

RESEARCH PAPER

Effects of nano-curcumin and curcumin on the oxidant and antioxidant system of the liver mitochondria in aluminum phosphide-induced experimental toxicity

Akram Ranjbar¹, Leila Gholami¹, Hassan Ghasemi², Nejat Kheiripour^{3*}

¹Medicinal Plants and Natural Products Research Center, Hamadan University of Medical Sciences, Hamadan, Iran

²Department of Clinical Biochemistry, Abadan School of Medical Sciences, Abadan, Iran

³Research Center for Biochemistry and Nutrition in Metabolic Diseases, Kashan University of Medical Sciences, Kashan, Iran

ABSTRACT

Objective(s): Aluminum phosphide (AIP) is commonly used pesticide which could cause poisoning mainly through the induction of oxidative stress. The present study aimed to evaluate the effects of nano-curcumin and curcumin on the oxidant and antioxidant system in the liver mitochondria using AIP-induced toxicity model.

Materials and Methods: In this study, 36 male albino Wistar rats were randomly divided into six groups (n=6). The control subjects and animals poisoned with AIP (2 mg/kg) received treatment with and without nano-curcumin (100 mg/kg) and curcumin (100 mg/kg) for seven days. Mitochondria were isolated from the liver and analyzed in terms of lipid peroxidation (LPO), total antioxidant capacity (TAC), total thiol groups (TTGs), superoxide dismutase (SOD), and catalase activity. In addition, mitochondrial viability was assessed.

Results: AIP caused a significant increase in the LPO levels, while significantly decreasing TAC, TTG, SOD, catalase activity, and mitochondrial viability compared to the controls (P<0.05). Moreover, nano-curcumin treatment significantly enhanced TAC, TTG, SOD, and mitochondrial viability (P<0.05). Curcumin could also improve TTG and mitochondrial viability (P<0.05).

Conclusion: According to the results, nano-curcumin exerted protective effects against AIP-induced experimental toxicity, and the effect was attributed to the antioxidant properties of this compound.

Keywords: Aluminum Phosphide, Curcumin, Mitochondria, Nano-curcumin, Oxidative Stress

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INTRODUCTION

Aluminum phosphide (AIP), also known as rice tablet, is used as a fumigant to protect stored grains against insects and rodents [1]. The toxic effects of AIP are promoted through the toxic phosphine gas (PH₃), which is produced via contact with water, air humidity or gastric acid [2, 3]. However, the poisoning mechanism of AIP remains unknown. Recent studies have demonstrated that PH₃ is involved in the inhibition of cytochrome c oxidase, which is followed by the release of free radicals [4]. In addition, the inhibition of the

electron transporter chain leads to the reduction of adenosine triphosphate (ATP), which in turn causes damage to various tissues, such as the digestive tract, liver, and kidneys.

Evidence suggests that AIP is able to decrease the activity of antioxidant enzymes, such as superoxide dismutase (SOD), catalase, and peroxidase, as well as the release of iron from transferrin that causes the production of iron-catalyzed reactive oxygen species (ROS) through the Fenton and Haber-Weiss reactions [5]. However, no efficient antidote is available for AIP, while several studies have demonstrated that antioxidant supplements (e.g., vitamin C, vitamin E, and glutathione) have beneficial effects

* Corresponding Author Email: nejatkh.bio@gmail.com

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on the improvement of AIP poisoning [6]. Natural antioxidant components are the important agents that have been recently used for the prevention and treatment of the complications associated with oxidative stress and ROS. Curcumin (1, 7-bis [4-hydroxy-3-methoxyphenyl]-1, 6-heptadiene-3, 5-dione; Cur) is an active component of turmeric powder, which is available in the form of dietary spices and is used in herbal remedies [7]. It is a yellow curry spice that has long been used as a traditional medicine [8]. The scavenging properties of curcumin for oxygen free radicals have been reported in various studies. Furthermore, some studies have denoted that curcumin could increase intracellular antioxidant capacity, thereby preventing the damage caused by lipid peroxidation and oxidative stress [9]. On the other hand, curcumin is an inexpensive herbal medicine with few side-effects. Despite the major antioxidant properties of curcumin, recent findings have indicated that using curcumin-encapsulated nanosystems could increase the stability and bioavailability of curcumin [10].

Nanotechnology has extensively influenced bioanalysis. Stable chemical and physical features make inorganic nanoparticles particularly effective in biological assays.

