Original Article

Nanocurcumin: A Promising Therapeutic Supplements Lessening Cadmium-Induced Lung Injury Of Adult Male Albino Rat. A Histopathological, Biochemical And Ultra-structural Study

Ahmed Bayoumi, Hanan Dawood Yassa, Radwa Mohamed, Mohamed El-sebaie, Ali Mousa and Doaa Hassan

Department of Anatomy and Embryology, Faculty of Medicine, Cairo University, Egypt

ABSTRACT

Background: Cadmium (Cd) is considered a health hazard due to its industrial use. Lungs and kidneys are the primary organs affected by its toxicity. Inflammatory response in lung tissue is mediated by Cd exposure until it becomes dysfunctional. Cd damages DNA indirectly by increasing reactive oxygen species generation, which interacts with DNA defense system leading to genomic instability. Curcumin (C) has multiple health benefits, as well as its Nano-formulation, Nano-curcumin (NC), which has potential to increase biological and pharmacological actions of curcumin.

Aim: To focus on Cd-mediated inflammatory response and cell apoptosis in lung tissue of adult male albino rat and use NC as a potential therapeutic agent.

Methods and methods: Thirty adult albino male rats were split into three groups, each with ten rats: control (group I), Cd treated (group II), and Cd+NC treated (group III). Rats were sacrificed and lungs were extracted for light microscopic and ultra-structural examination. IL-6 and TNF-α concentration were estimated and lung cellular survival genes; Bax and Bcl2 expression was evaluated by PCR.

Results: Alveolar damage with loss of normal lung architecture were seen in H&E-stained sections of group II confirmed by ultrastructural investigation revealing cellular cytoplasmic vacuolations with degenerated mitochondria. In group II, IL-6, TNF-α and Bax gene were increased, whereas Bcl2 gene was decreased with increased Bax/Bcl-2 genes ratio expression. NC treated group showed cellular restoration, decreased pro-inflammatory cytokines and decreased Bax/Bcl-2 genes ratio expression.

Conclusions: NC can be taken as a medicinal supplement to ease Cd poisoning risk in lungs.

Key Words: Cadmium, Cell apoptosis, Lung, Nano-curcumin, Pro-inflammatory cytokines.

Revised: 13 April 2022, **Accepted:** 28 April 2022

Corresponding Author: *Ahmed Bayoumi*, *Department of Anatomy and Embryology, Faculty of Medicine, Cairo University, Cairo, Egypt,* **Mobile:** *+201009131878,* **E-mail:** *ahmedhamed81@kasralainy.edu.eg.*

ISSN: 1110-0559, Vol. 05, No. 01

INTRODUCTION

Cadmium (Cd) is a very hazardous industrial and environmental pollutant. Because of its unusual qualities, it is frequently used in a variety of industrial applications[1]. Despite its industrial benefits, it is considered one among the most poisonous heavy metals and a health hazard[2]. People who reside near industrial sites have respiratory problems; gastrointestinal symptoms, reproductive failure up to infertility, CNS harm, and even DNA damage or cancer development[3].

In addition to the production of ROS, tissue oxidative stress causes Cd-induced cellular lung damage and even death, as well as driving the inflammatory response through the production of pro-inflammatory cytokines including IL-6 and TNF-α[4 - 5], which are hypothesized to act as an endogenous pyrogen, generating an increase in ROS and cell death by apoptosis[6].

Payne *et al.* focused on lung cellular apoptotic degenerative evidence and associated gene expression of Bax and Bcl-2 proteins, establishing a link between those genes' expression and the process of cellular survival or death, and demonstrating that those genes' expression played a role in regulating the process of lung cellular survival status. In lung tissue, Bcl-2 is engaged in preventing cellular apoptosis, whereas Bax is involved in promoting cellular death[7]. Furthermore, according to Lag *et al.*, Cd can cause cellular apoptosis by altering the expression of certain genes[8]. Based on prior research on Cd-induced lung damage, Marit *et al*. concluded that administration of antioxidant or anti-inflammatory medicines is likely to be a significant defense strategy against Cd[5].

Curcumin (C) is a traditional herbal medication that has numerous health benefits after systemic ingestion, including antioxidant, anti-inflammatory, and anticancer properties[9]. Its antioxidant

 DOI: 10.21608/jmh.2022.133055.1098

properties are owing to its capacity to protect cells from peroxidative damage[10]. It also has the ability to reduce the expression of IL-6 and TNF-α, which suppresses the production of inflammatory cytokine genes[11]. Due to inadequate absorption in the small intestine, quick breakdown, poor solubility, and rapid systemic elimination, curcumin's potential health effects are limited[12].

In the pharmacological and therapeutic disciplines, nanotechnology has emerged as a very promising topic. Because it enhances existing and well-known therapies by strengthening drug delivery systems, the medicine's effectiveness, blood circulation, and stability, as well as its harmful side effects, are all improved[13].

A novel application of the nano-therapeutics field is applied, to enhance the beneficial effectiveness of the substance[14]. Curcumin's size may be reduced to nanocurcumin (NC), which improves its solubility and permeability, as well as its biological effects[15]. As a result, the major purpose of this study is to evaluate whether there is a possible therapeutic benefit of a novel supplement, NC, in the treatment of Cd-induced lung injury.

MATERIALS AND METHODS

Chemicals:

Cadmium: Cadmium chloride (CdCl2) preparation was bought from Sigma Chemical Company (St Louis, MO, USA).

Nano Curcumin: a yellow-brown powder was obtained from Nanotech Egypt for the photo electronics communication centre. The biochemistry lab at Cairo University's Faculty of Medicine created an ethanol CI nano-emulsion. A drop of nano-curcumin suspension (10 l; 2 mg/ml) was deposited on a 3 mm copper grid and allowed to dry. It was measured using transmission electron microscopy (TEM). The primed nano-emulsion had a particle size of 97.5 - 106 nm (Figure 1).

