N-Acetylcysteine Attenuates Monocrotophos-induced Toxicity by Mitigating Oxidative Stress and Structural Changes in Rat Liver

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Abstract

The present study evaluated the effect of N-acetylcysteine (NAC) against sub chronic monocrotophos (MCP) exposure induced oxidative stress in rat liver. Albino wistar rats were divided into control, NAC treated, MCP and MCP treated groups. An oral dose of MCP (0.9 mg/kg b.wt) and NAC (200 mg/kg b.wt) was administered for 28 days. We observed high oxidative stress generation on MCP exposure in liver tissue as evident by significant increase in lipid peroxidation, protein oxidation and decreased glutathione content followed by altered activities of superoxide dismutase, catalase and acetylcholinesterase. Sub chronic MCP exposure caused an array of cellular and structural alternations in lipids and proteins of liver tissue as depicted by the FTIR, histopathological and electron microscopic analysis. N-acetylcysteine attenuated the loss of glutathione and prevented lipid peroxidation and protein oxidation. Pre-treatment of NAC also restored histological and ultra space structural alternations. So NAC protects oxidative stress and tissue damage induced by sub chronic MCP exposure in rat liver; suggesting the therapeutic and antioxidant potential of NAC.

Introduction

Monocrotophos (MCP) insecticide is used to control sucking and chewing pests of fruits and vegetables. Despite its restricted to use label, it is used extensively in various crops like rice, cotton, soybeans, sugarcane, maize, groundnut, etc. (Kumar et al. 2018; Dash and Osborne 2020). Due to its wide spread use, MCP possesses major environmental risk factors for nematodes, arthropods, fishes and mammals including humans. Monocrotophos has been detected in water, soil, as well as in agriculture products (Waite et al. 1992). Exposure of MCP and its residues is known to induce hepatotoxicity (Arora 2009), nephrotoxicity (Malik et al. 2021), neurotoxicity (Sankhwar et al. 2016), genotoxicity (Mahboob et al. 2002) in mammals and *in vitro* toxicity (Siddiqui et al. 1992). The toxic effects of MCP exposure are attributed to the synergic effects of acetylcholinesterase (AChE) inhibition and generation of reactive oxygen species (ROS).

The liver has long been considered as major target organ of MCP exposure and its metabolites thus it is mostly affected by MCP intoxication (Hanafy et al. 1991). Studies have demonstrated that MCP exposure induces morphological and physiological changes in liver (Yaduvanshi et al. 2010; Begum and Rajini 2011). The disturbance of balance between the degree of ROS generation and antioxidant capabilities is a key factor for MCP-induced hepatotoxicity. In fact, MCP-exposure has been reported to alter the enzymatic as well as non enzymatic antioxidants inside the liver suggestive of liver oxidative stress (Yaduvanshi et al. 2010). Monocrotophos mediated oxidative stress generation is associated with increased lipid peroxidation, protein oxidation, decreased antioxidant enzymatic activity and reduced glutathione content in liver (Desai et al. 1984; Siddiqui et al. 1990; Yaduvanshi et al. 2010; Begum and Rajini 2011). Chronic MCP exposure also mediates structural changes including degeneration of hepatocytes, fatty and hydropic changes, vacuolization, fibrosis, congestion and infiltration of red blood cells in rat liver tissue (Arora 2009).

