

Synergistic Hepatotoxic Effects of Cadmium and Allethrin in Mice: Role of Mitochondrial Oxidative Stress and Inflammatory Responses

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ABSTRACT

Background: Cadmium (Cd) is a toxic heavy metal that is found predominantly in the environment. Allethrin is an insecticide that is used extensively in insect killing sprays and coils.

Objective: People may be exposed to pesticides like Allethrin and heavy metals at the same time in environment, therefore, the present study was designed to assess the effect of co-exposure of Allethrin and Cd on hepatic tissues of mice.

Methods: Total 32 mice were randomly distributed into four groups, including control, Cd, Allethrin and Cd+Allethrin groups. The trial was conducted for 28 days and then the mice was killed and their blood and liver were taken for further analysis.

Results: The results showed that Cd and Allethrin elevated the level of mitochondrial ROS, lipid peroxidation, and inflammatory markers. Moreover, it lowered the activities of antioxidant enzymes and lowered body defense. In addition, they also elevated the levels of AST and ALT that further showed that liver was damaged. Importantly, it was observed that Cd and Allethrin co-exposed group showed the highest intensity of the hepatic damage.

Conclusion: These results propose that Cd and Allethrin are mechanistically linked and their damage intensity is increased when they are exposed together.

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Introduction

Environmental contamination with synthetic pesticides and heavy metals is a recurring global health concern due to the widespread exposure and cumulative biological impacts [1]. Cadmium (Cd), one of the heavy metals, is a very hazardous environmental contaminant that mostly enters organisms through contaminated food, water, and air. Due to its extended biological half-life, cadmium accumulates in organs with high metabolic activity, especially the liver and kidneys, and is linked to a number of harmful effects, including hepatic dysfunction [2]. The liver is a major target for cadmium

poisoning due to its crucial involvement in xenobiotic metabolism [3]. Cadmium predominantly causes liver damage through oxidative stress and impairment of cellular antioxidant defenses, according to recent experimental research [4]. It has been demonstrated that animals exposed to cadmium both acutely and subchronically had higher levels of hepatic malondialdehyde (MDA), lower levels of important antioxidant enzymes like glutathione (GSH), superoxide dismutase (SOD), and overall antioxidant capacity, and pathological liver alterations [5].

A prominent source of environmental exposure for humans is allethrin, a type I synthetic pyrethroid that is

frequently found in mosquito coils and household pest management products. Evidence indicates that some pyrethroids may cause oxidative stress and negative consequences in non-target creatures, despite the fact that they were first thought to be less dangerous to mammals due to their quick metabolic clearance [6]. Although there aren't many direct research on allethrin's hepatotoxicity, there is evidence that it can cause apoptosis and upset redox equilibrium. It has been demonstrated that long-term exposure to mosquito coil smoke containing allethrin increases hepatic enzyme activity (such as ALT and AST) and causes histological liver damage in mice, including hepatocyte necrosis and apoptosis [7]. These findings suggest that exposure to allethrin can cause liver oxidative damage, despite the fact that smoke contents contain a variety of substances other than pure allethrin [8]. Allethrin has also been demonstrated to cause oxidative stress, boost the generation of reactive oxygen species (ROS), and activate apoptotic pathways involving caspase-3 and Bax in mammalian cells [9]. Allethrin enhanced ROS production, lipid peroxidation, and apoptotic gene expression in one in vitro study employing human neuroblastoma cells, indicating pro-oxidative and programmed cell death pathways. The idea that allethrin may likewise affect oxidative and apoptotic pathways in liver tissues is supported by the similar cellular stress processes, even though hepatic cells were not used in this investigation [10].

When taken as a whole, these results suggest that oxidative stress pathways linked to pyrethroids and those generated by cadmium may share a mechanism. People may be exposed to pesticides like allethrin and heavy metals at the same time in environmental exposure situations, but the combined liver effects of these co-exposures are still little understood. Because simultaneous disruption of antioxidant systems may overwhelm cellular defenses and intensify oxidative damage, toxicological research has increasingly recognized that co-exposure to several chemicals that disturb redox equilibrium may worsen organ injury [11]. As integrative mechanisms connecting many toxicants to liver damage, oxidative stress and mitochondrial dysfunction are becoming better recognized. Overproduction of ROS can harm proteins and mitochondrial membranes, hinder electron transport, and lower ATP synthesis, making cells more susceptible to death. Furthermore, oxidative stress can trigger apoptotic and inflammatory signaling pathways that lead to hepatocellular dysfunction and degeneration [12].

