**Toxicology International,** *29(4) : 515-525; 2022. DOI: 10.18311/ti/2022/v29i4/30325*

Online ISSN : 0976-5131; Print ISSN : 0971-6580

**Toxicology International**

Contents available at: www.informaticsjournals.com/index.php/toxi



# N-acetylcysteine Mediated Regulation of MnSOD, UCP-2 and Cytochrome C Associated with Amelioration of Monocrotophos-Induced Hepatotoxicity in Rats

**Jagjeet Singh[1], Annu Phogat[1], Vijay Kumar[2] and Vinay Malik[1]***

*[1]Department of Zoology, Maharshi Dayanand University, Rohtak – 124001, Haryana India, vinaymalikzoo@mdurohtak.ac.in*

*[2]Department of Biochemistry, Maharshi Dayanand University, Rohtak – 124001, Haryana India*

ARTICLE INFO

**KEYWORDS:** Antioxidants, Hepatotoxicity, N-acetylcysteine, Pesticide

**ARTICLE HISTORY**

**Received:** 24.05.2022

**Revised:** 04.08.2022

**Accepted:** 08.08.2022

## Abstract

Pesticides are now a risk to the environment and public health. Monocrotophos (MCP) is known to cause organ toxicity and impart degenerative effects at cellular levels. N-acetylcysteine (NAC) is a natural antioxidant having various prophylactic properties. Male Wistar rats were given NAC (200 mg/kg b.wt), MCP (0.9 mg/kg b.wt) and NAC followed by MCP; intragastrically for 28 consecutive days. Regulation of MnSOD, UCP-2 and cytochrome c were analyzed by western blotting and polymerase chain reaction. Histology, electron microscopy and weight parameters were evaluated in the liver. MCP exposure significantly decreased body weight gain, relative liver weight, and structural changes. Altered MnSOD protein expression, decreased transcription of UCP-2 and MnSOD, and released cytochrome c indicated that oxidative stress is involved in MCP exposure. Treatment of NAC to MCP-exposed rats normalized the weight and structural changes, restored MnSOD and UCP-2 levels and prevented the release of cytochrome c. The present study suggests that the regulation of UCP-2, MnSOD and cytochrome c is involved in NAC efficacy against MCP toxicity. These findings illustrate that NAC can serve as a potential therapeutic agent for toxicity and oxidative stress in mammals.

## 1. Introduction

Pesticides are among the most commonly used chemicals in agriculture worldwide[1]. It is obvious that the extensive application of pesticides has contaminated the environment and posed a severe threat to the environment and human health. Moreover, systemic pesticides have potential future hazards; residues of these pesticides may accumulate in

*Author for correspondence

the food chain and lead to non-target toxicity in various species[2–4]. Organophosphates are the most common systemic pesticides extensively used worldwide for 40 years. The organophosphates are highly toxic and account for nearly 3 million poisoning in developing countries, reporting 200000 human causalities per year[5]. In case reports and research studies, these toxic chemicals induced mutagenic, carcinogenic and degenerative effects affecting the organ physiology and cellular functioning in the nervous system, gastrointestinal tract and reproductive organs of exposed mammals[6].

Monocrotophos (MCP) is one of the organophosphate pesticides restricted for use in some countries but widely used in commercial crops in developing countries. Being hydrophilic, MCP is highly mobile through the environment and can contaminate, which may cause unidentified chronic exposure in humans and other non-targeted organisms. Toxicological data from research studies have confirmed that acute and chronic exposure to MCP caused organ dysfunctioning, hormonal imbalance and structural damage inside the body[7–10]. Acute exposures are mostly intentional and account for mortality data, while chronic exposure is majorly unidentified and unintentional, which accounts for morbidity associated with MCP toxicity. Constant chronic exposure may lead to death. Studies have reported a two to fivefold increase in toxicity symptoms and nearly 20-30% in mortality in farm workers involved in MCP applications[11–13].

The liver is highly vulnerable to pesticide toxicity as it is involved in the metabolism and elimination of pesticides[14]; therefore, it has been reported as a prime organ for MCP toxicity also[15,16]. Recent studies have demonstrated severe biochemical and morphological alterations in rats exposed to MCP[17,18]. An accumulating body of evidence has suggested that MCP exposure causes metabolic alterations and induces physiological changes involving the impairment of glucose metabolism, showing MCP toxicity in hepatic tissue[19]. Studies have indicated that oxidative stress and antioxidant depletion can alter molecular biochemistry inside hepatic tissue, impairing liver physiology by eliciting biochemical and structural changes[13].

Research within the past few years indicated that antioxidants have a wide range of therapeutic applications in pesticide toxicity. Antioxidants assist the endogenous defence mechanism of the biological system via the activation of cellular biomolecules. N-acetylcysteine (NAC) has anti-oxidative, anti-apoptotic, and anti-inflammatory properties and suggests its therapeutic potential against pesticide exposure[20]. Studies have documented its ameliorate efficacy against chemical and pesticide exposure-induced hepatorenal toxicity, neurotoxicity and reproductive toxicity[21–23]. In a recent study paralleled to this one, NAC supplementation has been shown to attenuate MCP exposure-induced mitochondrial dysfunctions[24], inflammation and apoptosis[13]. However, there are limited studies about NAC-mediated amelioration on MCP-induced hepatotoxicity and the exact mechanism for its protective potential. So, the present study is aimed to evaluate the involvement of MnSOD, UCP-2 and cytochrome c in the efficacy of NAC on MCP-induced toxic effects and ultrastructural changes in rat hepatic tissue. The study could provide a new therapeutic approach to the field of NAC for clinical use.

## 2. Materials and Methods

### 2.1 Chemicals

Monocrotophos (Cat. No. 36173); N-acetylcysteine (Cat. No. A7250 ); 3',3',5,5'-Tetramethylbenzidine (TMB, Cat. No. T8665) and PCR primers were purchased from Sigma-Aldrich, St Louis, USA. RNA extraction kit, cDNA synthesis kit and Taq DNA polymerase were obtained from Fermentas, Germany. Primary and secondary antibodies were purchased from Santa Cruz Biotechnology, California, USA. All other chemicals used were of analytic grade. All the glassware and plastic ware were autoclaved and sterilized before use.