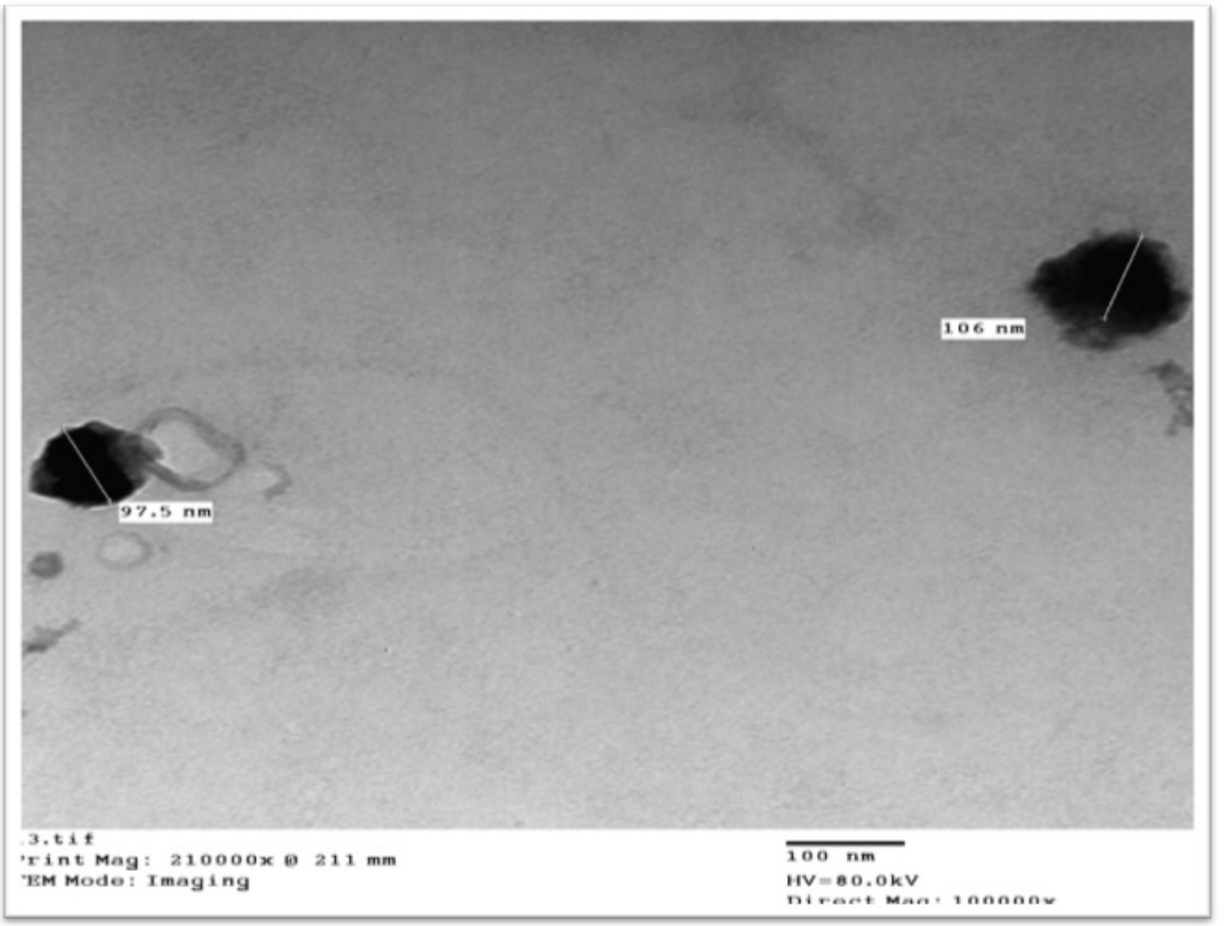

Figure 1: Curcumin nanoparticle size distribution and size characterization were calculated using TEMx100000.

Animals: Thirty adult male Sprague-Dawley albino rats weigh up 150 - 200 g, were procured from the animal house at Cairo University's Faculty of Medicine. Animal housing and care: Rats were allowed two weeks to acclimatize to their new settings before the experiment began. Rats were housed in metal cages, five per cage, and provided a daily meal as well as unlimited access to water in a suitable setting. They were kept at a temperature of 22° - 25° C and were subjected to a 12-hour dark/light cycle, good ventilation, and a temperature of 22° - 25° C.

Experimental Design:

Three experimental groups were formed from the thirty adult male rats utilized in this study (each with ten rats) at random for 31 days (duration of the study):

Control group I (group I): The rats were not given any kind of treatment.

Cd treated group (group II): For three days, the rats were given 1 mg/kg of Cd diluted in normal saline solution and supplied by subcutaneous injection[16].

Cd and NC treated group (group III): Cd was given to the rats at a rate of 1 mg/kg/day for three days[16]. On the fourth day, rats were given 5 mg/kg/day of NC emulsion dissolved in ethanol orally for four weeks[17].

Cd dose in this investigation was chosen based on an experimental methodology that resulted in clear lung damage in rats.

Sacrification and sample collection:

The rats were sacrificed at the finale of the experimentation using an intra-peritoneal overdose of pentobarbital sodium (80 g/g/kg). Blood samples were taken by a cardiac puncture and allowed to clot for biochemical analysis. The lungs dissected then preserved in 10 % neutral-buffered formalin for light microscopic, ultra-structural and biochemical tissue investigation in each group.

Biochemical analysis:

Estimation of the pro-inflammatory cytokines IL-6 and TNF-α: Serum IL-6 and TNF- were quantified by ELISA using Sunlong Biotech kits donated by IG technology firm in Egypt, agreeing to the manufacturer's instructions[18].