There is a significant knowledge gap about the effects of combined exposure to Cd and allethrin on liver health, despite a large body of literature describing the single-toxicant effects of Cd and evidence confirming oxidative stress from pyrethroid exposures. For a thorough risk assessment of environmental mixes, it is crucial to determine whether simultaneous exposure increases markers of oxidative stress, antioxidant depletion, and mitochondrial dysfunction. This study aims to clarify the underlying biochemical and molecular pathways and ascertain if co-exposure results in increased liver injury by comparing these parameters between single and combined exposures.

Methodology

Chemicals and Animals: Cd and Allethrin were obtained from Sigma Aldrich (Germany). 32 male mice were used for this research. The mice were kept in the animal research center of University of Agriculture, Faisalabad, Pakistan. The mice were provided with normal laboratory conditions with 50% relative humidity, 22-24°C temperature and 12h dark/light cycle. Commercial rodent feed was given to mice throughout the trial.

Experimental design: The mice were randomly allotted to 4 groups and administered daily with respective dosages for 28 days.

- Control group: This group received no treatment.
- Cd group: This group received 5mg/kg of Cd via oral gavage.
- Allethrin group: This group received 25mg/kg of Allethrin via oral gavage.
- Cd + Allethrin group: This group received 25mg/kg of Allethrin and 5mg/kg of Cd via oral gavage.

After the experiment, the mice were dissected, their blood was taken in sterile syringes and liver was excised and stored for further analysis.

Oxidative Stress Parameters: The activities of CAT and SOD were estimated via the previously reported methods of Sinha [13] and Das and Nanda [14], respectively. The levels of lipid peroxidation were measured using MDA as per the techniques of Uchiyama and Mihara [15].

Mitochondrial ROS: Mitochondrial ROS in hepatic tissues of mice was estimated via membrane-permeable fluorescent probe DCFH-DA as per the methods of Gan et al. [16].

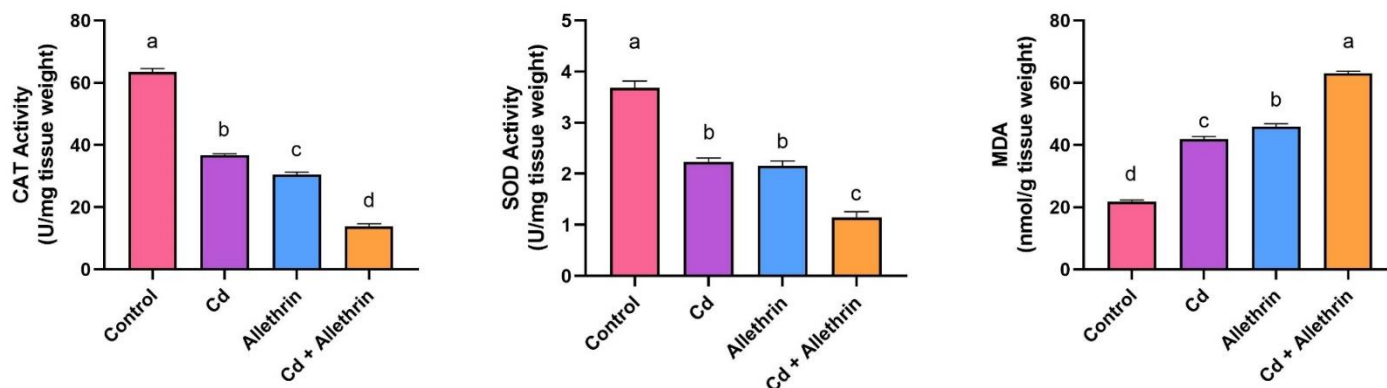


Figure 1. The impact of Cd and Allethrin on CAT, SOD and MDA. Different superscripts show significant difference ($P < 0.01$) among the groups.

The levels of mitochondrial ROS was expressed as a percentage in fluorescence relative to the control group.

ELISA: The levels of inflammatory markers (TNF- α and IL-1 β) and hepatic function markers (AST and ALT) were estimated via mouse ELISA kits (Abcam, UK).

Statistical Analysis: The results are presented as Mean $\pm$ SE. One way ANOVA followed by Tuckey's test was applied for the estimation of significant differences among the groups. $P < 0.01$ was considered significant.