The present study aimed to assess the preventative effects of curcumin and nano-curcumin against AIP-induced oxidative stress in the liver mitochondria.

MATERIALS AND METHODS

In this study, reagents and chemicals were provided by Sigma-Aldrich (St. Louis, MA, USA), including acetate buffer, ferric chloride, 2,4,6-tripyridyl-S-triazine (TPTZ), 2-thiobarbituric acid (TBA), tetraethoxypropane (TEP), 5,5-dithiobis-2-nitrobenzoic acid (DTNB), trichloroacetic acid (TCA), 2, 4, 6-tripyridyl-S-triazine (TPTZ), Tris base, n-butanol, hydrochloric acid (HCL), and ethylenediamine tetraacetic acid (EDTA). In addition, curcumin and nano-curcumin were purchased from Exir Nano Sina Co., Iran. All the applied chemicals were of an analytical grade.

Experimental design

In total, 36 male Wistar rats (weight: 220-250 g) were obtained from the animal colony of Hamadan University of Medical Sciences, Iran. The animals were preserved in standard conditions with the temperature of $22\pm 1^\circ\text{C}$, humidity of 45-55%, and

12-hour light/dark cycle. The rats were randomly divided into six groups ($n=6$). The animals in the control group received saline solution, and the animals in the AIP group were administered with an AIP solution (2 mg/kg). In addition, the animals in the control and AIP treatment groups were administered with nano-curcumin or curcumin (100 mg/kg). The treatments were performed via oral gavage and continued for seven consecutive days. At the next stage and 24 hours after the last injection, the fasting rats were anesthetized with ketamine (50 mg/kg), and liver tissue samples were collected from all the rats.

The study protocol was approved by the Medical Ethics Review Board of Hamadan University of Medical Sciences (code: IR.UMSHA.REC.1397.282)

Separation of the mitochondria from the liver cells

The isolation of the liver mitochondrial tissues was performed by centrifugation as described previously [11]. After sacrificing the animals, their liver tissues were removed, washed with cold buffer, and cut into small pieces. Afterwards, the liver pieces were homogenized in an ice-cold isolation buffer containing sucrose (70 mM), mannitol (200 mM), HEPES (10 mM), EGTA (1 mM), and 0.1% BSA (pH $\frac{1}{4}$ 7.4). Following that, the samples were centrifuged ($1500\times g$) at the temperature of 4°C for 10 minutes, and the pellets were discarded. The supernatant was centrifuged ($10,000\times g$) for 10 minutes, and the superior layer was meticulously discarded. The mitochondrial pellets were washed through suspension in the isolation buffer and centrifuged again ($10,000\times g$) for 10 minutes. Finally, the mitochondrial pellets were suspended in Tris buffer containing Tris-HCl (0.05 M), sucrose (0.25 M), KCl (20 mM), MgCl_2 (2.0 mM), and Na_2HPO_4 (1.0 mM) at the pH of 7.4 and temperature of 4°C . In order to achieve the highest quality of mitochondrial isolation, all the stages were carried out on ice.

Measurement of total protein

Total protein concentration was measured using the Bradford method and concentrated coomassie blue reagent. In addition, bovine serum albumin was used as the standard agent [12].

Measurement of lipid peroxidation

TBA was used to measure the rate of lipid peroxidation, which reacted with the lipid peroxide

molecules. The liver mitochondria were mixed with 20% TCA, and the precipitate was dispersed in H_2SO_4 (0.05 M). TBA (0.2% in 2 M sodium sulfate) was added and heated for 30 minutes in a boiling water bath. Thiobarbituric acid reacting substance adducts were extracted using n-butanol, and the absorbance was measured at 532 nanometers. The reaction was performed at acidic pH and high temperature, and the maximum absorption was measured as a pink complex at 532 nanometers [13].

Measurement of total antioxidant capacity

TAC was measured using the ferric-reducing ability of plasma (FRAP) method. FRAP is a test that shows antioxidant capacity and quantifies the ability of the plasma to reduce the ferric ion to the ferrous ion. In this process, the FRAP reagent was prepared, which contained 25 milliliters of acetate buffer (300 mM; pH: 3.6) with 16 milliliters of acetic acid per one portion of the buffer solution, 2.5 milliliters of the TPTZ solution obtained from TPTZ (10 mM) in HCl (40 mM), and 2.5 milliliters of $FeCl_3 \cdot 6H_2O$. Following that, 10 microliters of the sample was diluted with distilled water and added to 300 microliters of the reagent, which was provided recently, and incubated at the temperature of 37 °C for 10 minutes. The interaction between Fe_{2+} and TPTZ produced a blue complex with the optimum absorbance measured at 593 nanometers [14].

