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Research Article

Antioxidative Effects of Nanocurcumin and Curcumin Against Aluminum Phosphide-induced Serum Oxidative Stress

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Abstract

Objectives: Aluminum phosphide (AIP) is the commonly used pesticide in agriculture, which induces oxidative stress in almost all major body systems and organs. The aim of this study was to evaluate the efficacy of nanocurcumin and curcumin on serum oxidative stress level in subacute toxicity with AIP.

Materials and Methods: In this study 36 male Wistar rats (220-250 g) were randomly divided into six groups. Control (C) receiving normal saline; group AIPreceiving AIP (2 mg/kg daily); group Cur receiving curcumin (100 mg/kg daily); group Nano-cur receiving nanocurcumin (100 mg/kg daily); group AIP+ Cur receiving AIP (2 mg/kg daily) and curcumin (100 mg/kg daily); and group AIP and Nano-cur receiving AIP (2 mg/kg daily) and nanocurcumin (100 mg/kg daily). Serum total antioxidant capacity (TAC), lipid peroxidation (LPO), total thiol groups (TTG), and catalase (CAT) activity were measured.

Results: AIP administration led to a significant increase in LPO, and decreased the CAT activity, TAC, and TTG compared to the control group ($P<0.05$). Curcumin and nanocurcumin caused a significant decrease in the levels of LPO compared to the AIP-exposed groups ($P<0.05$). Moreover, in the nanocurcumin-treated groups, compared to a poisoned group, TAC and TTG increased significantly ($P<0.01$). There were no significant changes in CAT activity improvement.

Conclusion: Nanocurcumin and curcumin improved the AIP-induced oxidative damage.

Keywords: Nanocurcumin, Curcumin, Aluminum Phosphide, Serum

Background

Aluminum phosphide (AIP) is known as "rice pill" in Iran and is extensively used due to its protective effects on grain storage (1,2). AIP releases phosphine (PH3) gas in humid environments, which is a very toxic and dangerous gas for humans and animals (2). PH3 is highly poisonous and is a protoplasmic toxin that interferes with enzymes and proteins (2). The main mechanism of AIP-induced toxicity is the disruption of the balance between the production and neutralization of active radicals, which is known as oxidative stress. Superoxide dismutase (SOD) and catalase (CAT) are the most important enzymes involved in fighting against oxidative stress (3-6). Swallowing 1 g of zinc phosphide in humans causes poisoning signs and symptoms. Death has been reported as a result of oral administration of 4 g zinc phosphide. The usual lethal dose of AIP in an adult person with an average weight of 70 kg is estimated at 500 mg (2,7). Antioxidant defense mechanisms have been designed to neutralize or minimize the harmful effects of free radicals (8). Antioxidants such as glutathione peroxidase (GPx), CAT, SOD, uric acid, bilirubin, and thiol group molecules are produced within the body (9). Exogenous antioxidants, such as vitamin E or alpha-tocotrienols need to be supplied via diet (10).

In general, the ability of an antioxidant to neutralize reactive oxygen species and free radicals depends on various factors, the most important of which are intrinsic chemical activity of free radicals such as vitamin E or alpha-tocotrienol. These antioxidants are more active on the radicals of lipophilic membrane or lipoprotein part, the site of free radical production and its activity, antioxidant site, concentration and mobility in the environment, fate of antioxidant radicals, and interference with other antioxidants, as well as uptake, distribution, and metabolism of antioxidants (11,12). Curcumin or diferuloylmethane is a biologically active ingredient of curcuma longa belonging to ginger family that has the potential to treat a wide range of diseases such as cancer, lung diseases, neurodegenerative diseases, kidney disease, metabolite diseases, heart diseases, etc. (13). Curcumin has

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anti-oxidative, anti-inflammatory, and anti-cancer effects. It is also considered as a chemical inhibitor of cancer and tumor growth due to its high ability in absorbing and accumulating free radicals, as well as inhibiting its inflammation. Curcumin at high concentrations collects free radicals, and at low concentrations activates or inhibits one or more message transmission pathways in the cell. Curcumin can exhibit its antioxidant activity by intensifying glutathione synthesis (14).

Curcumin is hardly dissolved in water and is extremely sensitive to changes in physiological pH. Furthermore, it is poorly absorbed in the gastrointestinal tract, and in acidic environments, it makes the serum curcumin levels be less than 1% of consumed curcumin despite its high intake. Several ways of increasing the bioavailability of curcumin are under investigation, such as the application of adjuvants like piperine, liposomal curcumin, structural curcumin analogs such as EF-24, curcumin phospholipid complex, as well as nanoparticle form of curcumin. Nanotechnology and encapsulation of curcumin in nano-emulsions (nanocurcumin) can improve the medical properties of this substance (15). The present investigation was conducted to compare the efficacy of nanocurcumin as well as curcumin on serum level of oxidants in subacute toxicity with AIP.