Genomic analysis of Bax and Bcl2 gene mRNA expression:

It was assessed using the quantitative reverse transcriptase-polymerase chain reaction (qRT-PCR) approach[19]:

Extraction of RNA:

Using a Qiagene cells/tissue extraction kit, total RNA was extracted from tissue according to the manufacturer's recommendations (Qiagene, USA).

cDNA synthesis:

The cDNA 25l master mix was made using first strand buffer (10x) 5l, 10 mM dNTPs, RNase inhibitor (40 U/l), MMLV-RT enzyme (50 U/l), and DEPC-treated water. In a programmed thermal cycler (Biometra, Germany), the resulting mixtures was incubated for one hour at 37 °C, followed by 10 minutes of enzyme inactivation at 95 °C, and then cool at 4°C. The cDNA was then kept at – 20 °C.

Real-time qPCR using SYBR Green I (Table 1):

Table 1: Primers Used in Real-Time Polymerase Chain Reaction Gene Expression Analysis:

Gene	Primer sequence	Product length
Bcl-2	F: 5'TATATGGCCCCAGCATGCGA3' R: 5'GGGCAGGTTTGTCGACCTCA3'	136
Bax	F: 5'ATCCAAGACCAGGGTGGCTG3' R:5'CACAGTCCAAGGCAGTGGGA3'	150

An Applied Biosystem with software version 3.1 (StepOneTM, USA) was used to perform real-time qPCR amplification and analysis. The annealing temperature was tuned for the qPCR test with the primer sets. All cDNA samples were in duplicate, including previously produced samples (for AFP gene expression), internal controls (for GAPDH gene expression as a housekeeping gene), and non-template controls (water to ensure the lack of DNA contamination in the reaction mixture). For sample determination, each 25 litre reaction mixture contains 12.5 litres of SYBR Green (Ferments), 1 litre of each primer (10 mol/L), and 1 gramme of cDNA (1 g/mL). Taq polymerase was activated for 5 minutes at 95 °C, then 40 two-step amplification cycles were performed: 10 s denaturalizing at 95 °C, 50 s annealing at 55 °C. The quantitative results were expressed in the Cycle threshold (Ct) of the

examined gene (AFP) and the housekeeping gene after the RT-PCR run (GAPDH). As a result, using the previously reported approach RQ = 2 – (Ct), the relative quantitation (RQ) of target gene expression was measured and linked to the housekeeping gene.

Light Microscopic examination:

For light microscopy, Hematoxylin and eosin staining of formalin-fixed lung tissues sectioned 5 mm thick (H&E) and the light microscope was equipped with a digital camera (FUJIX HC-2000; Fuji Photo Film, Tokyo, Japan) for examination and photographing (VANOX AHBS3; Olympus, Tokyo, Japan)[20].

Transmission electron microscopy (TEM) examination:

Fresh micro-specimens (12-mm) were achieved immediately after lung dissection and equipped in 3% glutaraldehyde for ultrastructural examination using a transmission electron microscope JEM 100 CXII electron microscope at 80 KV and photographed with a CCD digital camera Model XR- 41 at Cairo University[21].

Statistical analysis:

The biochemical data were analysed using the SPSS version 16.0 statistical tool. The information is given in the form of a mean standard deviation. The differences between the groups were determined using a one-way analysis of variance (ANOVA). The following *P* values were generated and interpreted: $p > 0.05$ was deemed statistically insignificant, p 0.05 was considered statistically significant, and p 0.001 was considered extremely significant.

RESULTS

Histopathological Results:
Light microscopic Results

The alveoli in the control group's H&E-stained lung sections seemed normal and spongy under light microscopy. The interalveolar septa were thin and sometimes thick, and the alveolar sacs were transparent. Blood capillaries and interstitial cells were plainly visible in the interalveolar septa. There were two types of cells at the interalveolar septa: type I pneumocytes with flattened nuclei and type II pneumocytes with rounded nuclei. The bronchioles and alveolar ducts were well-known with normal respiratory epithelium, and smooth muscle fibers (Figures 2 A, B and C).

In the Cd-treated animals, severe alveolar damage was detected in the form of collapsed alveoli and over-dilation of some alveolar spaces, as well as a loss of normal lung architecture. In several alveoli, intra-alveolar exudate was found. Interalveolar septa were thickened and many interstitial cells were seen. In addition, there was a substantial infiltration of inflammatory cells, with macrophages protruding into the alveolar sac lumen on occasion. With areas of interstitial hemorrhage, the interstitial blood vessels were congested and dilated. The bronchiole was surrounded by fragmented smooth muscles fibers, and the mucosal lining was shaded with some cellular debris in the lumen (Figures 3 A, B and C).

In Cd + NC treated group, there was mild alveolar injury and a relative healing of most of the affected alveoli. Mildly thickened interalveolar septa were discovered, along with a few inflammatory cell infiltrations, indicating a reduced tissue inflammatory response. The alveolar appearance was quite typical, with a clear lumen. Almost normal bronchiole with intact respiratory epithelium and smooth muscle fibers were seen (Figures 4 A, B and C).