### 2.2 Animals Care and Experimental design

Albino male rats (Wistar strain, 165–185 g) were procured from diseases free small animal house in Lala Lajpat Rai University of Animal Sciences, Hisar. Rats were housed in the central animal house of Maharshi Dayanand University, Rohtak, under standard conditions (temperature 25 ± 1° C, relative humidity 50 ± 10%; 12 h alternate light-dark cycle) with free access to water and feed pellets. All protocols involving animals were according to the guidelines of the Committee for the purpose of Control and Supervision of Experiments on Animals, India and approved by the Institutional Animal Ethical Committee vide approval letter 1767/RE/S/14/CPCSEA/210-19 dated 28.11.2019.

Rats were randomly distributed into four groups containing five rats in each. The control group received 1 mL of distilled; N-acetylcysteine treated group received NAC (200 mg/kg b.wt); Monocrotophos exposed group received MCP (0.9 mg/kg b.wt); the NAC+MCP co-treated group received NAC 2 h before MCP intragastrically. Each dose was given intragastrically for 28 days.

The dose and 2 h prior administration of NAC was based on the study of Ahmad *et al.*, and Galal *et al.*,[25,26]. The dose of MCP was based on the study of Nagaraju *et al*[18].

After dissection, the liver was collected and processed further.

## 2.3 Body and Liver Weight Assessment

The body weights of all animals were measured each week and just before dissection. Weights of liver tissue were measured once after the dissection of the experimental animals. Diet consumption was recorded for the individual animal of each group for at least 10 days.

## 2.4 Preparation of Mitochondria

For mitochondria isolation, dissected livers were washed in normal saline and homogenized in five volumes of buffer (0.25 M sucrose, 1 mM EDTA and 5 mM tris; pH 7.4). After debris removal, the tissue homogenate was centrifuged at 9000×g for 10 min at 4°C. The resulting pellets were resuspended in 0.5 mL of the buffer and centrifuged at 13000×g for 10 min. The obtained pellets were washed appropriately and resuspended in the buffer.

## 2.5 Western Blot Analysis

Liver tissue was homogenized with lysis buffer (30mM Tris-HCl, pH 7.4, buffer containing 10mM EGTA, 5mM EDTA, 1% Triton X-100, 250 mM sucrose and protease inhibitor) and centrifuged at 2100x g for 10 min and the supernatant was collected and used further. A part of liver tissue was used for mitochondrial and cytosolic fraction preparation for cytochrome c protein expression analysis. 20 µL sample containing 80 µg protein was subjected to 12% SDS-PAGE followed by blotting on the nitrocellulose membrane. The nitrocellulose membrane was blocked with 5% skim milk protein in Phosphate Buffer Saline (PBS) for 2 h at room temperature, followed by incubation with primary antibodies of MnSOD with 1:400 dilution for 4 h. After this, the membrane was washed twice with PBST and PBST for 10 minutes each at room temperature. The membrane was incubated with corresponding secondary antibodies with 1:10000 dilutions for 1.5 h at room temperature. After washing twice with PBS and PBST for 10 minutes each, the expression analysis for immunoreactive MnSOD protein was visualized by TMB and analyzed using Image J software.

## 2.6 RNA Extraction and Semi-Quantitative RT-PCR

Total RNA was isolated from liver tissue using the RNAsure kit (NP-84105, Genetix, India) described by the manufacturer. RNA was reverse-transcripted into cDNA using the cDNA synthesis kit (K1621, Genetix, India). cDNA products were amplified on a gradient thermal cycler (Biorad C-1000, California, USA) using respective primers, shown in Table 1.

## 2.7 Histology and Electron Microscopy

For histology, dissected liver tissues were fixed in formalin for 24 h and dehydrated in ascending grades of alcohol (30%, 50%, 70%, 90% and 100% for 15 minutes each). After

**Table 1.** The specific primers sequence used for PCR quantification of mRNA levels

| Gene | Accession | Direction | Sequence (5' to 3') |
|---|---|---|---|
| β-actin | V01217.1 | Forward | TTGCCCTAGACTTCGAGCAA |
| | | Reverse | AGACTTACAGTGTGGCCTCC |
| MnSOD | NM_017051.2 | Forward | ACAGGCCTTATTCCACTGCT |
| | | Reverse | CTACAAAACACCCACCACGG |
| UCP-2 | NM_019354.2 | Forward | AGACCATTGCACGAGAGGAA |
| | | Reverse | AGAAGTGAAGTGGCAAGGGA |

dehydration, the fixed tissues were saturated with xylene and wax and embedded in paraffin wax. Tissue sections (5 µm thicknesses) were sliced from paraffin blocks using a microtome and sliced sections were fixed on slides using egg albumin. Slides were cleared in xylene and processed for Haematoxylin and Eosin staining. The stained slides were mounted with Dibutylphthalate Polystyrene Xylene and covered with glass slips. Tissue sections were observed under the microscope (Nikon Eclipse Ci-L, Tokyo, Japan) and were photographed.

For electron microscopy, liver tissues (1-2 $mm^3$) were primarily fixed in 2.5% glutaraldehyde and finally fixed in osmium tetraoxide. The fixed tissues were embedded in epoxy resin after graded dehydration in ethanol. Then epoxy resins were dried at 55°C for 48 h and cut into thin sections using an ultra-microtome. Prepared sections were mounted on the grid, stained and examined under the transmission electron microscope (Tecnai TF-30, FEI-Thermo Fisher) and captured images.

### 2.8 Protein Determination

The protein content of each sample was quantified using bovine serum albumin as standard[27].

### 2.9 Statistical Analysis

Data were analyzed using SPSS (version 22.0) for statistical significance by one-way analysis of variance (ANOVA) followed by Tukey's post hoc test. The results were expressed as mean ± SD. A p-value < 0.05 was considered significant.