Results

Oxidative Stress Parameters: [Figure 1](#) shows the impact of Cd and Allethrin on hepatic oxidative stress parameters of mice. The results showed that Cd and Allethrin administration significantly lowered antioxidant defense and increased lipid peroxidation in hepatic tissues. The activities of CAT and SOD reduced significantly ($P < 0.01$) in Cd and Allethrin treated groups as compared to the control, while the levels of MDA increased. Moreover, Cd + Allethrin co-treated group showed highest deleterious impacts on these parameters as the activities of antioxidants were significantly ($P < 0.01$) lowered and levels of MDA were significantly elevated in this group as compared to the control, Cd and Allethrin treated groups.

Mitochondrial ROS: Cd and Allethrin treatment also elevated the percentage of mitochondrial ROS. As it appears in [Figure 2](#), the mitochondrial ROS in Cd, Allethrin and Cd + Allethrin groups was significantly (P

< 0.01) higher as compared to the control. Where Cd + Allethrin co-treated group showed the highest levels of mitochondrial ROS.

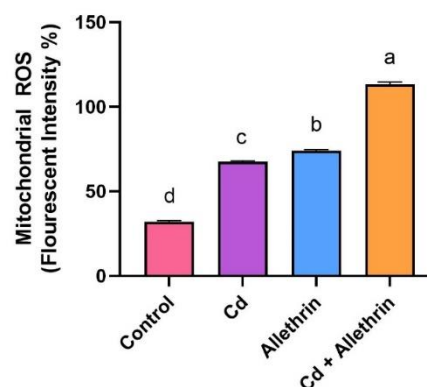


Figure 2. The impact of Cd and Allethrin on mitochondrial ROS. Different superscripts show significant difference ($P < 0.01$) among the groups.

Inflammatory Parameter: [Figure 3](#) reveals that Cd and Allethrin treatment elevated the inflammatory response of the body in hepatic tissues. The results showed that Cd and Allethrin showed significant ($P < 0.01$) elevation in the levels of TNF- α and IL-1 β , as compared to control group. Moreover, Cd + Allethrin group showed the most significant ($P < 0.01$) difference in the levels of these parameters as the inflammatory response was the highest in this group as compared to others.

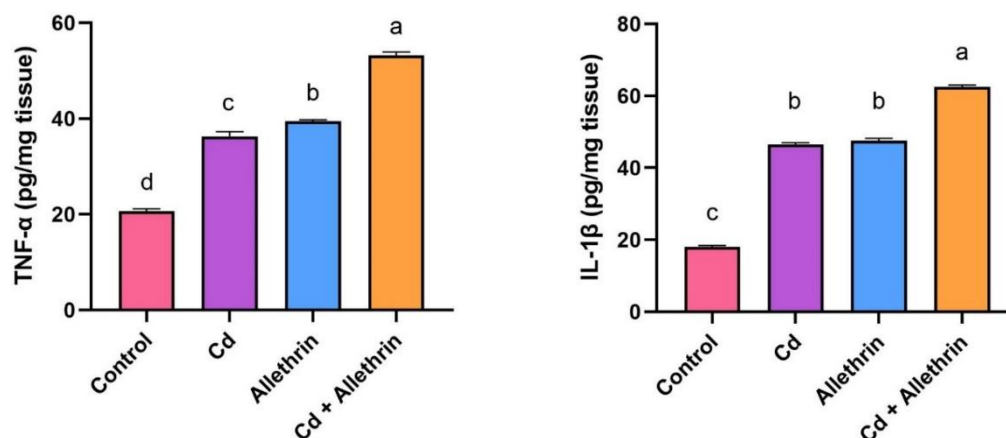


Figure 3. The impact of Cd and Allethrin on TNF- α and IL-1 β . Different superscripts show significant difference ($P < 0.01$) among the groups

Hepatic function markers: The results showed that Cd and Allethrin treatment brought significant ($P < 0.01$) upsurge in AST and ALT levels in Cd and Allethrin groups as compared to the control. Importantly, the co-treatment with Cd + Allethrin brought the highest change as shown by significantly ($P < 0.01$) upregulated levels of hepatic parameters as matched to the control, Cd and Allethrin groups (Table 1).

Table 1. The impact of Cd and Allethrin on AST and ALT.