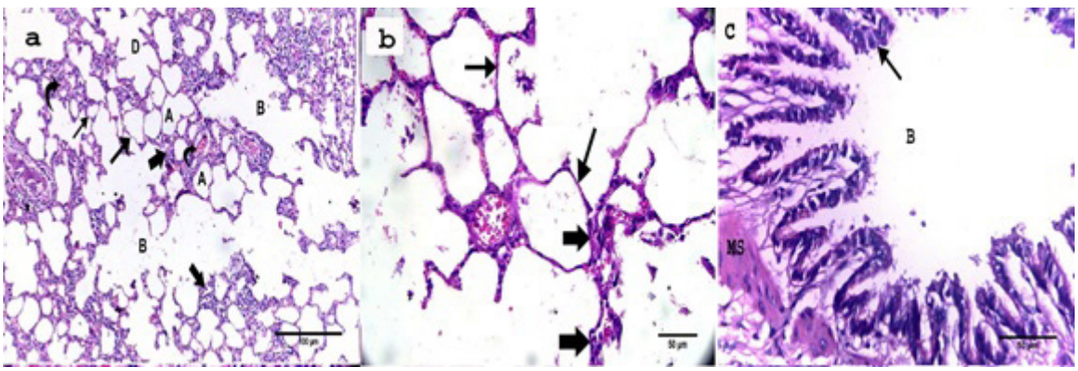

Figure 2: Photomicrographs of H & E-stained lung sections; (2a, 2b and 2c): Control group 2a, 2b: showed normal spongy appearance of the lung alveoli, alveolar sacs (A) separated by thin (thin arrow) and thick portions of interalveolar septa (thick arrow), alveolar ducts (D) and respiratory bronchioles (B). Blood capillaries and interstitial cells within the thin interalveolar septa (curved arrows), the alveoli lined by both; type-I pneumocytes with flattened nuclei (thin arrows) and type-II pneumocytes with rounded nuclei (thick arrows) were seen. 2c: Bronchioles (B) with normal respiratory epithelium (arrow), and smooth muscle fibers (MS). (Figs 2a: H&E X100., Figs 2b H&E X400 and 2c: H&E X1000).

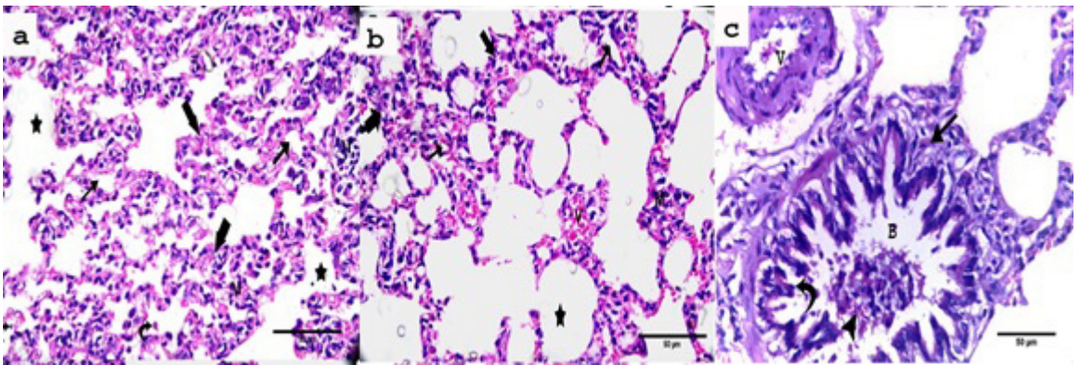

Figure 3: Photomicrographs of H & E-stained lung sections; (3a, 3b and 3c): Cd group 3a, 3b: showed interalveolar septa thickening (thin arrow) and collapsing alveoli (thick arrow), some over dilated alveolar spaces (star). Interstitial cells fill the thickened interalveolar septa. Inflammatory cells (M) with some bulging into the alveolar sac lumen (curved arrows) and congested dilated interstitial blood vessels (V) were also seen. 3c: The bronchiole (B) surrounded by fragmented smooth muscles fibers (arrow), shedding of mucosal lining (curved arrow), cellular debris in the lumen (arrowhead), and congested dilated blood vessel (V). (Figures 3a,3b H&E X400 and 3c: H&E X1000).

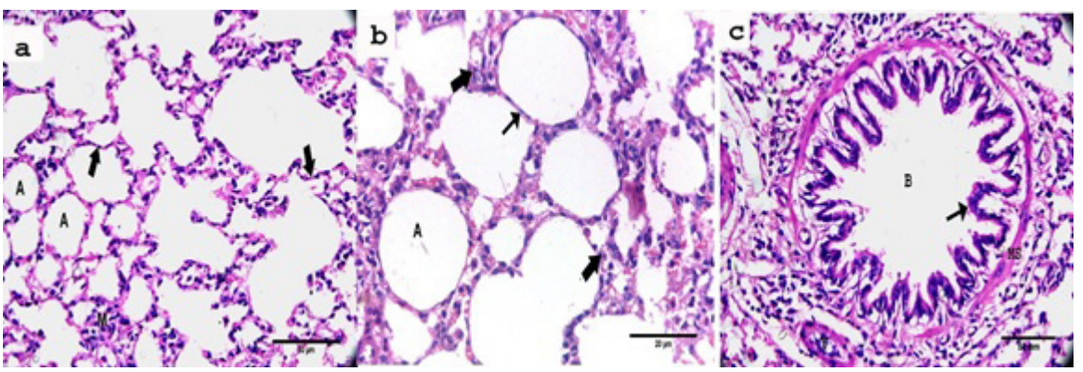

Figure 4: Photomicrographs of H & E-stained lung sections; (4a, 4b and 4c): Cd + NC group: showed restoration of injured alveoli in the form of mild thickened interalveolar septa (thin arrows), thick septa still present (thick arrow) with a few infiltrations of inflammatory cells (M), relatively normal alveolar appearance with clear lumen (A), and relative normal bronchiole with intact respiratory epithelium and smooth muscle fibers were seen (Figures 4a, 4c: H&E X400 and Figures 4b: H&E X1000).

Ultrastructure Results

The ultrastructure investigation of the control group revealed type II pneumocytes with an irregular euchromatic nucleus, normal lysosomes, mitochondria, interstitial blood capillaies and microvilli, in agreement with the light microscopy study. Blood capillaries in the interstitial space were also observed (Figures 5 A and B). The Cd-treated group, on the other hand, had deteriorated appearances such as hyperchromatic nuclei and cytoplasm with destructed mitochondria, degenerated lamellar bodies, and vacuolations (Figures 6 A and B). However, in the Cd + NC treated group as there were cells with nearly normal irregular nuclei, preserved mitochondria, and still degenerated lamellar bodies (Figures 7 A and B).