Antioxidants are chemical compounds capable of inducing the normal response of a biological system to reduce chemical-induced toxicity. Antioxidants primarily include natural extracts of plants and animals having chemically active groups such as thiols, polyphenols, isoquinolines, etc. These compounds are assumed to protect the biological system from oxidative stress by scavenging free radicals resulting from metabolites of synthetic chemical pesticides. N-acetylcysteine (NAC) is a thiol-containing reducing agent and possess many pharmaceutical characteristics such as anti-apoptotic, anti-oxidative, anti-inflammatory and anti-neoplastic properties (Malik et al. 2007; Rakshit et al. 2009; Wang et al. 2009; Jiao et al. 2016; Mokhtari et al. 2017). N-acetylcysteine has been reported to exert an array of ameliorative pharmacological effects against pesticide exposure mediated hepatic-renal toxicity, pulmonary toxicity, neurotoxicity and reproductive toxicity (Shohrati et al. 2008; Falluel-Morel et al. 2012; Abdel-Daim et al. 2019; Owumi et al. 2021). In the literature survey, no study has reported the role of NAC against MCP-induced chronic hepatotoxicity. Further due to the persistence of MCP in fruits and vegetables, humans are susceptible to long-term exposure even if the contamination is below acceptable limits. Thus, the present study was designed to evaluate the effects of NAC against MCP-induced chronic biochemical alteration and structural changes in liver tissue of Wistar rats.

Materials And Methods

Chemicals

Monocrotophos and NAC were purchased from Sigma-Aldrich, St Louis, USA. 5,5-dithiobis-2-nitrobenzoic acid (DTNB), Thiobarbituric acid (TBA), Ethylene diamine tetraacetic acid (EDTA) 2,4-dinitrophenylhydrazine (DNPH), and Trichloroacetic acid (TCA) were from Sisco Research Laboratory, Mumbai, India. Glassware and plastic ware used in the study were autoclaved and sterilized before use.

Animals and their care

Albino rats of Wistar strain weighing 150-180 g were procured from the approved animal house source and kept in polyvinyl carbon cages in well-ventilated room. All protocols related to rats experiments were according to the guidelines of the Committee for Control and Supervision of Experiments on Animals, India. Permission to use of animals was duly approved by the Institutional Animal Ethical Committee.

Experimental Design

A total of 20 rats were randomly assigned to the following four groups containing 5 rats in each group:

1. **Control group:** Rats received an equal volume of distilled water (vehicle) through oral gavage for 28 days, as administered to the rats of the MCP-treated group.
2. **N-acetylcysteine treated group:** Rats received NAC (200 mg/kg b.wt) mixed in distilled water through oral gavage for 28 days.
3. **Monocrotophos-treated group:** Rats received MCP (0.9 mg/kg b.wt, i.e. 1/20th of LD₅₀) dissolved in distilled water through oral gavage for 28 days.

4. **Monocrotophos + NAC treated group:** Rats received MCP (0.9 mg/kg b.wt) dissolved in distilled water through oral gavage and NAC (200 mg/kg b.wt) mixed in water through oral gavage.

Dose and time of NAC treatment was based on previous studies that have shown the selected dose significantly effective against stress conditions (Ahmad et al. 2013; Galal et al. 2019). Dose of MCP was selected based on previous studies that have reported the selected dose to cause hepatic toxicity in rats (Nagaraju et al. 2020).

Rats were euthanized via carbon dioxide asphyxiation after last dose administration. Liver tissue was dissected out, washed in 0.9% ice-cold normal saline and was used for preparation of tissue homogenate. A part of liver tissue was used for histology and electron microscopy also.

Acetylcholine esterase (AChE) activity assay

Acetylcholine esterase activity in serum and liver tissue homogenate was estimated using method of Ellman et al. (1961). The reaction mixture was read at 412 nm for 2 min. Results were expressed as nmol product formed/min/mg protein.

Estimation of lipid peroxidation

Liver tissue homogenate was used to calculate lipid peroxidation following method of Wills (1971). Proteins were precipitated using TCA and supernatant was mixed with 0.67% TBA followed by placing it in boiling water bath to develop colour. The amount of TBARS formed was determined and used to express peroxidation of lipids as nmol malondialdehyde (MDA)/mg protein.

Estimation of protein carbonylation

The protein carbonyl in liver tissue homogenate was quantified after derivatization with DNPH (Levine et al. 1990). The protein carbonyl content was calculated results were expressed as nmol carbonyl/mg protein.

Antioxidant enzyme assays

Catalase (CAT) activity in liver homogenate was assayed by monitoring the enzyme-catalyzed degradation of H_2O_2 at 240 nm for 3 min (Johansson and Borg 1988). Results were expressed as $\mu\text{mole H}_2\text{O}_2$ decomposed/min/mg protein.