Measurement of total thiol groups

At this stage, DTNB was used as the reagent to estimate the total thiol molecules in the liver mitochondria. In this process, DTNB reacted with the thiol molecules, generating a yellow complex with good absorbance at 412 nanometers [15].

Measurement of superoxide dismutase and catalase activity

SOD and catalase activity were determined using a BioVision competitive ELISA kit in accordance with the instructions of the manufacturer.

Measurement of mitochondrial viability

As a testing endpoint of cytotoxicity, mitochondrial viability was determined using the 3-(4,5-dimethylthiazol-2-yl)-2,5 diphenyltetrazolium bromide (MTT) assay. Mitochondria are the major site for the reduction of the tetrazolium dye MTT to its insoluble formazan. This assay is used to measure the conversion of MTT into purple

formazan through the succinate dehydrogenase of the intact mitochondria of living cells [16].

Statistical analysis

Data analysis was performed in SPSS version 16.0 (SPSS Inc., Chicago-USA) and GraphPad Prism version 6.0 (GraphPad Software, San Diego-USA). One-way analysis of variance (ANOVA) and Tukey's post-hoc test were applied, and Shapiro-Wilk test was used to determine the normal distribution of the data in the groups. The obtained results were expressed as mean and standard deviation, and P-value of less than 0.05 was considered statistically significant.

RESULTS

Lipid peroxidation (LPO)

According to the findings, AIP caused a significant increase in LPO compared to the control group ($P < 0.01$). In the nano-curcumin and curcumin groups, LPO decreased compared to the AIP group, while the difference was not considered significant (Fig 1).

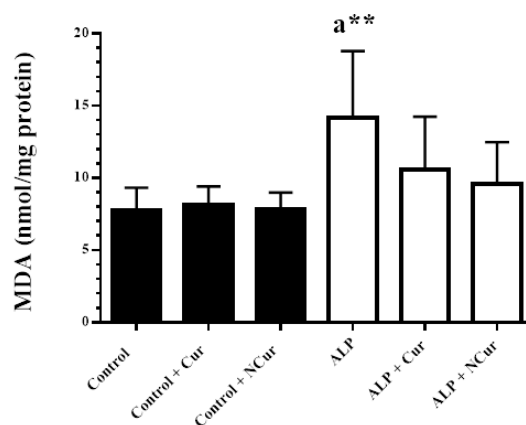


Fig 1. Measurement of MDA in Liver Mitochondria of Rats (MDA: malondialdehyde; AIP: aluminum phosphide; Cur: curcumin; NCur: nano-curcumin)
aSignificant difference with control group; bsignificant difference with PQ group; ** $P < 0.01$

Total antioxidant capacity (TAC)

AIP ($P < 0.01$) and AIP co-administered with 100 mg/kg of curcumin ($P < 0.05$) significantly decreased TAC compared to the control group. Moreover, TAC was observed to increase significantly in the nano-curcumin group (100 mg/kg) compared to the AIP group. On the other hand, the changes in the groups administered with AIP and curcumin (100 mg/kg) were not considered significant

compared to the AIP group, while a significant difference was observed in the mitochondrial viability of this group compared to the controls (P<0.05) (Fig 2).

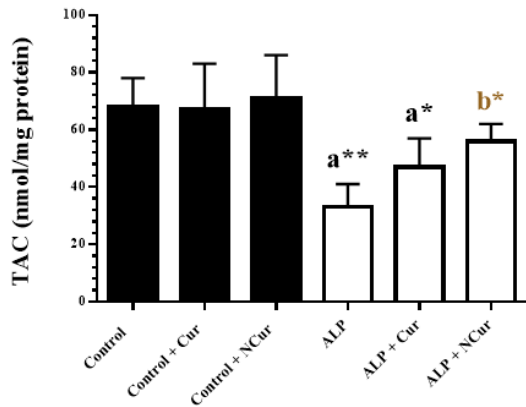


Fig 2. Measurement of TAC in Liver Mitochondria of Rats (TAC: total antioxidant capacity)
 aSignificant difference with control group; bsignificant difference with PQ group; *P<0.05, **P<0.01

Total thiol molecules (TTM)

According to the obtained results, treatment with AIP significantly reduced TTM compared to the controls (P<0.05). However, the administration of nano-curcumin and curcumin significantly improved the TTM levels compared to the AIP group (P<0.05) (Fig 3).