Materials and Methods

We used 36 male Wistar rats (220-250 g) in the present research. All the animals were kept in standard conditions, 12-hour dark/light cycle (25 ± 2°C) and then randomly assigned to six groups (n=6). The groups were divided to: control (C) receiving normal saline; group AIPreceiving AIP (2 mg/kg daily); group Cur receiving curcumin (100 mg/kg daily); group Nano-cur receiving nanocurcumin (100 mg/kg daily); group AIP+ Cur receiving AIP (2 mg/kg daily) and curcumin (100 mg/kg daily); and group AIP and Nano-cur receiving AIP (2 mg/kg daily) and nanocurcumin (100 mg/kg daily). Physical characteristics of curcumin-loaded soft gel nanoparticles were as follows: Nano micelle size (nm) <50; Encapsulation (%) >99; Assay (%) =90 – 110; pellet durability index (PDI) = 0.2 – 0.8; and Zeta potential= -2 to -15. The doses of AlP, curcumin, and nanocurcumin were selected according to the dosages used in a previous study (16). The animals were treated by oral gavage for 7 days. The rats were anesthetized by ketamine (50 mg/kg) at the end of treatment. After collecting samples from rats, the sera were separated, frozen in liquid nitrogen, and stored in -70°C.

Assay for Oxidative Stress Biomarkers

Assessment of Lipid Peroxidation

We used thiobarbituric acid (TBA) test to quantify lipid peroxidation (LPO), which reacts with peroxide molecules. Samples were thoroughly mixed with trichloroacetic acid (TCA) (20%) and the resulting precipitate was dissolved in 0.05M sulfuric acid. Subsequently, TBA (0.2%) was added to sodium sulfate (2M). The mixture was heated up to boiling in a bath for 30 minutes. We extracted thiobarbituric acid reactive substances (TBARS) adducts using n-butanol, and the absorbance was determined in 532 nm (17).

Total Antioxidant Capacity Evaluation

In the presence of 2,4,6-tris(2t-pyridyl)-s-triazine (TPTZ), total antioxidant capacity (TAC) was measured using ferric reducing ability of plasma technique that relies on the aptitude of plasma in reducing Fe^{3+} to Fe^{2+}. An interaction between Fe^{2+} and TPTZ generates a complex in blue color having maximum absorbance at 593 nm (18).

Evaluation of Total Thiol Groups

Dithinitrobazoic acid (DTNB) reagent was used to assess total thiol groups (TTG) in plasma, which reacts with TTG to generate a yellow complex with maximum absorbance in 412 nm (19).

Evaluation of Catalase Activity

The determination of CAT activity was done using a BioVision competitive ELISA Kit based on the instructions provided by the company.

Statistical Analysis

We performed statistical analysis using SPSS software (Chicago-USA,16.0 SPSS Inc) as well as GraphPad Prism version 7.0 (GraphPad Software, San Diego-USA). One-way ANOVA along with post-hoc Tukey's tests were considered for further analysis of biochemical results. All the data were indicated as mean ± SD. The differences between different groups were significant at $P<0.05$.

Results

Lipid Peroxidation

According to the results of the present study, serum LPO level increased significantly in AIP-exposed group compared to the control group ($P<0.01$). Treatment with curcumin ($P<0.05$) and nanocurcumin ($P<0.01$) led to an improvement in LPO compared to the poisoned group. However, curcumin treatment did not increase the degree of recovery up to the level of the healthy control group, and showed a significant difference with the latter group ($P<0.01$) (Figure 1).

Total Antioxidant Capacity

The results revealed that TAC in the group exposed to AIPwas significantly decreased in comparison to the control group ($P<0.01$). Treatment with nanocurcumin improved the TAC relative to the poisoned group ($P<0.05$). It should be noted that the treatment with curcumin did not