Groups	AST (U/L)	ALT (U/L)
Control	26.58 $\pm$ 0.46 ^d	22.62 $\pm$ 0.51 ^d
Cd	100.97 $\pm$ 1.18 ^c	67.97 $\pm$ 0.89 ^b
Allethrin	112.14 $\pm$ 0.87 ^b	62.52 $\pm$ 0.77 ^c
Cd + Allethrin	143.34 $\pm$ 1.17 ^a	89.38 $\pm$ 0.93 ^a

Different superscripts show significant difference ($P < 0.01$) among the groups

Discussion

The results showed that Cd and Allethrin caused significant oxidative stress in the hepatic tissues. It lowered antioxidant activities and increased lipid peroxidation and mitochondrial ROS. ROS are produced via indirect damage and are the major contributors to chemical induced damages in biological systems. It creates oxygen free radicals that disrupt redox balance in the cells and cause lipid peroxidation [17]. If it is not controlled it may lead to apoptosis [18]. ROS is mostly produced in mitochondria and are vulnerable to ROS [16]. Therefore, ROS may lead to mitochondria associated apoptosis. In this study, Cd + Allethrin group

produced highest oxidative damage, which proposes that their toxicity is even enhanced when they interact with each other. The results of our study are consistent with Shaw et al. [19] and Jalouli et al. [20], who reported that Cd and Allethrin cause oxidative stress, respectively.

In addition to lipid peroxidation, Cd and Allethrin caused significant elevation in hepatic inflammation as evidenced by upregulated levels of TNF- α and IL-1 β , the biomarkers of inflammation. ROS triggers NF- κ B, which further induces the expressions of its downstream inflammatory markers [21]. Therefore, the elevated levels of TNF- α and IL-1 β in the current study shows that Cd and Allethrin caused significant inflammatory damages in the hepatic tissues.

For the further validation of oxidative and inflammatory damage, the levels of serum hepatic markers were determined. The higher levels of AST and ALT in Cd and Allethrin groups further validated our claims that they caused remarkable damage. AST and ALT are common markers for the determination hepatic function [22]. Reportedly, oxidative stress damages the hepatocytes that leads to the elevation of these markers in blood [23]. The highest levels of these markers were observed in Cd + Allethrin group, which showed that the impact of Allethrin and Cd further increased when they are in combination.

Conclusion

In conclusion, Cd and Allethrin caused significant elevation in the levels of oxidative stress, inflammatory and serum markers. Most importantly, the group treated with Cd and Allethrin showed the highest intensity of

damage. Therefore, it may be possible that Cd and Allethrin interact with each other and their toxic potential is further enhanced. Therefore, environmental protective measures should be taken to avoid the damage that may occur due to their combined exposure. However, further in silico and detailed mechanistic research is required to validate these outcomes and propose a possible treatment to the damage caused by them.

Authors’ contributions

ICMJE criteria	Details	Author(s)
1. Substantial contributions	Conception, OR	1
	Design of the work, OR	2
	Data acquisition, analysis, or interpretation	3
2. Drafting or reviewing	Draft the work, OR Review critically for important intellectual content	1,2 3
3. Final approval	Approve the version to be published	All
4. Accountable	Agree to be accountable for all aspects of the work	All

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

The Ethics Review Committee of University of Agriculture, Faisalabad, Pakistan approved the study.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

References

[1]. Alengebawy A, Abdelkhalek ST, Qureshi SR, Wang MQ. Heavy metals and pesticides toxicity in agricultural soil and plants: Ecological risks and human health implications. *Toxics*. 2021;9(3):42.

[2]. Genchi G, Sinicropi MS, Lauria G, Carocci A, Catalano A. The effects of cadmium toxicity. *Int J Environ Res Public Health*. 2020;17(11):3782.

[3]. Souza-Arroyo V, Fabián JJ, Bucio-Ortiz L, Miranda-Labra RU, Gomez-Quiroz LE, Gutiérrez-Ruiz MC. The mechanism of the cadmium-induced toxicity and cellular response in the liver. *Toxicol*. 2022;480:153339.

[4]. Ma Y, Su Q, Yue C, Zou H, Zhu J, Zhao H, et al. The effect of oxidative stress-induced autophagy by cadmium exposure in kidney, liver, and bone damage, and neurotoxicity. *Int J Mol Sci*. 2022;23(21):13491.

[5]. Liu Q, Zhang R, Wang X, Shen X, Wang P, Sun N, et al. Effects of sub-chronic, low-dose cadmium exposure on kidney damage and potential mechanisms. *Annals Translat Med*. 2019;7(8):177.