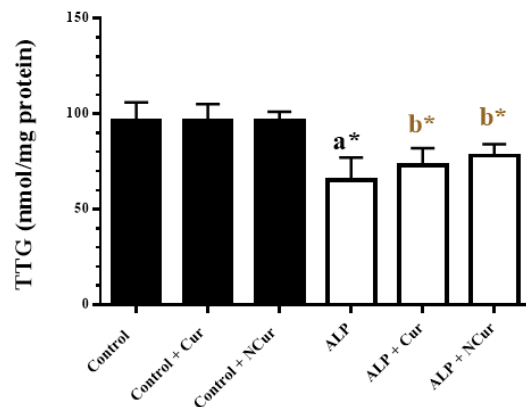


Fig 3. Measurement of TTM in Liver Mitochondria of Rats (TTG: total thiol groups)
 aSignificant difference with control group; bsignificant difference with PQ group; *P<0.05

SOD and catalase activity

A significant reduction was observed in SOD (P<0.01) and catalase activity (P<0.05) in the AIP group compared to the controls. Furthermore, the administration of nano-curcumin (100 mg/kg)

significantly increased the SOD enzyme activity compared to the AIP group (P<0.05) (Figs 4 & 5).

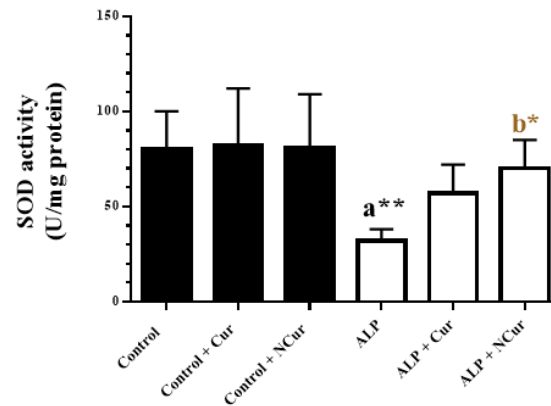


Fig 4. Measurement of SOD in Liver Mitochondria of Rats (SOD: super oxide dismutase)
 aSignificant difference with control group; bsignificant difference with PQ group; *P<0.05, **P<0.01

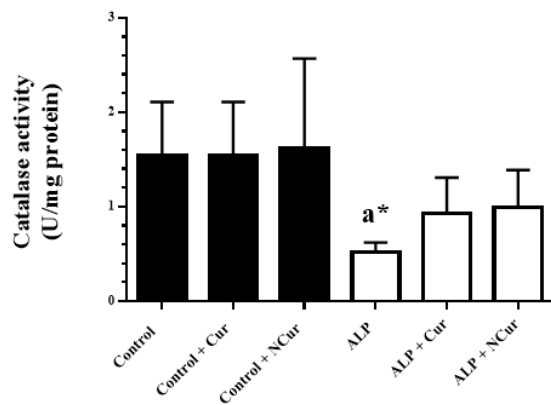


Fig 5. Measurement of Catalase in Liver Mitochondria of Rats
 aSignificant difference with control group; bsignificant difference with PQ group; *P<0.05

Mitochondrial viability

The obtained results regarding mitochondrial viability in the liver (Fig 4) indicated that AIP induced significant reduction in mitochondrial viability compared to the control group (P<0.01). Interestingly, the treatment with nano-curcumin (P<0.01) and curcumin (P<0.05) caused a significant increase in mitochondrial viability compared to the AIP group.

Moreover, the co-administration of AIP and curcumin (100 mg/kg) induced a significant difference in the mitochondrial viability compared to the controls (P<0.05) (Fig 6).

