Evaluation of Protective Effects of Curcumin and Nanocurcumin on Aluminium Phosphide-Induced Subacute Lung Injury in Rats: Modulation of Oxidative Stress through SIRT1/FOXO3 Signalling Pathway

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ABSTRACT

Objective Aluminum phosphide (AlP) is widely used to protect stored food products and grains from pests and rodents. The availability of AlP, especially in Asian countries it has become a desirable factor to commit suicide. The phosphine produced from ALP is a very reactive radical and a respiratory inhibitor that causes oxidative damage. There is no dedicated antidote or effective drug to manage AlP-induced lung toxicity. The present study aims to evaluate and compare the protective effects of curcumin and nanocurcumin on ALP-induced subacute lung injury and determine the underlying mechanism.

Methods Rats were exposed to AlP (2 mg/kg/day, orally) + curcumin or nanocurcumin (100 mg/kg/day, orally) for 7 days. Then rats were anesthetized and lung tissues were collected. Oxidative stress biomarkers, genes expression of antioxidant enzymes, participated genes in the SIRT1/FOXO3 pathway, and lung histopathology were assessed by biochemical and ELISA methods, Real-Time PCR analysis, and H&E staining.

Results Curcumin and nanocurcumin produced a remarkable improvement in AlP-induced lung damage through reduction of MDA, induction of antioxidant capacity (TAC, TTG) and antioxidant enzymes (CAT, GPx), modulation of histopathological changes, and up-regulation of genes expression of SIRT1, FOXO3, FOXO1 in lung tissue.

Conclusion Nanocurcumin had a significantly more protective effect than curcumin to prevent AlP-induced lung injury via inhibition of oxidative stress. Nanocurcumin could be considered a suitable therapeutic choice for AlP poisoning.

Introduction

Aluminum phosphide (AlP), known as rice pill in Iran, is widely used to protect stored food products and grains from pests and rodents [1]. Although AlP is a high-risk agent for humans and animals, its use is rapidly increasing because of its wide availability, low cost, high potency against various pests, and lack of persistence in the environment [2]. Due to the availability of AlP in Asian pesticide markets, it has become an unwanted death or desirable factor to commit suicide. Moreover, documents indicate that AlP is one of the most common reasons for poisoning mortalities in Iran, India, Oman, Sri Lanka, and Morocco. Furthermore, it is reported more than 90 % of these deaths are due to suicide [3, 4]. The lung is the main target organ in AlP poisoning and pulmonary toxicity is one of the important causes of mortality in AlP-poisoned cases [3]. Dyspnea, tachypnea, and pulmonary edema are the dominant lung disturbances from AlP exposure [5]. After AlP ingestion, it reacts to water or hydrochloric acid in the stomach and induced releases of the fatal phosphine (PH_3) gas which is readily absorbed into the bloodstream and lung epithelium. Although the exact mechanism of AlP toxicity has not yet been known, studies on animals revealed that PH_3 is a respiratory inhibitor and causes oxidative damage. PH_3 is a very reactive radical that infiltrates into the intracellular spaces and increases the H_2O_2 generation from mitochondria, which leads to oxidant-induced cellular damage. PH_3 also increases lipid peroxidation (LPO) and reactive oxygen species (ROS). PH_3 is a potent metabolic toxin that affects all body organs, especially the lung, and induces a wide range of effects, including cellular poisoning, oxidative stress, and pulmonary toxicity [6, 7]. It is noteworthy that there is no dedicated antidote or effective drug to manage AlP-induced lung toxicity. Only through shared experience in managing AlP poisoning can we increase the chance of survival [3].

The damage induced by free radicals is an important etiological factor related to many diseases. Plants with medicinal properties have long been used to treat various diseases. The World Health Organization (WHO) estimates that 80 % of the world's population depends on herbal medicine to keep their health. Bioactive compounds derived from plants are accessible, effective, inexpensive, and safe and are an important source for treating diseases. Antioxidant activities and free radical scavengers of several plants have also been documented [8, 9].

The silent information regulator 1 (SIRT1) is a histone deacetylase of nicotinamide adenine dinucleotide (NAD^+), which has received much attention for its resistance property against oxidative stress through the sirtuin 1 (SIRT1)/forkhead box protein O3 (FOXO3) pathway [10].

Since oxidative stress is the major contributor to AlP-induced cellular toxicity in the lung, it seems that plants with their antioxidant properties can be a proper approach for the treatment of poisoning caused by AlP. Natural antioxidant components such as curcumin are the important agents that have been recently used to prevent and treat the complications associated with oxidative stress in AlP toxicity. Nanotechnology has extensively influenced bioanalysis. Stable chemical and physical features make curcumin nanoparticles particularly effective in biological assays. In addition, by structural modifications, researchers have found several synthetic derivatives that have better bioavailability. Some of them show comparable or even improved pharmacokinetic/pharmacodynamics profile compared to curcumin [11].