[6]. Li H, Lydy MJ, You J. Pyrethroids in indoor air during application of various mosquito repellents: Occurrence, dissipation and potential exposure risk. *Chemosphere*. 2016;144:2427-35.

[7]. Badmus JA, Fatoki JO, Adedeji AL, Mustapha IK, Ovili BC, Oyedijo OO, et al. Evaluation of the divergent effects of pyrethroid and D-allethrin-based coils fumes in male Wistar rats. *Environmental Analysis, Health Toxicol*. 2025;40:e2025020.

[8]. Peter OC, Dave OO, Kalu AN, Chieze CP, Obinna EC, Ajibola AH, et al. Toxicological Assessment of D-Allethrin Containing Mosquito Coils: Impact on Serum Electrolyte Balance and Antioxidant Defence Mechanism in Wistar Rats. *Applied Sci Computing Energy*. 2025;3(1):194-203.

[9]. Guven C, Sevgiler Y, Taskin E. Pyrethroid insecticides as the mitochondrial dysfunction inducers. *Mitochondrial Dis*. 2018;293:322.

[10]. Kaur S. In vitro evaluation of cytotoxic and genotoxic potential of bifenthrin and its amelioration with ascorbic acid. *Diss. Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana*. 2017.

[11]. Wu K, Chen Y, Huang W. Combined molecular toxicity mechanism of heavy metals mixtures. *Toxicological assessment of combined chemicals in the environment*. 2025:125-72.

[12]. Le Bras M, Clement MV, Pervaiz S, Brenner C. Reactive oxygen species and the mitochondrial signaling pathway of cell death. *Histol Histopathol*. 2005;20(1):205-19.

[13]. Sinha AK. Colorimetric assay of catalase. *Analyt Biochem*. 1972;47(2):389-94.

[14]. Das BS, Nanda NK. Evidence for erythrocyte lipid peroxidation in acute falciparum malaria. *Trans Royal Soc Trop Med Hyg*. 1999;93(1):58-62.

[15]. Uchiyama M, Mihara M. Determination of malonaldehyde precursor in tissues by thiobarbituric acid test. *Analyt Biochem*. 1978;86(1):271-8.

[16]. Gan L, Wang Z, Si J, Zhou R, Sun C, Liu Y, et al. Protective effect of mitochondrial-targeted antioxidant MitoQ

against iron ion ^{56}Fe radiation induced brain injury in mice. *Toxicol Applied Pharmacol.* 2018;341:1-7.

- [17]. D'Autréaux B, Toledano MB. ROS as signalling molecules: mechanisms that generate specificity in ROS homeostasis. *Nature Rev Mol cell Biol.* 2007;8(10):813-24.
- [18]. Shankar B, Kumar SS, Sainis KB. Generation of reactive oxygen species and radiation response in lymphocytes and tumor cells. *Rad Res.* 2003 ;160(4):478-87.
- [19]. Shaw R, Hati R, Sharma S, Deep S, Chaube R. Protective role of Curcumin in cadmium-induced physiological and reproductive toxicity in male BALB/c mice. *Phyto-medicine Plus.* 2025;5(2):100803.
- [20]. Jalouli M, Mofti A, Elnakady YA, Nahdi S, Feriani A, Alrezaki A, et al. Allethrin promotes apoptosis and autophagy associated with the oxidative stress-related PI3K/AKT/mTOR signaling pathway in developing rat ovaries. *Int J Mol Sc.* 2022;23(12):6397.
- [21]. Pang Y, Wu D, Ma Y, Cao Y, Liu Q, Tang M, et al. Reactive oxygen species trigger NF- κ B-mediated NLRP3 inflammasome activation involvement in low-dose CdTe QDs exposure-induced hepatotoxicity. *Redox Biology.* 2021;47:102157.
- [22]. Ulasoglu C, Enc FY, Kaya E, Yilmaz Y. Characterization of patients with biopsy-proven non-alcoholic fatty liver disease and normal aminotransferase levels. *J Gastrointest Liver Dis.* 2019;28(4):427-31.
- [23]. Al Saihati HA, Badr OA, Dessouky AA, Mostafa O, Farid AS, Aborayah NH, et al. Exploring the cytoprotective role of mesenchymal stem cell-derived exosomes in chronic liver fibrosis: insights into the Nrf2/Keap1/p62 signaling pathway. *Int Immunopharmacol.* 2024;141:112934.