Biochemical Assays

In this paper, the biochemical analysis of pro-inflammatory cytokines in animal serum is given (Table 2). When equaled to the control group, the Cd group had a significant ($p \leq 0.05$) rise in tissue levels of IL-6 and TNF-α indicating a severe inflammatory response. Also, when compared to the Cd treated group, those inflammatory cytokines were significantly ($p \leq 0.05$) lower in the Cd + NC treated group, but still higher than the control group, indicating a decrease in the inflammatory response.

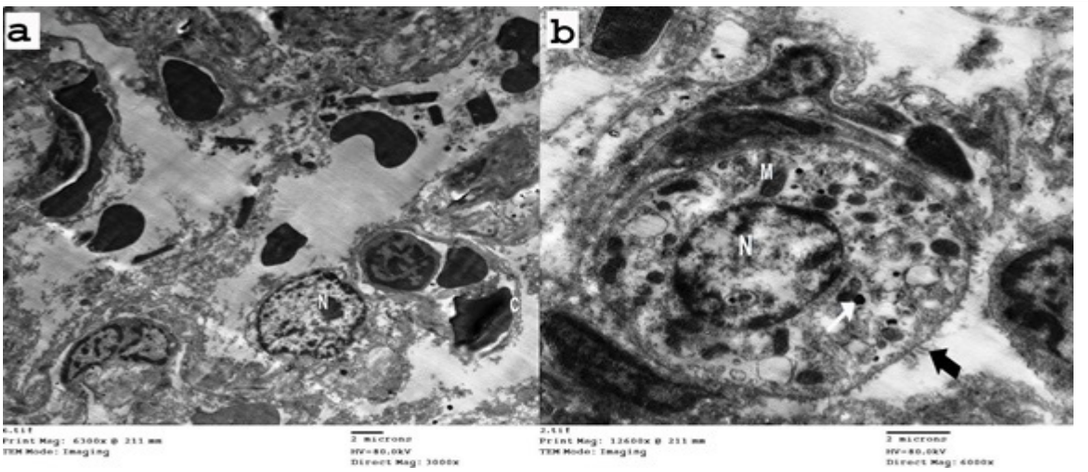

Figure 5: (5 A, 5 B): control group higher magnification showing pneumocyte type II with irregular euchromatic nucleus (N), lysosome (white arrow), normal mitochondria (M), microvilli (thick arrow) and interstitial blood capillaries (C). (Figure 5 A: TEMX3000, Figure 5 B: TEMX6000).

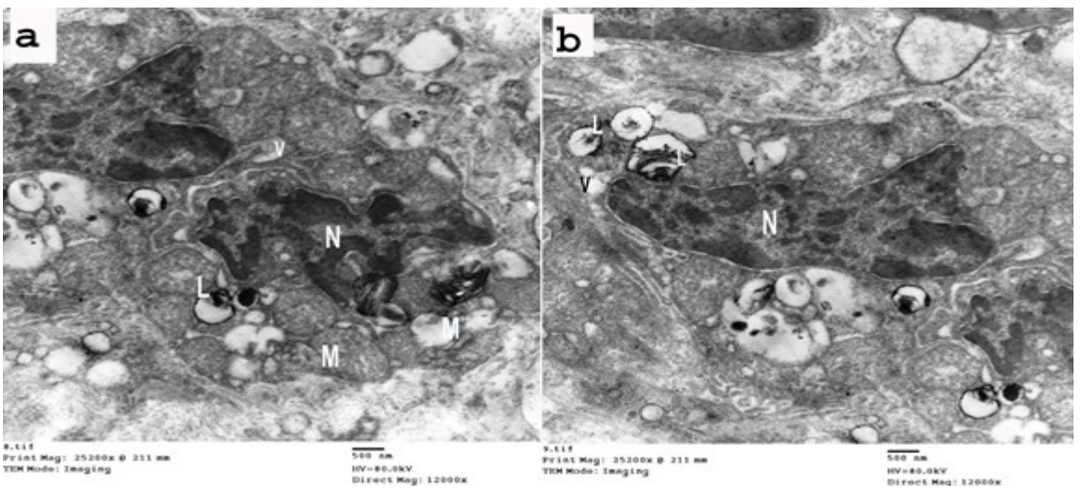

Figure 6: (6a, 6b): Cd group higher magnification showing pneumocyte type II with hyperchromatic nuclei (N) destructed dilated mitochondria (m) degenerated lamellar bodies (L) and cytoplasmic vacuoles (V). (Fig. 6a: TEMX12000, Fig. 6b: TEMX12000).

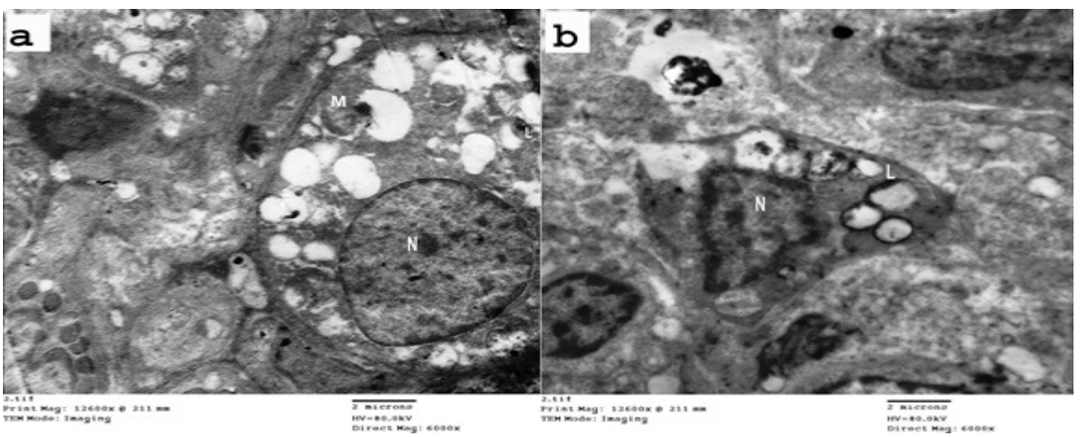

Figure 7: (7a, 7b): Cd + NC group higher magnification showing type II pneumocyte with nearly normal irregular nuclei (N), preserved mitochondria (M) and slightly degenerated lamellar bodies (L). (Fig. 7a: TEMX6000, Fig. 7b: TEMX6000).