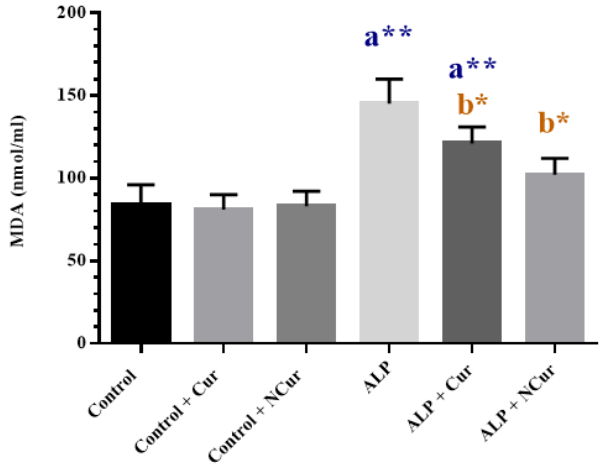

Figure 1. LPO Level in Rats (n = 6 in each group). Data have been reported as Mean ± SD. AIP Aluminum Phosphide, Cur: Curcumin, NCur: nanocurcumin, a: The significance compared to control group, b: The significance compared to AIP-exposed control group (* $P<0.05$, ** $P<0.01$).

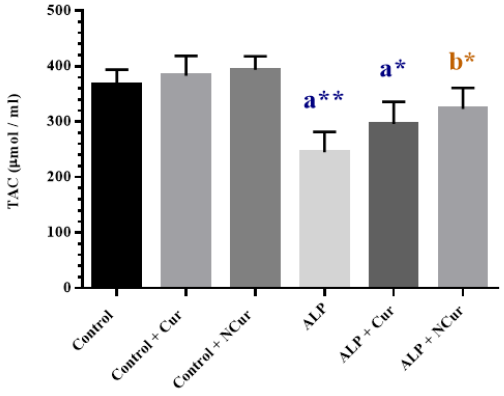

Figure 2. TAC Level in Rats (n = 6 in each group). Data have been reported as Mean ± SD. AIP: Aluminum Phosphide, Cur: Curcumin, NCur: nanocurcumin, a: The significance compared to control group, b: The significance compared to AIP-exposed control group (* $P<0.05$, ** $P<0.01$).

improve TAC compared to the poisoned group (Figure 2).

Total thiol groups

Our data showed that the level of TTG in serum tissue decreased considerably in the AIP-exposed group in comparison with the healthy control group ($P<0.001$). The treatment with nanocurcumin and curcumin led to significant difference in TTG levels compared to AIP-exposed group ($P<0.05$) (Figure 3).

Catalase Activity

Our data showed that the CAT activity of serum was reduced remarkably in the AIP-exposed group as compared to the normal group ($P<0.001$). The treatment with nanocurcumin and curcumin resulted in a major change of the activity of CAT enzyme compared to the poisoned group ($P<0.05$) (Figure 4).

Discussion

There is no specific antidote for the treatment of rice pill poisoning, and treatment mainly involves taking symptomatic and supportive measures (20). According to some studies, the organs that require more oxygen, including brain, heart, liver, and kidney are more susceptible and vulnerable to this toxin. Due to the harmful effects of toxins, especially AIP, which can release free radicals and cause damage to body tissues, this study was conducted to compare the antioxidant effects of nanocurcumin and curcumin on the oxidative damage induced in the serum of AIP-exposed rats. The results of this study showed the toxicity of AIP in inducing oxidative stress. Moreover, the results showed that AIP disrupted the antioxidant balance

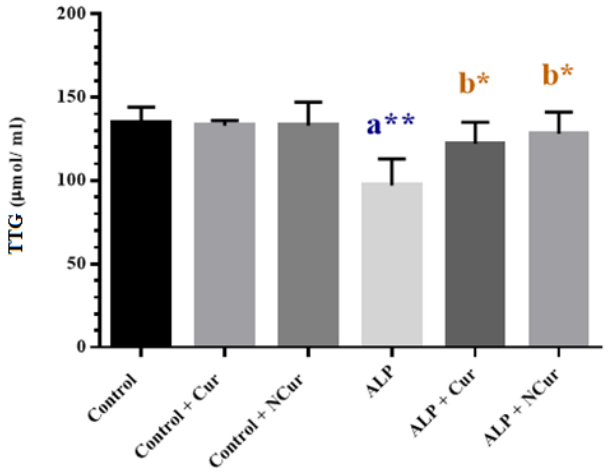

Figure 3. TTG in Rats (n = 6 in each group). Data have been reported as Mean ± SD. AIP: Aluminum Phosphide, Cur: Curcumin, NCur: nanocurcumin, a: The significance compared to control group, b: The significance compared to AIP-exposed control group (* $P<0.05$, ** $P<0.01$).