Curcumin is the most active fat-soluble polyphenolic compound derived from the curcuma longa plant. Curcumin has a wide range of biological effects such as free radical scavenging [12, 13], anti-inflammatory [14], anti-neurodegenerative [15], and hepato-protective [16]. The literature revealed that curcumin significantly reduces lung damage caused by various causes [17–21]. However, the effectiveness of curcumin is limited due to its low oral bioavailability through its poor water solubility and absorption and rapid metabolism and elimination. So, it is needs high curcumin doses to achieve therapeutic effects [22]. Nanocarriers can improve the water solubility of curcumin biopharmaceutical properties. Curcumin nanoparticles can increase clinical application efficacy, bioavailability, biological half-life, and tissue distribution; therefore, their efficacy in clinical application [11, 23]. Documents demonstrated nanocurcumin to be superior to curcumin in antioxidant, anti-proliferative, neuro-protective, and anti-inflammatory effects in experimental studies [23–25].

Since no relevant study has discovered the underlying mechanism of curcumin property on AlP-induced lung injury in rats. The objective of the present study was to evaluate and compare the role of curcumin and nanocurcumin on AlP-induced sub-acute lung injury and determine the underlying mechanism.

Materials and Methods

Chemicals

All reagents and chemicals were obtained from Sigma-Aldrich (USA). The AlP was prepared from Samiran Pesticide Formulating Company, Tehran, Iran. Nanocurcumin used in this study was a nanomicelle containing curcumin obtained from Exir Nano Sina Company, Tehran, Iran (IRC: 1228225765). The encapsulation of curcumin in nanomicelle is about100 %, with sizes around 10 nm.

Animal treatment

A total of 38 Wistar male rats with weight average of 235 ± 15 g were obtained from the Animals Lab of Hamadan University of Medical Sciences. The rats were kept in standard animal cages plastic at room temperature (25 ± 2 °C) and humidity (60 ± 5 %) with 12 hours light/dark cycle. The rats were fed by standard chow and tap water *ad libitum* during the experiments study. After one week of adaptation to the laboratory conditions, the rats were divided randomly into 6 equal groups of 8 rats in each group: 1- Control (normal saline), 2- AlP (2 mg/kg/day), 3- Curcumin (100 mg/kg/day), 4- Nanocurcumin (100 mg/kg/day), 5- ALP + curcumin (2 mg/kg/day + 100 mg/kg/day), 6- AlP + nanocurcumin (2 mg/kg/day + 100 mg/kg/day). All materials were given orally by gavage for 7 days. All procedures were approved in advance by the Ethical Committee for Hamadan University of Medical Sciences (Code: IR.UMSHA.REC.1397.284). Moreover, the doses of AlP, curcumin, and naocurcumin were chosen according to our pilot study.

Sample preparation

After complete anesthesia of rats, the lung tissues were immediately removed and dissected over an ice-cold glass and then stored

at − 80 °C. The homogenization of lung tissues was carried out in a Teflon glass homogenizer. Then to prepare lysis buffer, HEPES 10 mM + KCl 10 mM + MgCl2 1.5 mM + EDTA 1 mM + Triton X-100 % 0.2 + DTT 0.5 mM were mixed and pH of the solution was adjusted to 7.9. After that, 100 mg of homogenized tissue was mixed with 1 ml lysis buffer including anti protease and was incubated for 20 min in ice. The homogenates were centrifuged at 14 000 (4 °C) for 30 min. The supernatant solution was separated and stored at − 80 °C to measure of biochemical and molecular parameters in lung tissue.

Biochemical parameters

Measurement of antioxidant parameters

The total antioxidant capacity (TAC) was measured at 593 nm according to the method reported by Benzie et al. [26]. A standard curve was made by adding the FRAP reagent to a range of Fe^{2+} solutions of known concentrations (Fe_2SO_4 ranging: 0 to 800 µM) which allows the samples Fe^{2+} concentration to be calculated to determine antioxidant capacity. Measurement of lipid peroxidation (LPO) measurement was done based on the protocol reported by Ohkawa et al. Briefly, conjugation ability of malondialdehyde (MDA) with 2-thiobarbituric acid (TBA) to form a pink complex with a maximum absorbance at 532 nm using UV-Visible spectrophotometer [27]. The analysis of total thiol groups (TTG) was done spectrophotometerly at a wavelength of 412 nm using a UV-Visible based on the protocol reported by Rao et al.in the lung tissues [28].