Superoxide dismutase (SOD) activity in liver homogenate was determined by measurement of the nitrobluetetrazolium reduction (Sun et al. 1988). The reaction mixture was read at 560 nm for 3 min. Results were expressed as U/mg protein.

Glutathione (GSH) estimation

Glutathione content in liver tissue homogenate of rats was estimated using DTNB as a substrate (Ellman 1959). The yellow colour developed was read immediately at 412 nm and the results were expressed as nmol/mg protein.

Fourier Transforms Infrared (FTIR) Analysis

Liver tissue samples were prepared for FTIR as described earlier (Akkas et al. 2007) and spectra was recorded in the region $4000 - 400 \text{ cm}^{-1}$ on an FTIR spectrometer (Alpha, Bruker Optics, Ettlingen, Germany). The obtained spectra was further analyzed in two separate regions ($3800 - 2700 \text{ cm}^{-1}$ and $1800 - 1400 \text{ cm}^{-1}$) using ORIGIN® 19 software (Origin Lab Corporation, Massachusetts, USA)

Histopathology study

For histological analysis dissected liver tissues were stored in 70% ethanol after 24 h of fixation in formalin followed by dehydration in different grades of alcohol. After dehydration, the fixed tissues were embedded in paraffin wax after saturating with xylene and wax. Tissue sections ($5 \mu\text{m}$ thickness) were cut using microtome and fixed on slides using egg albumin and heat. Slides were dipped in xylene to deparaffinise the tissue sections and processed for Hematoxyline (stain nucleus) and Eosin (stain cytoplasm), and Van Geison (stain connective tissues) staining; and mounted with Dibutylphthalate Polystyrene Xylene and cover glasses. Slides were observed under the Nikon microscope and were photographed.

Electron microscopy

For electron microscopy tissues of the liver ($1-2 \text{ mm}^2$) were fixed in 2.5% glutaraldehyde and post-fixation storage was done in phosphate buffer at room temperature. After primary fixation tissues were fixed in secondary fixative (osmium tetroxide) and 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 ultra-microtome. Prepared sections were stained and examined under transmission electron microscope at Sophisticated Analytical Instrumentation Facility, All India Institute of Medical Science in New Delhi, India.

Protein Determination

Protein content in each sample was determined using bovine serum albumin as standard (Lowry et al. 1951).

Statistical analysis

All data was analyzed for statistically significance with help of one-way analysis of variance (ANOVA) followed by Tukey's post hoc test. Results with values for $p \leq .05$ were considered as statistically significant.

Results

Acetylcholine esterase activities

Significant depletion of AChE activities in serum and liver tissue was observed in animals exposed to MCP insecticides as compared to control rats. Pre-treatment of NAC with MCP for 28 days significantly elevated both serum and liver AChE activities as compared to MCP administrated group, while NAC alone treated group showed no significant change in AChE activity both in serum and liver tissues compared to control rats (Fig 1 & 2).

Lipid peroxidation

Monocrotophos exposure for 28 days significantly caused peroxidation of lipids in rats as compared to the control group. Administration of NAC to the MCP treated group significantly prevented lipid peroxidation as compared with MCP group. N-acetylcysteine alone treatment showed no significant change in lipid peroxidation of liver tissue as compared with control groups (Fig 3).

Protein Oxidation

Protein oxidation was significantly increased in MCP exposed rats. In contrast, prior administration of NAC to MCP-treated animals significantly declined protein oxidation as compared to those of MCP intoxicated animals while NAC alone treatment showed no significant change in protein oxidation as compared to those of the control group (Figure 4).

Antioxidant enzyme activities

Monocrotophos exposure for 28 days significantly decreased the activities of SOD and CAT as compared to the control group. Pre treatments of NAC to MCP intoxicated animals significantly restored both SOD and CAT activities as compared to MCP alone exposed group. Administration of NAC alone showed no significant changes in antioxidant enzyme activities as compared to the control group (Table 1).