## 3. Results

### 3.1 Bodyweight Parameters

The changes in body weight and organ weight of experimental animals during the study are given in Table 2. The exposure to MCP alone significantly diminished body weight gain. The treatment of NAC to MCP-exposed rats normalized the increase in body weight gain. Monocrotophos alone exposed animals were recorded with the lowest body weight at the end. In contrast, treatment of NAC in the MCP-exposed animals restored final body weight compared to MCP-alone exposed animals.

Monocrotophos exposure has significant detrimental effects on liver weight and relative liver weight. The treatment of NAC to MCP-exposed rats effectively restored liver weight and relative liver weight in co-treated animals. No changes were observed in the feed consumption of all the experimental groups (Table 2).

### 3.2 Western Blot Analysis

In the western blotting of MnSOD and cytochrome c, the MCP alone exposure significantly reduced the level of MnSOD protein compared to control animals and released cytochrome c from mitochondria to cytosol inside liver cells. Administration of NAC to MCP-exposed animals significantly restored the MnSOD protein level in the liver tissue of co-treated rats and prevented the release of cytochrome c from mitochondria. NAC alone treated animals and control animals showed a similar

**Table 2.** Effects of N-acetylcysteine (NAC) and Monocrotophos (MCP) on body weight, liver weight and feed consumed in rats

| | Initial BW | Final BW | Weight Gain | Liver weight | Relative liver weight | Feed consumed |
|---|---|---|---|---|---|---|
| Control | 181.6 ± 4.10 | 212.6 ± 4.83 | 31 ± 5.04 | 9 ± 0.19 | 4.23 ± 0.06 | 14.0 ± 1.58 |
| NAC | 178 ± 4.3 | 210.4 ± 8.64 | 32.4 ± 6.99 | 8.94 ± 0.24 | 4.25 ± 0.08 | 14.4 ± 2.13 |
| MCP | 174.2 ± 3.83 | 199.8 ± 3.27** | 25.6 ± 3.51* | 8.03 ± 0.49** | 4.01 ± 0.14* | 14.1 ± 1.69 |
| NAC+MCP | 176.8 ± 5.71 | 205.6 ± 3.91# | 28.8 ± 7.05# | 8.57 ± 0.29 # | 4.16 ± 0.14 | 13.9 ± 1.76 |

Results are expressed as mean ± SD, n = 5. BW: body weight; Weight is expressed in grams, and feed consumed is gram/per day. Significant differences are indicated by * ($p<0.05$), ** ($p<0.005$) compared with the control group; # ($p<0.05$) compared with the MCP-exposed group.

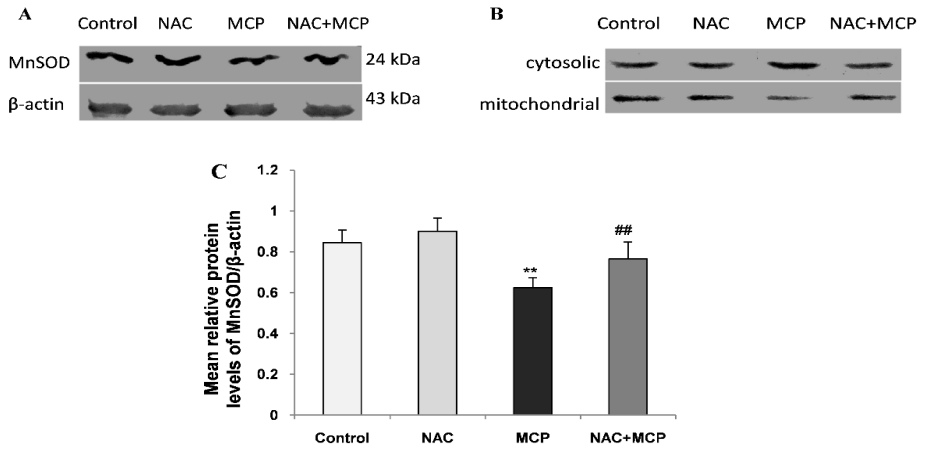



**Figure 1.** Western blots showing the protein levels in experimental groups. **(A)** MnSOD, **(B)** cytochrome c. **(C)** Relative protein levels of MnSOD with respect to β-actin. The values represented are mean ± SD (N=3). **p<0.01 as compared with control and ##p<0.01 as compared with MCP.

level of MnSOD protein and localization of cytochrome c (Figure 1A-C).

## 3.3 PCR Analysis

The mRNA expression of MnSOD and UCP-2 in liver tissue exposed to MCP alone was significantly reduced compared to control rats. The treatment of NAC to MCP-exposed rats significantly restored mRNA expression of MnSOD and UCP-2 in co-treated rats compared to MCP-alone exposed rats. Treatment of NAC alone showed nearly similar mRNA expressions to that of control animals (Figure 2A-C).

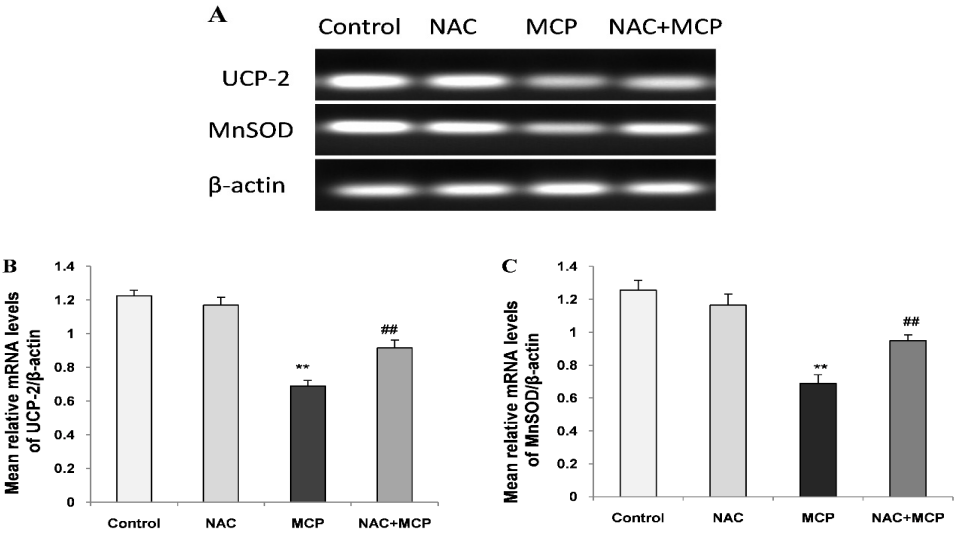



**Figure 2.** **(A)** Images of mRNA expression of MnSOD and UCP-2 in experimental groups. Relative mRNA expression of **(B)** MnSOD and **(C)** UCP-2 with respect to β-actin. The values represented are mean ± SD (N=3). **p<0.01 as compared with control and ##p<0.01 as compared with MCP.