Molecular parameters

Determination of mRNA expression of SIRT1, FOXO1, FOXO3, catalase (CAT), glutathione peroxidase (GPx) in the lung tissue

The genes expressions levels in the homogenized lung tissue were measured with Real-Time PCR. Total RNA extraction was performed manually from lung tissues by RNX-Plus reagent (Cinnagen, Tehran, Iran) in accordance with the manufacturer's protocol. The extracted RNA (1 µg) was reverse-transcribed into cDNA by the PrimeScript RT reagent kit (TaKaRa Biotechnology, Japan). Quantitative Real-Time PCR was performed with SYBR premix Ex TaqTM II (TaKaRa Biotechnology, Japan) on a Roche Light Cycler 96 System (Roche Life Science Deutschland GmbH, Sandhofer, Germany) using β-actin as a loading control. To normalize relative gene expression levels, β-actin was used. To investigate the fold change in gene expression, $2^{-\Delta\Delta CT}$ formula was used [29]. The characteristics of forward and reverse primer Sequence (5' →3') was listed as follows:

β-Actin forward: CCCGCGAGTACAACCTTCT,
β-Actin reverse: CGTCATCCATGGCGAACT
SIRT1 forward: CAGTGTCATGGTTCCTTTGC,
SIRT1 reverse: CACCGAGGAACTACCTGAT
FOXO 1a forward: CGAGTGGATGGTGAAGAGTG
FOXO 1a reverse: CGAATAAACTTGCTGTGTAGGG
FOXO3a forward: CTCCCGTCAGCCAGTCTATG
FOXO3a reverse: GCTTAGCACCAGTGAAGTTCC
CAT forward: CCCAGAAGCCTAAGAATGCAA
CAT reverse: TCCCTTGGCAGCTATGTGAGA
GP_X forward: CACTGTGGCTGAGCTGTTGT
GP_X reverse CCAAGCAATTCAAGCCTCT

Histological parameters

Measurement of hydroxyproline (HYP)

The lung tissue samples were immediately weighed and were freeze-dried (Helsicc) to reach constant dry weight by the following equation: percentage dry matter content = (dry weight/wet weight) × 100 [26]. The HYP content was measured according to the manufacturer's instructions of the Kiazist commercial kit. The collagen content was estimated from the HYP concentration of tissue.

Histological studies

Immediately after isolation, lung tissues were immersed in 10 % neutral buffered formalin solution. Lung aliquots were diluted in ethanol graded concentrations, immersed in xylene and placed in paraffin. Sections were cut at 5 um thicknesses on a rotary microtome and then fixed and stained by hematoxylin and eosin. The sections were then photographed with a digital camera (Nikon E800, Japan) connected to a microscope. For each rat, the tissues changes were studied by achieving five serial coronal sections at 400× magnification. An experienced histologist who was unaware of the treatment conditions, performed the histological evaluation [21].

Statistical analysis

Statistical analysis was performed with SPSS 16.0 (SPSS Inc., Chicago, Ill., USA) statistical software. The data were analyzed by one-way analysis of variance (ANOVA) followed by Tukey's post hoc test for multiple comparisons. The results were expressed as the mean ± Standard deviation (SD). P-value < 0.05 was regarded as the significant change in all the experiments.

Results

Oxidative stress parameters in lung tissue

The effects of curcumin and nanocurcumin on TAC level

According to ▶ **Table 1**, the TAC level of lung tissue in the AlP group was significantly decreased compared with the control group ($P < 0.01$). The TAC levels were significantly increased in the groups of AlP + curcumin ($P < 0.05$) and AlP + nanocurcumin ($P < 0.01$) compared with the AlP group.

The effects of curcumin and nanocurcumin on LPO level

As shown in ▶ **Table 1**, the level of lung tissue MDA in the AlP group was significantly increased compared to the control group ($P < 0.01$). The lung MDA levels were significantly decreased in the groups of AlP + curcumin ($P < 0.05$) and AlP + nanocurcumin ($P < 0.01$) compared with the AlP group. Also, MDA level in rats that received AlP + nanocurcumin had more ($p < 0.01$) decrease than rats that received AlP + curcumin.

The effects of curcumin and nanocurcumin on TTG level in lung tissues

As indicated in ▶ **Table 1**, the TTGs level of lung tissue was significantly decreased in the AlP group compared with the control group ($P < 0.01$). In the AlP + curcumin and AlP + nanocurcumin groups, the lung TTGs levels were significantly increased compared with the AlP group ($P < 0.01$).