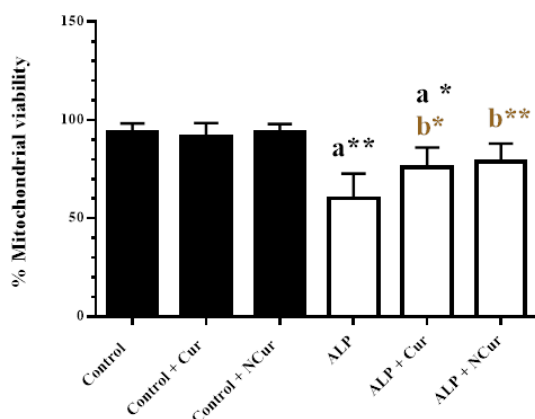


Fig 6. Measurement of Liver Mitochondria Viability in Rats
 aSignificant difference with control group; bsignificant difference with PQ group; *P<0.05, **P<0.01

DISCUSSION

AIP is known as rice tablet and is an effective pesticide, which is commonly used for protection of stored grains against various rodents and pests. Poisoning by AIP leads to PH3 production, which causes oxidative stress in various tissues [17]. Oxidative stress plays a key role in the development of several diseases, and no effective antidotes have been identified for the treatment of AIP poisoning. Therefore, evaluation of the influence of other treatments is important in this regard [3]. In the present study, we also investigated the antioxidant capacity of curcumin and nano-curcumin against liver mitochondrial toxicity induced by AIP in a rat model. According to the obtained results, AIP had toxic effects on the liver mitochondria through the induction of oxidative stress, which was indicated by the increased LPO. Moreover, AIP decreased the TAC and TTM content, as well as SOD and catalase activity, in the liver mitochondria [18-21]. The oxidative damage caused by phosphine has been confirmed in various tissues in several animal models [17].

Recent studies have indicated that the phosphine-induced production of H_2O_2 is the most remarkable oxidant agent that could reduce glutathione and enzymatic antioxidant systems (e.g., catalase and SOD) [17]. Moreover, various studies have demonstrated that PH3 poisoning could significantly increase malondialdehyde (MDA) and H_2O_2 levels in rats exposed to AIP [20, 22-24]. In this regard, Chugh et al. increased MDA and decreased SOD activity in the serum of patients one and two days after

AIP poisoning [25].

Natural free radical scavengers and antioxidant agents such as curcumin and phytochemicals derived from turmeric (*Curcuma longa*) could attenuate the cellular damage mediated by oxidative stress caused by toxic agents such as AIP [26, 27]. Curcumin is a yellow compound isolated from a rhizome, which is a member of the curcuminoid family and has been used in traditional medicine for centuries. To date, no toxicity has been attributed to this natural compound. Several studies have indicated that curcumin has broad biological functions, especially antioxidant and anti-inflammatory properties [28]. Furthermore, our findings demonstrated that curcumin exerts hepatoprotective effects through the reduction of oxidative stress and induction of antioxidant activities by TAC, TTM, and SOD in the liver mitochondria, especially when applied encapsulated.

These findings are consistent with the previous studies, reporting that curcumin is a potent antioxidant that attenuates oxidative stress in experimental models [27, 29-31]. According to the literature, the beneficial effects of curcumin are associated with its structure. Curcumin has been reported to have two phenolic rings, which result in its potent antioxidant activity. In addition, curcumin is a unique compound owing to its remarkable antioxidant, phenolic, and beta-ketone segments in its structure [32]. However, studies have denoted that the protective effects of curcumin are less significant compared to its nano capsule forms since curcumin has considerably low solubility and bioavailability [27]. On the other hand, more recent findings have shown that nano-curcumin could be used to compensate for some of the limitations of curcumin (e.g., low solubility and bioavailability) [33, 34]. The results of the present study also confirmed the efficiency and better outcomes of curcumin nanoparticles compared to curcumin alone.

Curcumin has a potential therapeutic role in disease treatment through the inhibition of LPO and oxidative damage of DNA and proteins. Furthermore, curcumin has been reported to reduce the expression of inflammatory genes, such as *COX-2*, *LOX*, and *NOX* [35]. An *in-vivo* study in this regard indicated that curcumin could stimulate antioxidant enzymes (e.g., SOD, catalase, and glutathione peroxidase), thereby leading to ROS detoxification [36]. The results of the present study also confirmed that curcumin

is an effective antioxidant, which participates in the neutralizing or scavenging of free radicals, followed by the inhibition of oxidative toxic stress. Moreover, it was well documented that nano-curcumin was more effective than curcumin. It is also notable that the co-administration of nano-curcumin and curcumin was associated with no adverse effects compared to the controls.

CONCLUSION

According to the results, exposure to AIP induced significant oxidative toxicity in the liver mitochondria. Oxidative stress factors could be improved using nano-curcumin, suggesting that nano-curcumin treatment could protect the liver against the adverse effects of AIP through the scavenging of free radicals and stabilizing the oxidative status.

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