### 3.4 Histology

Histological sections of liver tissues of Control and NAC alone treated animals showed normal architecture and arrangements of hepatocytes, blood sinusoids and central vein. Monocrotophos alone exposure caused dilation of the central vein, infiltration of cells, pyknotic nucleus, and broadening of sinusoidal spaces. Whereas treatment of NAC to MCP-exposed animals significantly prevented infiltration of cells in the central vein, pyknotic nucleus, broadening of sinusoidal spaces and retained the shape of the central vein (Figure 3).

### 3.5 Transmission Electron Microscopy

Ultrastructural analysis in liver tissues of control and NAC alone treated animals showed normal shape and size of both nucleus and mitochondria. Exposure to MCP alone caused disruption of mitochondria, altered nucleus shape, vacuolization and loss of endoplasmic reticulum. Treatment of NAC to MCP-exposed animals significantly reduced mitochondrial disruption and vacuolization and maintained nucleus shape and endoplasmic reticulum in co-treated animals (Figure 4).

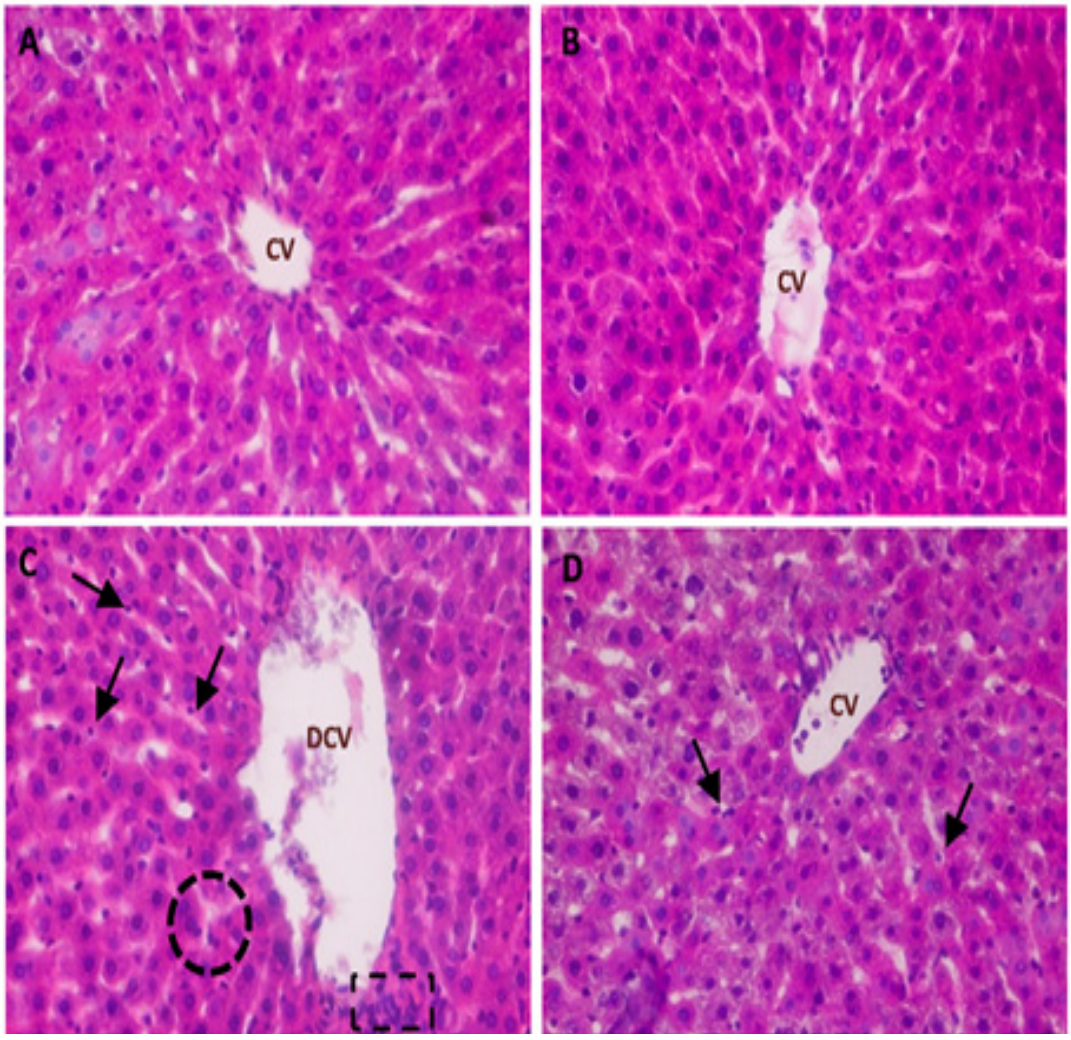



**Figure 3.** Photomicrograph of the liver tissue sections (H and E staining) (400X) **(A)** control rats. **(B)** NAC-treated rats. **(C)** MCP-exposed rats. **(D)** NAC+MCP co-treated rats. Arrow points pyknotic nucleus, the circle shows the broadening of sinusoidal spaces and the rectangle shows the infiltration of cells. CV-Central Vein; DCV-Dilation of Central Vein