Glutathione content

Monocrotophos exposure significantly decreased the level of GSH content as compared to control rats. Treatment of NAC prior to MCP exposure significantly increased GSH content as compared to MCP exposed rats. Control and NAC alone administered group showed comparable GSH content (Table 1).

Fourier Transforms Infrared analysis of lipids and proteins

The FTIR absorption spectra between 4000 cm^{-1} to 400 cm^{-1} was analysed for protein and lipid structure in rat liver. Second derivative FTIR spectra was studied in two different regions i.e. $3800\text{-}2750\text{ cm}^{-1}$ and $1800\text{-}1400\text{ cm}^{-1}$ (Fig 5 & 6). The peak assignment (Table 3) shows changes in lipids and proteins among control and treated groups. As shown in Table 2, the peaks appeared at 3297 and 3080 cm^{-1} arise from N-H and =CH stretching of proteins (Amide A & B regions). Table 3 shows that there was a significant decrease in band area of N-H stretch of Amide A and B following MCP exposure. Olefinic acid band

observed at 3014 cm^{-1} mainly arise due to C-H stretching on HC=CH groups was also found to be decreased. Peak assigned to CH_3 asymmetrical ($\sim 2959\text{ cm}^{-1}$), CH_2 asymmetric ($\sim 2925\text{ cm}^{-1}$) and CH_2 symmetric ($\sim 2854\text{ cm}^{-1}$) stretch of lipids were also reduced significantly in area in MCP-exposed group (Table 3). Slight change in peak positions was also observed for these regions (Table 2). The treatment of NAC to MCP- exposed group restored the area of the peaks representing lipid and protein structure in the region ($3700\text{-}2750\text{ cm}^{-1}$) towards control values (Table 3).

In $1800\text{-}1400\text{ cm}^{-1}$ region (Fig 6), sub chronic MCP exposure significantly affected the peak area of C=O stretch ($\sim 1652\text{ cm}^{-1}$) of amide A; N-H and C-N stretch ($\sim 1541\text{ cm}^{-1}$) of Amide B (Table 3). Treatment of MCP also caused significant decrease in peak area of C=O stretch ($\sim 1745\text{ cm}^{-1}$), $-\text{CH}_2$ bend ($\sim 1456\text{ cm}^{-1}$) and COO- stretch ($\sim 1397\text{ cm}^{-1}$) of lipids and fatty acids (Table 3). Slight shifts in peak positions of these regions were observed in MCP exposure group (Table 2). Administration of NAC to MCP exposure group significantly prevented the structural alterations corresponding to proteins and lipids caused by MCP exposure, as evident from restoration of altered peak position and area values (Table 3).

Histopathological analysis

Haematoxylin and Eosin staining of liver tissue sections of rats exposed to MCP for 28 days showed histopathological abnormalities as compared to the control group. Control tissues exhibited normal architecture of bile duct, portal triads and hepatocyte. Hexagonal hepatocytes were arranged radiating from the central vein, separated by blood sinusoids with uniform cytoplasm. Monocrotophos exposure caused edema, sinusoidal disruption and dilation of the portal triad and central vein. Focal necrosis, inflammation, apoptosis in the hepatocyte, hyperchromatic nuclei were observed in MCP exposed rat liver sections. Administration of NAC effectively normalised the onset of histopathological changes caused by MCP exposure as evident by absence of focal necrosis, hyperchromatic nuclei, and apoptotic damage in hepatocyte, inflammation, edema and architecture. N-acetylcysteine only treated group showed normal histological architecture as that of the control group (Fig 7a-d and Table 4).

Van Geison's stain is used for differential staining of collagen and other connective tissues and detects fibrosis in tumours, and other pathological conditions. Van Geison's staining of liver sections of rats exposed to MCP showed hepatic necrotic plaques along with fibrotic generations. Replication of collagen fibres and degeneration of smooth muscles of the central vein was detected in MCP exposed liver tissues. In the group pre treated with NAC histopathological analysis exhibited a significant reduction in fibrotic generation and necrotic plaques as compared to MCP exposed group. Replication of collagen fibres and smooth muscle degeneration were reduced after pre-treatment of NAC to MCP exposed group. Control and NAC only treated group showed normal connective tissue arrangements in hepatic tissue sections (Fig 8a-d and Table 5).