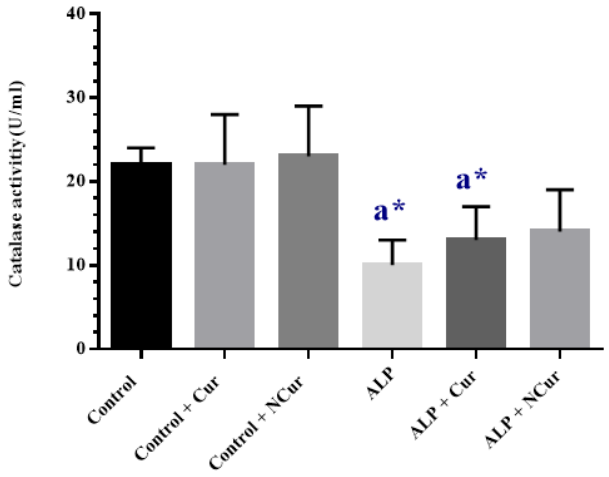

Figure 4. Catalase activity in rats (n = 6 in each group). Data have been reported as Mean ± SD. AIP: Aluminum Phosphide, Cur: Curcumin, NCur: nanocurcumin, a: The significance compared to control group, b: The significance compared to AIP-exposed control group (* $P<0.05$, ** $P<0.01$).

in the serum of poisoned rats, and it caused oxidative stress. Furthermore, curcumin and nanocurcumin improved the AIP-induced stress. In the serum of AIP-exposed rats, LPO increased significantly compared to healthy control group; and the poisoned group receiving curcumin and nanocurcumin showed a significant reduction in malondialdehyde level compared to AIP group.

Antioxidant capacity, thiol groups, and CAT activity in the poisoned group were significantly reduced compared to the healthy control group. The total antioxidant and TTG increased significantly in the nanocurcumin-poisoned group compared to AIP group.

Several studies have shown that curcumin has a wide range of biological functions, especially antioxidants and anti-inflammatory functions. Due to lipophilic specificity of curcumin, oral administration of curcumin is very low. However, in the nanocurcumin product, all curcumin is confined to the hydrophobic portion of the curcumin nanomicelles, which have a particle size of about 10 nm and increase the solubility of curcumin in water. Capsules containing curcumin nanomicelles are opened and dispersed in the acidic condition of stomach in fewer than 15 minutes after oral administration. Nanomicelles stay at least 6 hours in the acidic conditions of the stomach and do not disappear and reach the intestine intact. After reaching the intestine, nanomicelles facilitate the transfer of curcumin from the intact water layer on the surface of the gut cells, which is a barrier to the absorption of fat-soluble compounds, and enhance absorption of curcumin (21). Nitric oxide is also a short-life molecule that is part of the free radicals produced by the L-arginine nitric oxide synthase system that forms nitrogen radicals. It can damage DNA and cause disease. Studies have indicated that curcumin reduces nitric oxide levels and consequently causes oxidative stress (20).

Structurally, since curcumin has two phenolic rings in its molecule, it can have potent antioxidant activity. Curcumin is a unique substance since it has both phenolic ring and beta-diketone group on one molecule, and both of these groups cause antioxidant activity (22). Some researchers argued that curcumin gives hydrogen atoms for antioxidant activity of phenolic groups, while others argued that hydrogen originates from the central methylene group. Moreover, the effects of curcumin can be explained by the chelating properties of metals, including iron, which can produce free radicals in the Fenton and Haber–Weiss reaction by the central beta-diketone group or by the variability of hydrogen present on the central methylene group in curcumin. Other researchers argued that the antioxidant capacity of curcumin is not due to the central CH_2 group. Still, it is expected that phenolic groups should be present on both sides of the molecule (23). It has been proven that curcumin is considered a scavenger for free radicals due to having specific groups in its structure, and it can reduce the reactive oxygen species level. Curcumin prevents lipid oxidative stress and oxidative damages to DNA and proteins in disorders such as atherosclerosis and neurological diseases and such physiological conditions. It acts through affecting COX-2, LOX, and NOX pathways and the anti-inflammatory enzymes (24). According to several recent studies, antioxidant properties of curcumin on nano formula have been more than curcumin. Results of other studies demonstrated that curcumin nanomicelles may be better than curcumin against oxidative stress due to high lipophilicity and bioavailability (22, 25).

Conclusion

The results of this study showed that the use of curcumin and nanocurcumin reduced oxidative stress and increased serum antioxidant content in AIP-induced toxicity. However, it should be noted that the identification of accurate cellular and molecular mechanisms of this effect requires a full knowledge on the roles of curcumin and nanocurcumin in defense of toxicity.

Conflict of Interest Disclosures

The authors claim that they have no conflict of interests.

Ethical Issues

All the procedures were approved by the Medical Ethics Committee of Hamadan University of Medical Sciences (code: IR.UMSHA.REC.1397.283).

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