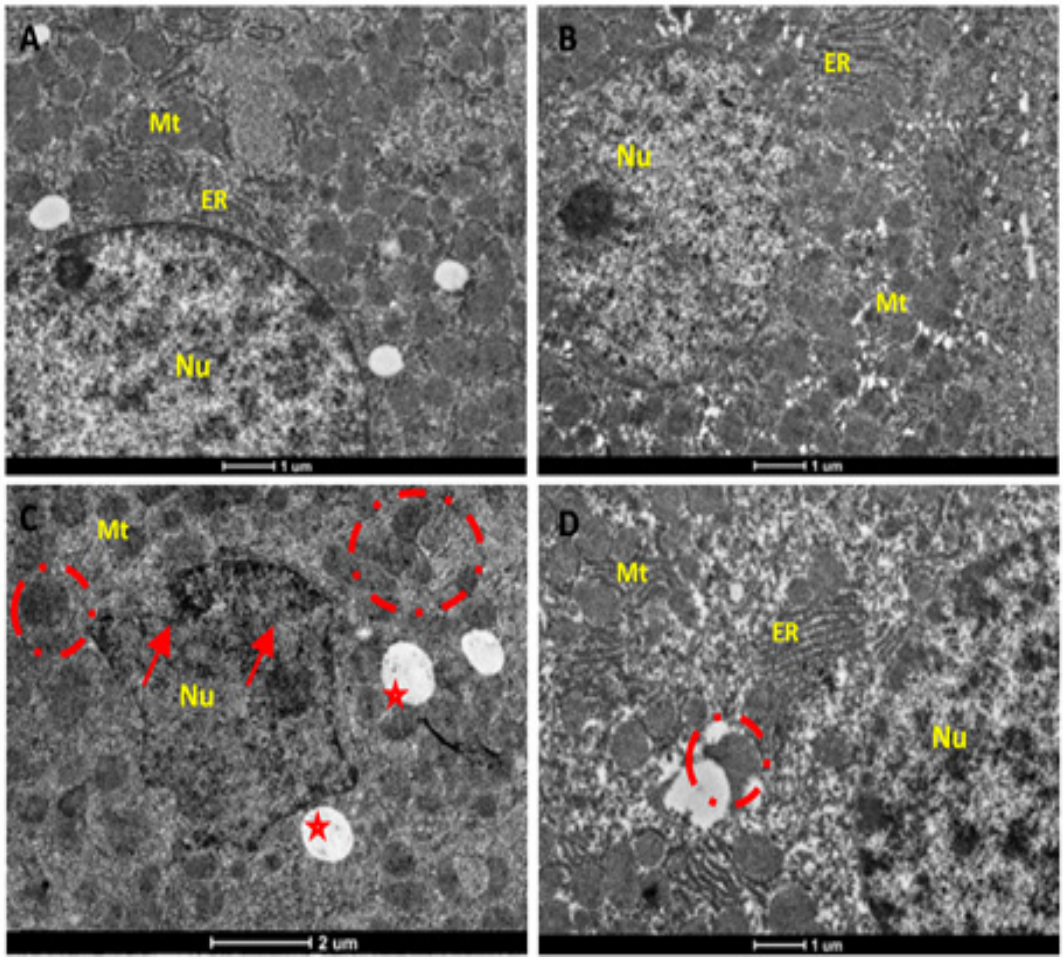



**Figure 4.** Transmission electron microscope images of the liver tissue sections representing ultrastructure of. **(A)** control rats. **(B)** NAC-treated rats. **(C)** MCP exposed rats. **(D)** NAC+MCP co-treated rats. The circle shows disruption of mitochondria, the arrow points to alteration in nuclear shape, star shows vacuolization. Nu-Nucleus, ER-Endoplasmic reticulum, Mt-Mitochondria

## 4. Discussion

Pesticides are in continuous use throughout the globe to control pests. Various pesticides have been detected in the physical resources of the environment, dairy and crop products[28,29]. Environmental, occupational and intentional exposures to pesticides are known to induce serious health issues and organ toxicity in non-targeted organisms, including humans. The liver is a major target organ for pesticide toxicity; several scientific studies have demonstrated that pesticides elicit severe oxidative stress and structural changes in the liver. Several *in-vivo* and *in-vitro* studies have shown the toxic effects of MCP exposure-mediated oxidative stress, biochemical alterations and structural changes in hepatic tissue. Recently, several investigations have evaluated the potential therapeutics of natural antioxidants on pesticide-mediated toxicity. A growing body of evidence has indicated NAC as an effective anti-oxidative therapeutic molecule for oxidative stress. It is evident that MCP exposure impaired activities of mitochondrial complexes by altering PGC-1α/Tfam signalling that caused mitochondrial dysfunction in rat liver, whereas NAC administration maintained mitochondrial functions[24]. Therefore, the present study evaluated the ameliorative efficacy of NAC to provide more insight into the molecular regulation of MCP-induced hepatotoxicity. The results suggest that MCP exposure altered MnSOD, UCP-2 and cytochrome c inside hepatocytes, reduced body and liver weight, causing structural damage and altered cellular functioning. Further, NAC effectively reduced

MCP-mediated structural damages and maintained body and liver weight via regulation of MnSOD, UCP-2 and cytochrome c.

Bodyweight is an indicator of the general health of the organism. Significant reduction in the body weight gain and relative liver weight of the exposure group may indicate the onset of hepatic toxicity following MCP exposure. Concomitantly, the average feed intake in the MCP exposure group compared to other experimental groups suggests impaired physiology, which is eventually reflected in animal body weight reduction. The present results agree with a previous study that suggested lipid and protein degradation and hepatic injuries are responsible for the observed decrease in body and organ weight on chlorpyrifos exposure[30]. In addition, an earlier study has also shown a reduction in body weight gain in rats following MCP exposure for 10 days; however, the relative liver weight was in contrast with the present study[31]. The significant recovery of body weight and liver weight in the NAC+MCP co-treated animals suggested attenuation of hepatic injuries and prevention of molecular damage in protein and lipids. The results of the present study agree with the earlier findings where pre-treatment of NAC prevented lipid peroxidation, protein oxidation, functional changes in lipid and protein structures and reduced structural alterations in hepatic tissues[13]. The anti-oxidative properties of NAC might be responsible for restoring the body and tissue weight.