The effects of curcumin and nanocurcumin on SIRT1, FOXO1a, and FOXO3a genes expression in lung tissue

The mRNA levels of SIRT1, FOXO1a, and FOXO3a in the AlP group were significantly ($p < 0.01$, $p < 0.05$, and $p < 0.01$, respectively) lower than the control group. The levels of SIRT1, FOXO1a, and FOXO3a mRNA in the AlP + curcumin group were not significant difference from AlP group. The mRNA level of SIRT1 in the AlP + nanocurcumin group had a significantly ($P < 0.05$) increase in comparison to the AlP group. We did not also find a significant difference among mRNA levels of FOXO1a and FOXO3a in the AlP + nanocurcumin group with AlP group (▶ **Fig. 1a-c**).

The effects of curcumin and nanocurcumin on CAT gene expression in lung tissue

As shown in ▶ **Fig. 2**, the mRNA level of CAT in the AlP group were significantly ($p < 0.001$) lower than the control group. This is while, treatment with curcumin and nanocurcumin significantly ($p < 0.05$ and $p < 0.01$, respectively) increased expression of this gene compared to the AlP group.

The effects of curcumin and nanocurcumin on GPx gene expression in lung tissue

mRNA expression of GPx was significantly ($p < 0.001$) reduced in AlP-exposed rats lungs. However, we found a significant ($p < 0.05$) increase in the mRNA level of GPx in the AlP + nanocurcumin group in comparison to the AlP group (▶ **Fig. 3**).

The effects of curcumin and nanocurcumin on HYP level in lung tissues

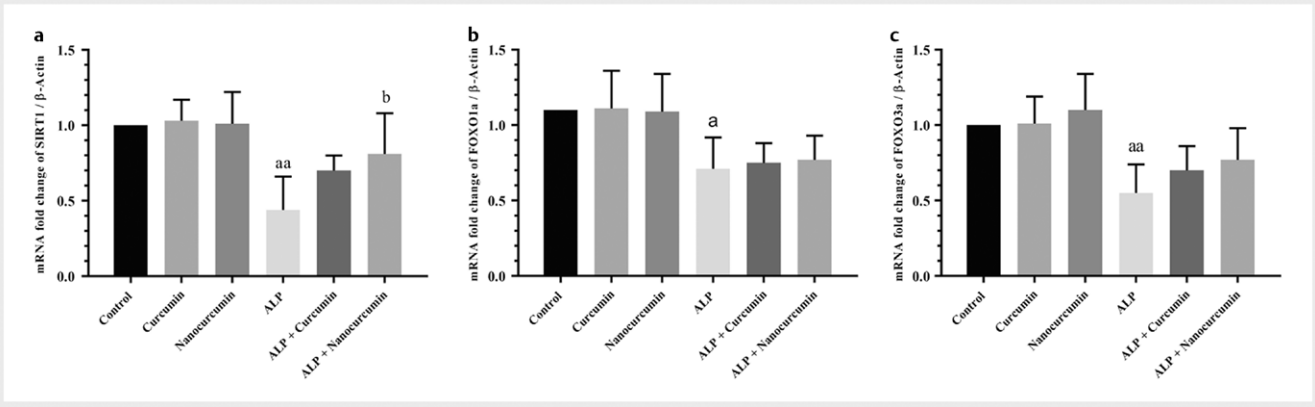
According to ▶ **Fig. 4**, the lung HYP level was significantly increased in the AlP group compared with the control group ($P < 0.01$). The lung HYP level in the AlP + nanocurcumin group was significantly decreased compared with the AlP group ($P < 0.05$). Also, the level of HYP was not significantly different in the AlP + curcumin group compared with the AlP group. Also, HYP level in rats that received AlP + nanocurcumin had more ($p < 0.01$) decrease than rats that received AlP + curcumin.

The effects of curcumin and nanocurcumin on AlP-induced histological changes in lung tissue

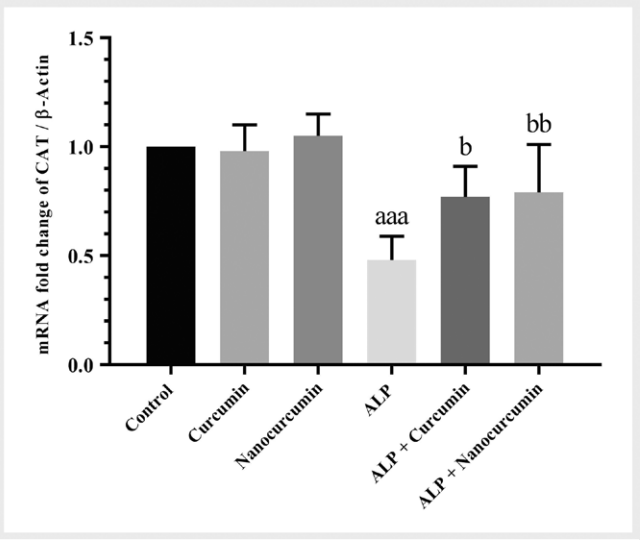
Evaluation of lung tissue following hematoxylin and eosin staining indicates the presence of multiple alveolus in the control group. A little of fibrous tissue is also seen in the control, curcumin and nanocurcumin groups. In the AlP group, the amount of fibrosis increased significantly compared to the control group, while the number of alveolus decreased compared to the control group. Administration of curcumin with AlP had no significant effect on reducing fibrosis, but it seems that in the group receiving nanocurcumin with