Table 2: Comparison of the means of TNF-α and IL-6 levels in all studied groups:

	Control group	Cd group	Cd + NC group
TNF-α	33.43 ± 2.57	124 ± 2.48*	65.9 ± 3.36* *
IL-6	15.07 ± 1.23	72.63 ± 4.17*	44.43 ± 8.29**

Data are displayed as mean± SE, *statistically significant as compared to control group; **statistically significant as compared to Cd group.

Genomic Assays

Lung tissue genomic analysis is presented in (Table 3). The lung cellular death was assessed by measuring the tissue gene expression of both the anti-apoptotic Bcl2 gene and the apoptotic Bax gene. The Cd group showed a significant ($p \leq 0.05$) increase in lung tissue Bax gene expression compared to the control group representing cellular apoptosis. Also, it was significantly ($p \leq 0.05$) decreased in the Cd + NC group compared to the Cd group to levels relatively similar to the control group. While the Cd group showed a significant ($p \leq 0.05$) decrease in lung tissue Bcl2 gene expression compared to the control group demonstrating anti-apoptotic cellular response. Also, it was significantly ($p \leq 0.05$) increased in the Cd + NC group compared to the Cd group to levels almost similar to the control group indicating lung cellular restoration. Furthermore, Cd exposure increased Bax/Bcl-2 ratio expression, causing cell death, but NC administration reduced Bax/Bcl-2 ratio expression, preventing cell apoptosis.

Table 3: Comparison of the means of Bax and Bcl-2 genes expression levels in all studied groups:

	Control group	Cd group	Cd + NC group
Bax	1.03 ± 0.01	6.61 ± 0.21*	3.04 ± 0.26**
Bcl-2	1.01 ± 0.01	0.32 ± 0.05*	0.82± 0.04* *
Bax /Bcl-2 Ratio	1.01 ± 0.01	20.65 ± 4.2	3.7 ± 1.2

Data are displayed as mean± SE, *statistically significant as compared to control group; **statistically significant as compared to Cd group.

DISCUSSION

We are always seeking for ways to live a healthier life and attempting to find natural preventive and therapeutic agents to avoid the harmful effects of heavy metals like Cd[1]. This work intends to identify a therapeutic opportunity in employing NC as a curative agent against Cd-induced lung damage in order to answer this complex problem. Cd toxicity and the therapeutic effect of NC are revealed in the current investigation by biochemical, genomic, and morphological alterations in lung tissue.

Excessive production of pro-inflammatory cytokines damages the lungs, resulting in apoptotic cell death[6]. The creation of cellular pro-inflammatory cytokines like IL-6 and TNF- was measured in this study and found to be significantly higher in Cd-treated rats. Similarly, cultured alveolar macrophages using Cd nanoparticles showed a rise in TNF-α[5, 22]. Also, high TNF-α production was suspected to cause increased ROS[6]. On the other hand, there was an increase in IL-6 production in lung cell cultures when Cd was added, but no variations in TNF-α production[4]. These biochemical findings matched the microscopic findings, which revealed a significant infiltration of inflammatory cells, particularly macrophages, in the lung alveoli. Accordingly, cellular exposure to Cd causes the release of a chemokine, which attracts neutrophils and macrophages to the site of inflammation[23].

Along with increased production of pro-inflammatory cytokines, the current study and similar previous studies showed cellular deterioration and damage of normal lung structure in the shape of modifications to alveolar gaps and walls[24], dilated, congested blood sinusoids, intercellular edema, and bleeding regions[25].

According to the current study, we study the pneumocytes type II ultrastructure, they are very crucial for the generation and secretion of lung surfactant, a fatty film aiding in the reduction of alveolar surface tension. The alveoli would collapse if they didn't have this covering, taking a lot of force for re-expansion. Ultrastructural investigation of Cd-treated groups revealed pneumocyte type II, with hyperchromatic nuclei, cellular cytoplasmic vacuolations, degraded mitochondrial cristae, and hollow lamellar bodies. These findings similarly were reported in the lungs after heavy metal exposure, indicating cellular deterioration[26, 27].

The Cd lung lesions were thought to be caused by an increase in the production of pro-inflammatory cytokines, which then exacerbated the oxidative stress pathway. Lung oxidative stress induces oxidative DNA damage and sequentially disables the DNA repair mechanism[28].

The genetic assessment of Bax and Bcl-2 proteins was employed in this work and similar earlier investigations to identify whether the cellular lung was in a state of life or death. The aforementioned findings backed up microscopical studies that demonstrated a significant increase in Bax gene

expression and a decrease in Bcl2 gene expression in Cd-treated lungs, indicating an increase in cellular death and a reduction in the defensive cellular response[8].

In accordance with a previous similar study, the purpose of using NC in this study was to find a therapeutically accessible supplement that might alleviate the suffering of patients with pulmonary lung disorders who had been exposed to Cd. Its anti-inflammatory and antioxidant properties, as well as its potential to control the cell death pathway, have shown promising results. The NC reduced the production of pro-inflammatory cytokines such as TNF and IL-6., C reduces TNF- in blood and lung tissue after inducing fibrosis in mice[29]. Similarly, C reduces TNF- in the lungs after radiation exposure. Furthermore, NC regulated the Bax/Bcl-2 pathway, which regulates cell death, in this study[27, 30].