Decreased protein levels of MnSOD in MCP-exposed rats might be associated with MCP-induced oxidative stress, which agrees with previous studies showing a decrease in MnSOD levels on organophosphate exposure[32]. Further, decreased mitochondrial cytochrome c levels and increased cytochrome c levels in cytosol revealed that MCP elicits apoptosis inside liver tissue. Cytochrome c release from mitochondria and its localization in the cytosol is a bio-indicator of cellular apoptosis. Apoptotic findings of the present study agree with previous studies that showed the release of cytochrome c is primarily involved in pesticide-mediated apoptosis[33]. Combining the decreased MnSOD protein levels and the release of cytochrome c indicates that mitochondrial alterations might be associated with MCP exposure-mediated cellular toxicity. Restoration of MnSOD and confinement of cytochrome c to mitochondria in the NAC+MCP co-treated group might be due to NAC's anti-oxidative and anti-apoptotic potential. Several studies have shown that NAC regulated mitochondrial signalling and showed anti-apoptotic effects against MCP exposure in hepatic tissue[13,24]. These findings agree with other studies also depicting antioxidants-mediated attenuation of pesticide exposure-induced apoptosis[26,34–36].

The change in protein levels of MnSOD may be attributed to alterations in its transcription. In the present study, it was observed that MCP exposure decreased mRNA expression of MnSOD, which is consistent with the observed decrease in protein levels of MnSOD; suggesting that MCP-induced oxidative stress affects both transcription and translation of MnSOD. In addition, a decrease in UCP-2 on MCP exposure suggests Reactive Oxygen Species (ROS) accumulation inside mitochondria. Activation of UCP-2 prevents oxidative stress by scavenging ROS[37]. The decreased UCP-2 transcription might have increased ROS production, which could have impaired mitochondrial functions and elicited oxidative injuries in hepatic cells. The treatment of NAC significantly restored MnSOD and UCP-2 mRNA expressions in MCP-exposed animals, suggesting improved efficacy of the mitochondrial antioxidant system. The present study results are in accordance with previous studies where antioxidants significantly improved anti-oxidative potential at sub-cellular levels[38,39]. Furthermore, these findings also indicate that the anti-oxidative potential of NAC regulates MnSOD and UCP-2 expressions inside hepatic tissue.

Histology and ultrastructural microscopy are tools to identify structural changes that can validate the biochemical and physiological alterations in tissues and cells. Histological findings illustrated severe alterations in tissue structure, which are evidence of oxidative injuries and apoptosis that are supposed to decrease body and tissue weight. In a recent similar study, histological findings have also shown inflammation and apoptosis following MCP exposure and attenuation of structural damages on NAC supplementation in the liver tissue of rats[13]. Structural protection imparted by NAC administration can be correlated with improved body and tissue weight reported in the present study. The histological findings of the present study are in agreement with earlier investigations depicting the involvement of pesticide-generated oxidative stress in hepatotoxicity and the protective effects of antioxidants against pesticide exposure-induced degenerative effects[40,41]. Ultrastructural changes like mitochondrial disruption, change in nuclear shape, vacuolization, and loss of endoplasmic reticulum are direct shreds of evidence of apoptosis and down-regulation of mitochondrial defence interconnected with a decrease in body and tissue weight reported in the

present study. However, NAC treatment prevented loss of endoplasmic reticulum and mitochondrial disruption; maintained mitochondrial membrane and nuclear shape inside hepatic cells, indicating the protective potential of NAC against MCP exposure and are also consistent with results of weight parameters, western blot of the present study. Further ultra-structural findings are in concordance with the recent studies concerning apoptosis, inflammation and mitochondrial dysfunction in MCP-exposed rats and their pre-treatment with NAC[13,24].

## 5. Conclusion

The present study evaluated the protective efficacy of NAC against MCP exposure-mediated hepatotoxicity. In view of the present findings, it can be concluded that NAC has anti-oxidative and anti-apoptotic effects and decreases the toxic effects of MCP in hepatic tissue. Monocrotophos caused hepatotoxicity by apoptosis and oxidative stress, which appears to be primarily responsible for MCP-induced structural changes and depletion of body and liver weights. The protective efficacy of NAC is mediated by preventing apoptosis via inhibition of cytochrome c release and transcriptional up-regulation of UCP-2 and MnSOD. NAC's anti-apoptotic properties and modulatory effects might be responsible for maintaining weight and cellular structures. The protective effects of NAC make it a potential therapeutic agent for liver toxicity due to pesticide exposure. The finding suggests that relative levels of MnSOD, UCP-2 and localization of cytochrome c are crucial for pesticide-mediated oxidative stress that can also act as a potential indicator for cellular damage. Further, other molecular mechanisms should also be assessed to ascertain potential biomarkers of cellular functions and toxicity.

## 6. Acknowledgements

Author Jagjeet Singh is a Senior Research Fellow of CSIR-New Delhi, India. The authors are thankful to the All India Institute of Medical Sciences, New Delhi, India, for the electron microscopy.