▶ **Table 1** Curcumin and nanocurcumin suppress ALP-induced oxidative changes in lung tissue in rat. Rats were exposed to ALP (2 mg/kg/day) + curcumin or nanocurcumin (100 mg/kg/day) for 7 days and then TAC, MDA, TTG was measured in lung tissues. Results are mean ± SD, n = 8. Difference between control and other groups is significant at $p < 0.01$ (^{aa}). Difference between ALP and other groups is significant at $p < 0.01$ (^{bb}) and $p < 0.05$ (^{b}). Difference between ALP + curcumin and other groups is significant at $p < 0.01$ (^{cc}). ALP: Aluminum phosphate.

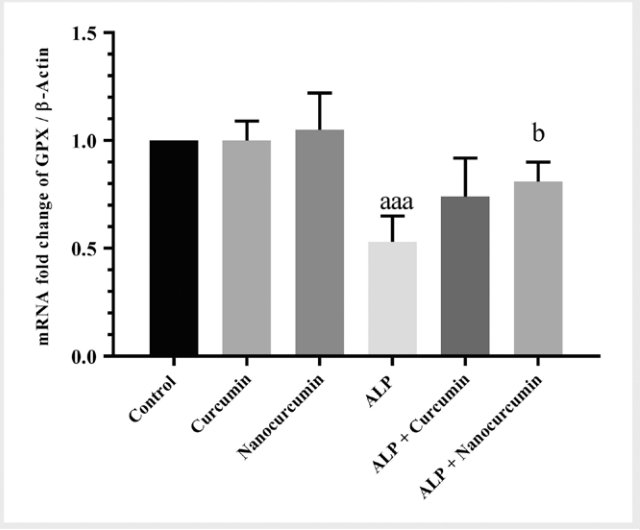
Groups	TAC (nmol/mg protein)	MDA (pmol/mg protein)	TTG (nmol/mg protein)
Control	2194.66 ± 195.05	10.7 ± 2.25	1354.83 ± 190.92
Curcumin	2218.83 ± 197.86	9.36 ± 1.12	1358.16 ± 182.4
Nanocurcumin	2254.5 ± 184.91	11.05 ± 2.39	1375 ± 221.08
ALP	1756.16 ± 183.93^{aa}	19.69 ± 4.24^{aa}	483.83 ± 53.21^{aa}
ALP + curcumin	2152.5 ± 174.31^{b}	14.64 ± 2.55^{b}	672.16 ± 43.19^{bb}
ALP + nanocurcumin	2235.66 ± 233.06^{bb}	9.95 ± 2.04^{bb,cc}	683.66 ± 69^{bb}



▶ **Fig. 1** Curcumin and nanocurcumin modulate SIRT1, FOXO1a and FOXO3a genes expression changes induced by ALP in lung tissue in rat. (**a**) SIRT1mRNA expression level. (**b**) FOXO1a mRNA expression level. (**c**) FOXO3a mRNA expression level. Results are mean ± SD, n = 8. Difference between control and other groups is significant at $p < 0.01$ (^{aa}) and $p < 0.05$ (^{a}). Difference between ALP and other groups is significant at $p < 0.05$ (b). ALP: Aluminum phosphate.



▶ **Fig. 2** Curcumin and nanocurcumin upregulate reduced gene expression of CAT induced by ALP in lung tissue. Results are mean ± SD, n = 8. Difference between control and other groups is significant at $p < 0.001$ (^{aaa}). Difference between ALP and other groups is significant at $p < 0.01$ (^{bb}) and $p < 0.05$ (^{b}). ALP: Aluminum phosphate.