In consistence with these findings, increased serum TNF- and overexpressed lung mRNA, and pretreatment with NC greatly decreased these effects[32].

Microscopical data demonstrated that rats given NC after being exposed to Cd had less lung tissue damage and fewer inflammatory cells than the control group, corroborating the biochemical findings. Similarly, NC reduced lung degeneration, necrosis, and cellular infiltration in hypoxic mice as it had a high protective antioxidant effect[32].

Curcumin supplementation improved these ultrastructure changes due to its antioxidant influence[33], which was proved in the current study, the NC treated group's ultrastructure shows type II pneumocytes with nearly normal irregular nuclei, preserved mitochondria, but still degenerated lamellar bodies.

The NC is considered as an encouraging supplement used as a therapeutic agent against the harmful Cd which is considered as one of our health's pollutants.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

REFEREANCES

1. Krichah R., Ben Rhouma K., Hallegue D., Tebourbi O., Joulin V., Couton D., Sakly M., 2003. Acute cadmium administration induces apoptosis in rat thymus and testicle, but not liver. Polish Journal of Environmental Studies 12 (5), 589–594. http://www.pjoes.com/Acute-Cadmium-Administration-Induces-Apoptosis-in-Rat-Thymus-and-Testicle-but-not,87596,0,2.html.

2. Suzuki Y., Morita I., Yamane Y., Murota S., 1989. Cadmium stimulates prostaglandin E2 production and bone resorption in cultured fetal mouse calvaria. Biochemical and Biophysical Research Communications 158 (2), 508–513. doi: 10.1016/s0006291-x(89)800786-.

3. Geetha M W., Xiuwen S D., Ted M., Elizabeth H., Yurong M., 2007. Costs of occupational injuries in construction in the United States. Accid Anal Prev, 39(6):125866-. doi: 10.1016/j.aap.2007.03.012.

4. Milena Kataranovskia, Ivana Mirkova, Sandra Belija, Miroslav Nikolicc, Lidija Zolotarevskid, Danica Cirica, Dragan Kataranovskia, 2009. Lungs: Remote inflammatory target of systemic cadmium administration in rats. Environ Toxicol Pharmacol. doi: 10.1016/j.etap.2009.04.008.

5. Marit L., Dmitrii R., Johan O., Oddmund B., Per E.S., Magne R., 2010. Cadmium-induced inflammatory responses in cells relevant for lung toxicity: Expression and release of cytokines in fibroblasts, epithelial cells and macrophages. Toxicology Letters, 193 (3): 252260-. doi: 10.1016/j.toxlet.2010.01.015.

6. Soccal PM, Gasche Y, Pache JC, *et al*. 2000. Matrix metalloproteinases correlate with alveolar-capillary permeability alteration in lung ischemia-reperfusion injury. Transplantation.70:998–1005. doi: 10.109700002-200010150-00007890/.

7. Payne CM, Bernstein C, Bernstein H. 1995. Apoptosis overview emphasizing the role of oxidative stress, DNA damage and signal-transduction pathways. Leuk Lymphoma.19:43^93. doi: 10.31091042819509059662/.

8. Lag M, Westly S, Lerstad T, Bjørnsrud C, Refsnes M, Schwarze P E., 2002. Cadmium-induced apoptosis of primary epithelial lung cells: involvement of Bax and p53, but not of oxidative stress Cell Biol Toxicol;18(1):2942-. DOI: 10.1023/a:1014467112463.

9. Manish B., Tripathi L M, Srivastava V M L, Anju P., Rakesh S., 2003.Modulation of inflammatory mediators by ibuprofen and curcumin treatment during chronic inflammation in rat. Immunopharmacol Immunotoxicol. 25:213–224. doi: 10.1081/iph-120020471.

10. Miquel J, Bernd A, Sempere JM, Diaz-Alperi J, Ramirez A. 2002. The curcuma antioxidants: pharmacological effects and prospects for future clinical use. A review. Arch Gerontol Geriatr.34:37–46. doi: 10.1016/s0167-7-00194(01)4943.

11. Ilya G., Christopher N R., Eva C V., Anna S G., Stephen J P., 2003.Curcumin ameliorates ethanol and nonethanol experimental pancreatitis. Am J Physiol. 284: G85–G95. doi:10.1152/ajpgi.00138.2002.

12. Anand, P., Kunnumakkara, A. B., Newman, R. A., & Aggarwal, B. B. (2007). Bioavailability of curcumin: problems and promises. Molecular pharmaceutics, 4(6), 807818-. doi: 10.1021/mp700113r

13. Kalaiarasi, R., Prasannaraj, G., & Venkatachalam, P. (2013). A rapid biological synthesis of silver nanoparticles using leaf broth of Rauvolfia tetraphylla and their promising antibacterial activity. doi:10.10441980-/iajpr.00998

14. Vicki H G. 2008. When size really matters: size-dependent properties and surface chemistry of metal and metal oxide nanoparticles in gas and liquid phase environments. J Phys Chem C.112:18303–13. https://doi.org/10.1021/jp806073t

15. Jazayeri-Tehrani SA, Rezayat SM, Mansouri S, Qorbani M, Alavian SM, Daneshi-Maskooni M, Hosseinzadeh-Attar MJ. 2017. Efficacy of nanocurcumin supplementation on insulin resistance, lipids, inflammatory factors and nesfatin among obese patients with non-alcoholic fatty liver disease (NAFLD): a trial protocol. BMJ Open. Jul 1;7(7): e016914. doi: 10.1136/bmjopen-2017016914-.

16. Cevat A. , Mehmet K., Mustafa E., Samil O., 2012 . Anti-apoptotic effects of curcumin on cadmium-induced apoptosis in rat testes. Toxicol Ind Health. Mar;28(2):12230-. doi: 10.11770748233711407242/.