## 7. References

1. Sarhan OMM, Al-Sahhaf ZY. Histological and biochemical effects of diazinon on liver and kidney of rabbits. Life Science Journal. 2011; 8(4):1183-1189.
2. Singh S, Kumar V, Kanwar R, Wani AB, Gill JPK, Ramamurthy PC, Singh J, Garg VK. Toxicity and detoxification of monocrotophos from ecosystem using different approaches: A review. Chemosphere. Elsevier; 2021; 130051. https://doi.org/10.1016/j.chemosphere.2021.130051 PMid:33676273
3. Chien MY, Yang CM, Chen CH. Organochlorine pesticide residue in Chinese herbal medicine. J Pestic Sci. 2022; 47(1):30-34. https://doi.org/10.1584/jpestics.D21-052. PMid:35414756 PMCid:PMC8931563
4. Phogat A, Singh J, Kumar V, Malik V. Toxicity of the acetamiprid insecticide for mammals: A review. Environ Chem Lett. 2022; 20(2):1453-1478. https://doi.org/10.1007/s10311-021-01353-1
5. Konradsen F. Acute pesticide poisoning-a global public health problem. Dan Med Bull. Citeseer. 2007; 54(1):58-59.
6. Lushchak VI, Matviishyn TM, Husak VV, Storey JM, Storey KB. Pesticide toxicity: A mechanistic approach. Excli J. 2018; 17:1101-1136. https://doi.org/10.17179/excli2018-1710.
7. Arora K. Toxic Effects of Monocrotophos (an organophosphate) on Histoarchitecture of Liver-Histopathological Studies. Int J Biotechnol Biochem. 2009; 5(4):445-450.
8. Sankhwar ML, Yadav RS, Shukla RK, Singh D, Ansari RW, Pant AB, Parmar D, Khanna VK. Monocrotophos induced oxidative stress and alterations in brain dopamine and serotonin receptors in young rats. Toxicol Ind Health. 2016; 32(3):422-436. https://doi.org/10.1177/0748233713500834. PMid:24105069
9. Devi S, Singh J, Kumar V, Malik V. Monocrotophos induced biochemical and histopathological alterations in the kidney tissues of Mice. Chem Biol Lett. 2019; 6(2):39-45.
10. Malik V, Singh J, Kumar A, Kumar V. Protective effect of coenzyme Q10 nanoparticles against monocrotophos induced oxidative stress in kidney tissues of rats. Biologia. 2021; 76:1849-1857. https://doi.org/10.1007/s11756-021-00732-x.
11. Loevinsohn M. Insecticide use and increased mortality in rural Central Luzon, Philippines. The Lancet. 1987; 329(8546):1359-1362. https://doi.org/10.1016/S0140-6736(87)90659-3. PMid:2884464
12. Hirshhorn N. Study of the occupational health of Indonesian farmers who spray pesticides. The Indonesian National IPM Program, FAO, Jakarta. 1993; 23:12-16.
13. Singh J, Phogat A, Prakash C, Chhikara SK, Singh S, Malik V, Kumar V. N-acetylcysteine reverses

monocrotophos exposure-induced hepatic oxidative damage via mitigating apoptosis, inflammation and structural changes in rats. Antioxidants (Basel). 2021; 11(1):90. https://doi.org/10.3390/antiox11010090. PMid: 35052593 PMCid:PMC8773366

14. Matsunaga K, Fukunaga S, Abe J, Takeuchi H, Kitamoto S, Tomigahara Y. Comparative hepatotoxicity of a herbicide, epyrifenacil, in humans and rodents by comparing the dynamics and kinetics of its causal metabolite. J Pestic Sci. 2021; 46(4):333-341. https://doi.org/10.1584/jpestics.D21-026. PMid:34908893 PMCid: PMC8640676
15. Yaduvanshi SK, Ojha A, Pant SC, Lomash V, Srivastava N. Monocrotophos induced lipid peroxidation and oxidative DNA damage in rat tissues. Pestic Biochem Phys. 2010; 97(3):214-222. https://doi.org/10.1016/j.pestbp.2010.02.004
16. Sunmonu TO, Oloyede OB. Monocrotophos-induced enzymatic changes as toxicity bio-markers in Wistar Rat liver. Agriculture and Biology Journal of North America. 2012; 2151:7525. https://doi.org/10.5251/abjna.2012.3.7.302.305
17. Karumuri SB, Singh H, Naqvi S, Mishra A, Flora SJS. Impact of chronic low dose exposure of monocrotophos in rat brain: Oxidative/nitrosative stress, neuronal changes and cholinesterase activity. Toxicol Rep. 2019; 6:1295-1303. https://doi.org/10.1016/j.toxrep.2019.11.005. PMid:31867220 PMCid:PMC6906705
18. Nagaraju R, Joshi AKR, Vamadeva SG, Rajini PS. Deregulation of hepatic lipid metabolism associated with insulin resistance in rats subjected to chronic monocrotophos exposure. J Biochem Mol Toxicol. 2020; 34(8):e22506. https://doi.org/10.1002/jbt.22506. PMid:32267039
19. Begum K, Rajini PS. Augmentation of hepatic and renal oxidative stress and disrupted glucose homeostasis by monocrotophos in streptozotocin-induced diabetic rats. Chem-Biol Interact. 2011; 193(3):240-245. https://doi.org/10.1016/j.cbi.2011.07.003. PMid:21821013
20. Elbini Dhouib I, Jallouli M, Annabi A, Gharbi N, Elfazaa S, Lasram MM. A minireview on N-acetylcysteine: An old drug with new approaches. Life Sci. 2016; 151:359-363. https://doi.org/10.1016/j.lfs.2016.03.003. PMid:26946308
21. Bavarsad Shahripour R, Harrigan MR, Alexandrov AV. N-acetylcysteine (NAC) in neurological disorders: mechanisms of action and therapeutic opportunities. Brain Behav. 2014; 4(2):108-122. https://doi.org/10.1002/brb3.208. PMid:24683506 PMCid:PMC3967529
22. Abdel-Daim MM, Dessouki AA, Abdel-Rahman HG, Eltaysh R, Alkahtani S. Hepatorenal protective effects of taurine and N-acetylcysteine against fipronil-induced injuries: The antioxidant status and apoptotic markers expression in rats. Sci Total Environ. 2019; 650:2063-2073. https://doi.org/10.1016/j.scitotenv.2018.09.313. PMid: 30290348
23. Ghafarizadeh A, Malmir M, Naderi Noreini S, Faraji T. Antioxidant effects of N-acetylcysteine on the male reproductive system: A systematic review. Andrologia. 2021; 53(1):e13898. https://doi.org/10.1111/and.13898
24. Singh J, Phogat A, Kumar V, Malik V. N-acetylcysteine ameliorates monocrotophos exposure-induced mitochondrial dysfunctions in rat liver. Toxicol Mech Methods. 2022; 1-29. https://doi.org/10.1080/15376516.2022.2064258. PMid:35403558
25. Ahmad I, Shukla S, Kumar A, Singh BK, Kumar V, Chauhan AK, Singh D, Pandey HP, Singh C. Biochemical and molecular mechanisms of N-acetyl cysteine and silymarin-mediated protection against maneb- and paraquat-induced hepatotoxicity in rats. Chem Biol Interact. 2013; 201(1-3):9-18. https://doi.org/10.1016/j.cbi.2012.10.027. PMid:23159886
26. Galal AAA, Ramadan RA, Metwally MMM, El-Sheikh SMA. Protective effect of N-acetylcysteine on fenitrothion-induced toxicity: The antioxidant status and metabolizing enzymes expression in rats. Ecotoxicol Environ Saf. 2019; 171:502-510. https://doi.org/10.1016/j.ecoenv.2019.01.004. PMid:30639957
27. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. J Biol Chem 1951; 193(1):265-275. https://doi.org/10.1016/S0021-9258(19)52451-6. PMid:14907713
28. Waite DT, Sommerstad H, Grover R, Kerr L, Westcott ND. Pesticides in ground water, surface water and spring runoff in a small Saskatchewan watershed. Environmental Toxicology and Chemistry: An International Journal. 1992; 11(6):741-748. https://doi.org/10.1002/etc.5620110603
29. Huang Y, Shi T, Luo X, Xiong H, Min F, Chen Y, Nie S, Xie M. Determination of multi-pesticide residues in green tea with a modified QuEChERS protocol coupled to HPLC-MS/MS. Food Chem. 2019; 275:255-264. https://doi.org/10.1016/j.foodchem.2018.09.094. PMid:30724195
30. Mansour SA, Mossa ATH. Oxidative damage, biochemical and histopathological alterations in rats exposed to chlorpyrifos and the antioxidant role of zinc.