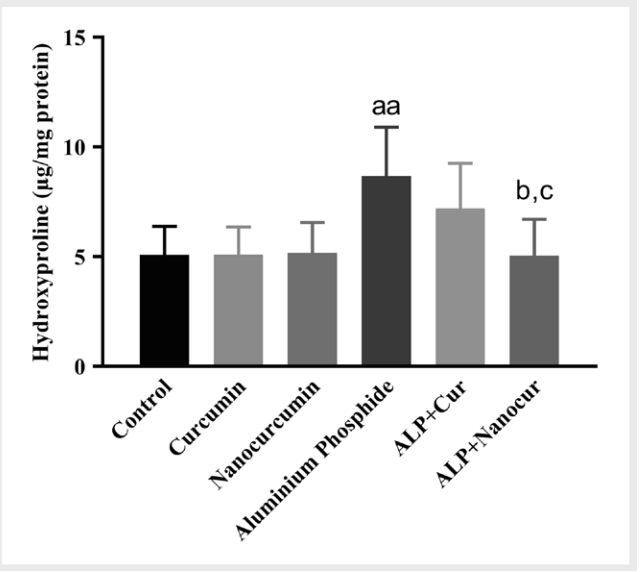


▶ **Fig. 3** Curcumin and nanocurcumin upregulate reduced gene expression of GPx induced by ALP in lung tissue. Results are mean ± SD, n = 8. Difference between control and other groups is significant at $p < 0.001$ (^{aaa}). Difference between ALP and other groups is significant at $p < 0.05$ (^{b}). ALP: Aluminum phosphate.

AlP, the rate of fibrosis decreased compared to the ALP group (▶ **Fig. 5a-f**).

Discussion

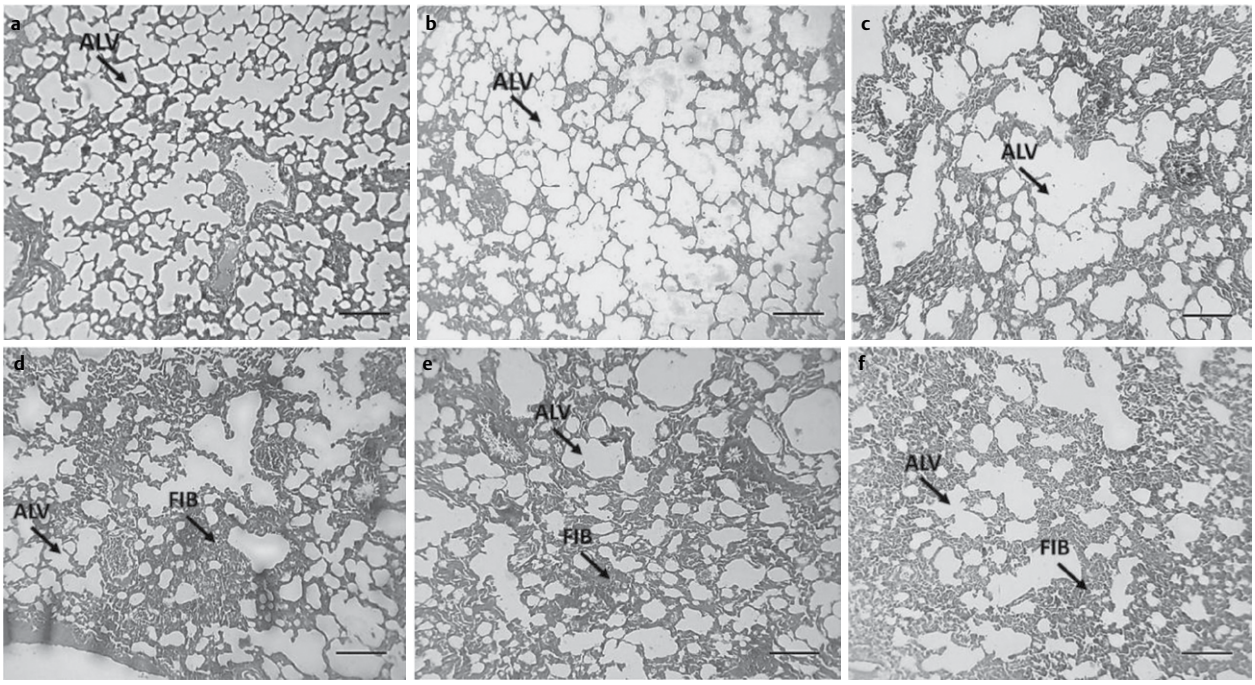
Lunge damage induced by AlP characterized by dyspnea, tachypnea, and pulmonary edema. There are many studies that examine the underlying mechanisms of toxicity caused by of AlP. Oxidative



▶ **Fig. 4** Curcumin and nanocurcumin reduce ALP-induced hydroxyproline level in lung tissue in rat. Rats were exposed to ALP (2 mg/kg/day) + curcumin or nanocurcumin (100 mg/kg/day) for 7 days and then hydroxyproline was measured in lung tissues. Results are mean ± SD, n = 8. Difference between control and other groups is significant at $p < 0.01$ (^{aa}). Difference between ALP and other groups is significant at $p < 0.05$ (^{b}). Difference between ALP + curcumin and other groups is significant at $p < 0.05$ (^{c}). ALP: Aluminum phosphate.

stress is one of the main causes revealed to be present in experimental models [5]. This research confirmed the presence of oxidative stress in poisoned rats by sub-acute exposure with AlP by assessing the changes of the biochemical, molecular, and histological parameters in lung tissues. This study was performed to evaluate and compare the protective effects of curcumin and nanocurcumin on AlP -induced subacute lung injury and to determine the underlying mechanism involved. The potential of curcumin and nanocurcumin to prevent oxidative damage make them a potential candidate to attenuate the toxic effects induced by AlP in the lung. The finding of this study showed curcumin and nanocurcumin reverse AlP-damaged indicators in rats' lung tissues.