17. Ahmed-Farid, O. A., Nasr, M., Ahmed, R. F., & Bakeer, R. M. (2017). Beneficial effects of curcumin nano-emulsion on spermatogenesis and reproductive performance in male rats under protein deficient diet model: enhancement of sperm motility, conservancy of testicular tissue integrity, cell energy and seminal plasma amino acids content. Journal of Biomedical Science, 24(1), 114-.

18. Koelman, L., Pivovarova-Ramich, O., Pfeiffer, A. F., Grune, T., & Aleksandrova, K. (2019). Cytokines for evaluation of chronic inflammatory status in ageing research: reliability and phenotypic characterisation. Immunity & Ageing, 16(1), 112-.

19. Ghasemi, A., Khanzadeh, T., Heydarabad, M. Z., Khorrami, A., Esfahlan, A. J., Ghavipanjeh, S. & Azimi, A. (2018). Evaluation of BAX and BCL-2 gene expression and apoptosis induction in acute lymphoblastic leukemia cell line CCRF-CEM after high-dose prednisolone treatment. Asian Pacific journal of cancer prevention: APJCP, 19(8), 2319.

20. Bancroft J., and Gamble M., 2008. Theory and Practice of Histological Techniques. 5th ed. P.: 126-612-601 & 127,150,171. Churchill-Livingstone, London, Edinburgh, New york, Philadelphia, St Louis, Sydney and Toronto.

21. John D., 2008. Theory and Practice of Histological Techniques, 6th edition. Elsevier Health Sciences, p. 121134-.

22. Jason L. Blum, Lauren K. Rosenblum, Gabriele Grunig, Mary Beth Beasley, Judy Q. Xiong, and Judith T. Zelikoff., 2014. Short-term inhalation of cadmium oxide nanoparticles alters pulmonary dynamics associated with lung injury, inflammation, and repair in a mouse model Inhal Toxicol. 26(1):48-58. doi: 10.310908958378.2013.851746/

23. Tomasz O., Irena B., Izabela G., Dariusz C., 2012. Pro-inflammatory properties of cadmium. Acta Biochim Pol; 59:475–482. https://pubmed.ncbi.nlm.nih.gov/23240106/

24. Amal I E., and Fawzy I E., 2013. Histopathology and cytotoxicity as biomarkers in treated rats with cadmium and some therapeutic agents. Saudi J Biol Sci; 20:265–280. DOI:10.1016/j.sjbs.2013.02.004

25. Ezemonye L I., and Enuneku A A., 2011. Histopathological alterations in the liver and lungs of Hoplobatrachus occipitalis exposed to sub lethal concentrations of cadmium. Aust J Basic Appl Sci; 5:1062–1068. http://www.ajbasweb.com/old/ajbas/2011/November-20111068-1062/.pdf

26. Huiying Z., Donna R N., James C B., Philip L S., 2012. Over-expression of human endosulfatase-1 exacerbates Cadmium-induced injury to transformed human lung cells in vitro. Toxicol Appl Pharmacol; 265:27–42. doi: 10.1016/j.taap.2012.09.008.

27. Yu Ji Cho , Chin Ok Yi, Byeong Tak Jeon, Yi Yeong Jeong, Gi Mun Kang, Jung Eun Lee, Gu Seob Roh, Jong Deog Lee, 2013. Curcumin attenuates radiation-induced inflammation and fibrosis in rat lungs. Korean J Physiol Pharmacol. Aug;17(4):267-74. doi:10.4196/kjpp.2013.17.4.267.

28. Anju R., Anuj K., Ankita L., Manu P. 2014. Cellular mechanisms of cadmium-induced toxicity: a review. Int J Environ Health Res; 24:378–399. doi: 10.108009603123.2013.835032/.

29. Zhen-you Jiang , Lin Zou, Shan-shan Shi, Yan-ru Lu, Jun Dong, Chun-hua Yang, Ye-cheng Lu, Guo-kui Dai, 2009. [Effects of curcumin on TNF-alpha and TGF-beta1 in serum and lung tissue of SiO2-induced fibrosis in mice Xi Bao Yu Fen Zi Mian Yi Xue Za Zhi. May;25(5):399401-. https://pubmed.ncbi.nlm.nih.gov/19426594/

30. Lingling Zhao , Qianru Gu , Lanting Xiang , Xidan Dong , Huimin Li , Jinyao Ni , Li Wan , Guoping Cai , Guorong Chen, 2017. Curcumin inhibits apoptosis by modulating Bax/Bcl-2 expression and alleviates oxidative stress in testes of streptozotocin-induced diabetic rats Ther Clin Risk Manag . Aug 28;13:10991105-. doi: 10.2147/TCRM.S141738.

31. Aygul Guzel , Mehmet Kanter, Ahmet Guzel, Ahmet Fikret Yucel, Mustafa Erboga, 2013. Protective effect of curcumin on acute lung injury induced by intestinal ischaemia/reperfusion. Toxicol Ind Health. Aug;29(7):63342-. doi: 10.11770748233711430984/.

32. Alhusaini, A., Alhumaidan, S., Almogren, R., Alsaif, S., Alsultan, E., & Hussein, I. (2021). Nano-Curcumin Protects Against Sodium Nitrite–Induced Lung Hypoxia Through Modulation of Mitogen-Activated Protein Kinases/c-Jun NH2-Terminal Kinase Signaling Pathway. Dose-Response, 19(3), doi: 10.11771559325821103314 8/.

33. Malhotra, A., Nair, P., & Dhawan, D. K. (2012). Premature mitochondrial senescence and related ultrastructural changes during lung carcinogenesis modulation by curcumin and resveratrol. Ultrastructural pathology, 36(3), 179184-. doi: 10.310901913123.2011.652765/.