O₂ to a suitable volume of its mitochondria in 50 mM sodium phosphate buffer at pH 7. The absorbance was then measured for 3 min at 240 nm, and specific activity was calculated in units per mg protein [17].

Measuring Superoxide Dismutase (SOD) activity

To measure Superoxide Dismutase (SOD) activity, 20 µL mitochondria was extracted with 0.1 mM EDTA in 0.3 mM sodium cyanide and 3 mM nitrobutetrazolium in a cuvette and stirred at 37°C for 5 min. Then, 0.12 mM riboflavin in 0.026 mM phosphate buffer with pH=7.8 was placed at room temperature for 10 min. The absorbance was read at 560 nm for 5 min, and the specific activity was determined in units per mg protein [18].

Measuring of mitochondria viability

The viability of mitochondria was evaluated by the MTT method. This method is based on the reduction of tetrazolium salt to purple formazan by the mitochondrial dehydrogenase enzyme in viable cells. The purple-colored formazan was measured at 560 nm, and the reference wavelength of 630 nm with ELISA reader [19].

Measuring mitochondrial membrane potential

Rhodamine B is an unusual rhodamine derivative. Besides emitting red fluorescence when excited at the standard rhodamine excitation wavelength of 546 nm [20], it emits a green fluorescence similar to that typically associated with fluorescein compounds when it is excited at a wavelength of 485 nm.

Statistical analysis

The study analysis was done in SPSS V. 16 and GraphPad Prism version 6.0 (GraphPad Software, San Diego-USA). Statistical analysis comprised a 1-way Analysis of Variance (ANOVA) followed by post hoc Tukey test. Shapiro-Wilk test was used to examine the groups’ normal distribution status. Results were expressed as the Mean±SD. P<0.05 indicates a statistically significant difference between groups.

Results

LPO levels of liver mitochondria

As shown in Figure 1, LPO in isolated mitochondria of liver tissue was significantly higher in the PQ group compared to the control group (P<0.05). Nano-curcumin treatment attenuates the LPO in isolated liver-mitochondria compared with the PQ group (P<0.05) (Figure 1).

TAC of liver mitochondria

Poisoning by PQ significantly decreased the TAC levels compared with the control subjects (P<0.01) (Figure 2).

The CAT activity of liver mitochondria

Our findings demonstrated that the level of CAT activity of mitochondria isolated from liver tissue in the PQ group significantly decreased compared with the control group (P<0.01). Treatment with curcumin and nano-curcumin did not significantly change the rate of CAT compared to the PQ group, and there were no significant differences with the healthy control group, which shows that they had normalized the CAT activity (P>0.05) (Figure 3).
The SOD activity of liver mitochondria

The results showed that the level of SOD activity of mitochondria isolated from liver tissue in the PQ group was significantly reduced as compared with the healthy control group (P<0.05). Treatment with nano-curcumin and PQ increased SOD activity compared with the PQ group (P<0.05), but no significant differences were seen between the treatment with curcumin and the control group, which shows that catalase enzyme activity has reached the normal level (Figure 4).

The viability of liver mitochondria

As shown in Figure 5, the viability of mitochondria isolated from liver tissue in the PQ group was significantly lower than that in the control group (P<0.001). Treatment with nano-curcumin improved the viability of isolated mitochondria of liver tissue as compared with the PQ group (P<0.05) (Figure 5).

The findings showed that the mitochondrial membrane potential significantly decreased in the PQ group compared with the control group (P<0.001). Treatment with nano-curcumin improved the membrane potential of mi-
toxchondria isolated from liver tissue as compared with the PQ group (P<0.05) (Figure 6).

Discussion

PQ-induced lung injury is characterized by fibrosis, pulmonary edema, and respiratory failure. The biochemical mechanism of the injury is complex and unclear; however, ROS may play a crucial role in mediating the cascade of biochemical changes induced by PQ. Accordingly, oxidative stress is an essential cause of PQ-induced lung injury [7]. The present study aimed to compare the effects of nano-curcumin and curcumin on the biological activity and membrane potential of mitochondria isolated from liver tissue in PQ subacute poisoning of male rats. The results showed the toxic effect of PQ in the liver mitochondria through induction of toxic oxidative stress as supported by increasing LPO and decreasing TAC, SOD, and CAT activity and mitochondrial dysfunction.

The results showed that nano-curcumin has more antioxidant effects as compared with curcumin. According to the results of this study, it seems that nano-curcumin co-administration with PQ has better effects on biochemical parameters compared with curcumin. Curcumin encapsulation in nanoparticles makes it easier to enter into the cells and enhance the first line of antioxidant defense against oxidative damages due to toxins such as PQ. Han et al. reported that the rats’ administration of PQ showed structural damage and liver tissue changes.

Also, PQ led to oxidative stress, increased expression of cytochrome P450 3A2 mRNA and mitochondrial damage, including mitochondrial membrane swelling, decreased mitochondrial cytochrome C, and increased levels of apoptosis-induced proteins in the cell [21]. Our results showed that PQ induces oxidative damage in liver mitochondria. Our previous study showed that curcumin and nano-curcumin improved liver mitochondrial function mitochondrial dysfunction in aluminum phosphide toxicity [22].

Based on our study results, nano-curcumin as an effective antioxidant is responsible for neutralizing or scavenging the free radicals and thus inhibiting the toxic oxidative stress. In summary, our data disclosed that exposure to PQ induced remarkable oxidative toxicity on liver mitochondria. Improvement in oxidative stress factors using nano-curcumin, suggest that nano-curcumin treatment could protect the liver against the oxidative injuries of PQ by scavenging ROS and stabilizing the oxidative status.

Ethical Considerations

Compliance with ethical guidelines

The Ethics Committee of Hamadan University of Medical Sciences approved this study (IR.UMSHA.REC.1397.281).

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Authors contribution's

Review, comment and approve of the final draft: All authors; Performing the experiment: Saba Nikdad, Nejat Kheiripour; data analysis and co-wrote the paper: Hassan Ghasemi; Supervision: Akram Ranjbar.

Conflict of interest

The authors declared no conflict of interest.

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