Based on previous studies, phosphine generated from AlP is a mitochondrial toxin that inhibits cellular respiration and induces oxidative stress resulting to free radicals' generation. Since phosphine generates free oxygen radicals in body tissues, it has been found the organs that need higher oxygen, such as lung indicates a higher sensitivity to damage caused by phosphine gas [3]. Many documents showed that AlP can induce considerable changes in oxidative stress biomarkers. LPO is main mechanism in ROS-induced cell injury. The unsaturated fatty acids in the macromolecules of body are sensitive to ROS attack and lipid peroxidation. MDA is one of the LPO products which show the degree of LPO and the intensity of free radicals- induced cell damage. Based on MDA content assessment in different animal and human samples, it has been shown that AlP significantly elevates levels of LPO. Moreover, AlP can increase ROS levels through disruption in the electron transfer chain and results to free radicals' overproduction along with a disturbance in the antioxidant systems [30–32]. Antioxidant agents



▶ **Fig. 5** Curcumin and nanocurcumin effects on ALP-induced histological changes in lung tissues. Rats were exposed to ALP (2 mg/kg/day) + curcumin or nanocurcumin (100 mg/kg/day) for 7 days and then lung tissues were stained with H&E. Appearance of lung tissues in different groups: (**a**) control, (**b**) curcumin, (**c**) nanocurcumin, (**d**) ALP, (**e**) ALP + curcumin, (**f**) ALP + nanocurcumin. Lung paranchyma is intact and well-preserved with multiple alveolus in (**a**) group. Extensive interstitial infiltration and fibrosis can be seen in (**d**) group. A significant improvement in lung tissue was seen in groups (**f**). The tip of the arrow in the shapes represents the alveolus (ALV) and fibrosis (FIB) areas. Magnification × 40. ALP: Aluminum phosphide.

are the most important defense system of human body to fight free radicals. In this regard enzymatic system, including CAT, superoxide dismutase (SOD), and thiol containing enzymes as GPx, are important enzymes for the antioxidant power preservation in body [30]. Previous studies have shown that AlP induces a decrease in levels of antioxidant agents such as TAC, TTG, CAT, and GPx [2, 30]. Similar to previous studies, in the present study we have shown that MDA level were significantly increased, this is while TTG, TAC, and CAT levels and GPx gene expression were significantly decreased in the lung tissues of rats after sub-acute exposure to AlP. The protective effects of curcumin and nanocurcumin were investigated by measuring changes in important factors. Our results revealed that curcumin and nanocurcumin administration protect the lung tissue from oxidative stress induced by AlP. It is remarkable rats exposed to AlP + nanocurcumin had better improvement than AlP + curcumin group on oxidative stress biomarkers and antioxidant system in lung tissue. In confirmation of our results, previous studies showed that curcumin is a natural antioxidant which not only can effectively trap free radicals and endogenous oxidizing active agents, but it can also increase the efficacy of antioxidant enzymes such as SOD, CAT, and GPx, and levels of TAC, and TTG. Moreover, curcumin induces inhibitory effects on free-radical producing enzymes [23, 33]. Nanocurcumin revealed excellent pharmacokinetics profiles compared to curcumin in many studies [34]. For example, Zhang et al., found that nanocurcumin markedly suppressed the oxidative stress in rats. nanocurcumin significantly elevated the activities of CAT, SOD, and GPx compared with curcumin in rat [35]. Nanocurcumin also showed better free radical scavenging activity and anti-lipid peroxidation effect compared to native curcumin in HepG2, Hep3B and PLC/PRF/5 [23].

SIRT1 is a histone deacetylase of nicotinamide adenine dinucleotide (NAD^+), and is a member of the mammalian sirtuins family, which is widely studied. SIRT1 is mostly present in the nucleus, where it has an important role in transcriptional inhibition through histone deacetylation [10]. There is widely interaction between SIRT1 and reactive stress. SIRT1 has received much attention for its role in resistance to oxidative stress. One of the mechanisms involved in SIRT1/FOXOs pathway [10].