Pestic Biochem Physiol. 2010; 96(1):14-23. https://doi.org/10.1016/j.pestbp.2009.08.008

31. Sunmonu TO, Oloyede OB. Performance and haematological indices in rats exposed to monocrotophos contamination. Hum Exp Toxicol. 2010; 29(10):845-850. https://doi.org/10.1177/0960327110363441. PMid:20194576
32. Wani WY, Gudup S, Sunkaria A, Bal A, Singh PP, Kandimalla RJL, Sharma DR, Gill KD. Protective efficacy of mitochondrial targeted antioxidant MitoQ against dichlorvos induced oxidative stress and cell death in rat brain. Neuropharmacology. 2011; 61(8):1193-1201. https://doi.org/10.1016/j.neuropharm.2011.07.008. PMid:21784090
33. Kaur P, Radotra B, Minz R, Gill K. Impaired mitochondrial energy metabolism and neuronal apoptotic cell death after chronic dichlorvos (OP) exposure in rat brain. NeuroToxicology. 2007; 28(6):1208-1219. https://doi.org/10.1016/j.neuro.2007.08.001. PMid:17850875
34. Li N, Ragheb K, Lawler G, Sturgis J, Rajwa B, Melendez JA, Robinson JP. DPI induces mitochondrial superoxide-mediated apoptosis. Free Radic Biol Med. 2003; 34(4):465-477. https://doi.org/10.1016/S0891-5849(02)01325-4. PMid:12566072
35. Zhu Y, Kalen AL, Li L, Lehmler HJ, Robertson LW, Goswami PC, Spitz DR, Aykin-Burns N. Polychlorinated-biphenyl-induced oxidative stress and cytotoxicity can be mitigated by antioxidants after exposure. Free Radic Biol Med. 2009; 47(12):1762-1771. https://doi.org/10.1016/j.freeradbiomed.2009.09.024. PMid:19796678 PMCid:PMC2785439
36. Allam A, Abdeen A, Devkota HP, Ibrahim SS, Youssef G, Soliman A, Abdel-Daim MM, Alzahrani KJ, Shoghy K, Ibrahim SF, Aboubakr M. N-Acetylcysteine Alleviated the Deltamethrin-Induced Oxidative Cascade and Apoptosis in Liver and Kidney Tissues. Int J Environ Res Public Health. 2022; 19(2):638. https://doi.org/10.3390/ijerph19020638. PMid:35055458 PMCid:PMC8775898
37. Prakash C, Soni M, Kumar V. Biochemical and Molecular Alterations Following Arsenic-Induced Oxidative Stress and Mitochondrial Dysfunction in Rat Brain. Biol Trace Elem Res. 2015; 167(1):121-129. https://doi.org/10.1007/s12011-015-0284-9. PMid:25764338
38. Soni M, Prakash C, Sehwag S, Kumar V. Protective effect of hydroxytyrosol in arsenic-induced mitochondrial dysfunction in rat brain. J Biochem Mol Toxicol. 2017; 31(7). https://doi.org/10.1002/jbt.21906. PMid:28225195
39. Alhusaini A, Fadda LM, Ali HM, Hasan IH, Ali RA, Zakaria EA. Mitigation of acetamiprid-induced renotoxicity by natural antioxidants via the regulation of ICAM, NF-kB and TLR 4 pathways. Pharmacol Rep. 2019; 71(6):1088-1094. https://doi.org/10.1016/j.pharep.2019.06.008. PMid:31629938
40. Ahmad A, Kumari P, Ahmad M. Apigenin attenuates edifenphos-induced toxicity by modulating ROS-mediated oxidative stress, mitochondrial dysfunction and caspase signal pathway in rat liver and kidney. Pestic Biochem Physiol. 2019; 159:163-172. https://doi.org/10.1016/j.pestbp.2019.06.010. PMid:31400778
41. Firouzian F, Pourshoja P, Nili-Ahmadabadi A, Ranjbar A. Hepatoprotective effect of N-acetylcystein loaded niosomes on liver function in paraquat-induced acute poisoning. Pestic Biochem Physiol. 2019; 160:146-153. https://doi.org/10.1016/j.pestbp.2019.08.001. PMid:31519249