FOXOs belong to a subgroup of Fork head family of transcriptional factors which among them FOXO1 and FOXO3 are the most usual. In stress conditions, FOXOs move into the nucleus and enhance their protein expression, thus engaging in various cellular functions such as oxidative stress inhibition. The connection between SIRT1 and FOXO represents an evolutionarily mechanism for resistance to oxidative stress. FOXO1 and FOXO3a, are essential for SIRT1- dependent cell viability against oxidative stress. It has been shown Sirt1 and FOXO3 form a complex by oxidative stress stimulation, and SIRT1 deacetylates FOXO3 to induce oxidative stress resistance. SIRT1 increases FOXO1 DNA binding ability through deacetylating FOXO1 and reduces the oxidative stress response [10]. Positive feedback mechanisms regulate SIRT1 and FOXO1 genes expression. Documents revealed that expression of SIRT1

mRNA in the damaged lung tissue was significantly reduced compared to healthy lung tissues and deacetylated FOXO3 level was also significantly decreased in injured lung tissue compared to healthy control. Moreover, it has been seen the FOXO1 expression and activity were down-regulated in oxidative stress-induced injuries [36, 37]. In the present study, similar to previous studies we showed a significant decrease in genes expression of SIRT1, FOXO1, and FOXO3 in the lung tissues of rats after sub-acute exposure to AlP. In this study, it was also demonstrated that following sub-acute exposure to AlP, the lung tissues exhibited a significant decrease in levels of tissue CAT, and GPx which these changes were parallel to the decrease SIRT1 level and decreased deacetylated FOXO3 protein expression. Following curcumin and nanocurcumin treatment, SIRT1 mRNA expression were elevated, which induced deacetylated FOXO3 protein level, and then induced CAT and GPx production. Due to the reversal of the damaged indicators mentioned above, it is suggested that the SIRT1/FOXO3 signaling pathway have an important role in lung tissue antioxidant capacity amelioration and oxidative stress reduction following sub-acute exposure to AlP. Many studies note that curcumin significantly increases SIRT1 activation and reduces oxidative stress. In fact, curcumin has potential to prevent or treat injuries which mainly associated with oxidative stress by increasing of SIRT1 level [38–41]. It was also seen curcumin up-regulates FOXO1 mRNA [37, 42].

Hydroxyproline is representative of fibrosis and indicates collagen that builds up in lung tissue. Previous studies showed that the hydroxyproline level significantly elevates in lung tissues that have been damaged by various factors [43, 44]. Our result, similar to previous studies showed that lung hydroxyproline content significantly increased after sub-acute exposure with AlP. Collagen produced by fibroblasts is a basic component for preserving lung architecture. In the present study, histopathological assessments of lung tissues demonstrated significant structural and fibrotic alteration created by sub-acute exposure with AlP, leading to collagen deposition and lung tissue fibrosis. Many studies in the past confirmed these findings [43, 44]. In this study, by using curcumin, we found a relative but insignificant improvement in hydroxyproline content in rats' lung tissues exposed with AlP, whereas treatment with nanocurcumin significantly improved hydroxyproline content in lung tissues after exposure with AlP. Many studies showed that curcumin decreases the hydroxyproline level in lung tissues that have been damaged by various factors [43, 45, 46]. In addition, curcumin was observed to have an anti-fibrotic effect [43, 46, 47]. In the present study, we found collagen deposition and fibrosis significantly reduced after nanocurcumin treatment in rat's lung tissues exposed to AlP. In comparison, we did not find significant improvement in AlP –induced histopathological changes in lung tissue by curcumin.

In the present study we found nanocurcumin had better improvement than curcumin on AlP-damaged indicators in lung tissue. Similar to our findings, in many in vitro and in-vivo studies, nanocurcumin performed superior therapeutic benefits than curcumin in various diseases. These further therapeutic effects can be expected from nanocurcumin due to its increased bioavailability, biological half-life, and tissue distribution [23]. For example, a previous study revealed that nanocurcumin was better than curcumin in improving lung injury and fibrosis induced by paraquat [21]. It was also seen that nanocurcumin protected better than curcumin the liver against the adverse effects of AlP by the scavenging of free radicals [48].

Conclusion

The findings of this research provide insights that curcumin and nanocurcumin produced remarkable improvement in AlP‍S‍induced lung damage through reduction of MDA level, elevation of antioxidant capacity (TAC, TTG) and antioxidant enzymes (CAT, GPx) activity, modulation of histopathological changes and up-regulation of genes expression of SIRT1, FOXO3, FOXO1 in lung tissue. This is the first comparative study to demonstrate the beneficial effects of curcumin nanoformulation in reversing the lung injury induced by AlP in-vivo along with studying involved mechanisms. Our findings indicate a better protective effect of nanocurcumin than curcumin to prevent lung injury after AlP challenge. Our findings suggest that nanocurcumin could be considered as a suitable therapeutic choice for AlP poisoning.

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Conflict of Interest

No potential conflict of interest was reported by the authors.

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