Transmission Electron Microscopy

Monocrotophos exposed liver tissue sections showed marked alterations in nuclear structure as compared to controls tissue sections. Ovulated and hyperchromatic nucleus and break in the nuclear envelope were observed as major alterations in MCP exposed rats. Pre-treatment of NAC to MCP exposed rats' maintained normal spherical nucleus and intact nuclear envelope as compared to MCP exposed animal tissue sections. Control and NAC treated group showed normal shape and size of the nucleus and nuclear envelop (Fig 9a-d and Table 6).

Discussion

Monocrotophos is a class I highly toxic organophosphate pesticide. Despite its restricted to use a label, its unplanned application in agriculture is responsible for environmental exposure to humans and mammals. The residue of MCP have been reported in fruits, vegetables, dairy products, soil and water(Leuthold 2013; Mishra 2013; Kumar et al. 2014; Singh et al. 2021). Monocrotophos exposure is known to induce oxidative stress, reduction of antioxidant levels and causes apoptosis that could lead to multi-organ failure and genetic alterations(Velmurugan et al. 2013; Sankhwar et al. 2016; Mandal et al. 2020). Recent studies have suggested liver as the prime targeted organ for pesticide related toxicity(Yilmaz et al. 2017; Milošević et al. 2018; Refaie et al. 2020). N-acetylcysteine is precursor of amino acid and natural source of sulfhydryl group(Dean et al. 2011; Bhardwaj and Saraf 2017). N-acetylcysteine has also shown effective protection against paraquat acute poisoning(Firouzian et al. 2019), deltamethrin mediated in-vitro cytotoxicity(Romero et al. 2012) and against fipronil induced hepatic injuries(Abdel-Daim et al. 2019). Based on the strong antioxidant potential of NAC against pesticide toxicity, we also evaluated the antioxidant potential of NAC against sub-chronic MCP exposure mediated hepatotoxicity in rats. The major finding of the study indicated the antioxidant properties of NAC; oral pre-treatment of NAC for 28 days ameliorated oxidative stress and restored antioxidant levels by regulating enzymatic activities in hepatic tissue and protected tissue damage.

Monocrotophos is a well-known AChE inhibitor and causes cholinergic toxicity inside the body. The present study shows that MCP exposure causes decline of AChE activity both in serum and liver homogenate. Inside body, MCP is readily bio-transformed to reactive metabolites which block esteratic site of acetylcholine esterase and inhibit AChE activity. Previous studies have also shown AChE inhibition in mammalian tissues following MCP exposure(Dwivedi et al. 2010; Joshi and Rajini 2012). Pre-treatment of NAC to MCP exposure recovered tissue and serum AChE level. Our results are in agreement with the findings of previous study that has reported AChE recovery on NAC treatment in carbofuran toxicity in rats(Kamboj et al. 2008). The present study reports non cholinergic effect of NAC against MCP induced oxidative stress in mammals.

Oxidative stress is a central mechanism of tissue injury. Oxidative stress arises when the critical balance between oxidants and antioxidants is disrupted inside the body(Oldham and Bowen 1998; Birben et al. 2012; Pisoschi and Pop 2015). The disruption between oxidants and antioxidants could be attributed to free radical accumulation or antioxidants depletion or both. These free radicals may lead to further biotransformation and attack lipids and proteins' side chains. Quantification of lipid peroxidation and

protein oxidation is a tool to investigate oxidative stress-mediated toxicity(Suntres 2002; Tarantino et al. 2009). In the present study, MCP exposure for 28 days was observed to cause lipid peroxidation and protein oxidation in rat liver that was significantly reversed by NAC administration along with MCP. The effects of NAC against MCP-induced damage to lipids and proteins were also evident in FTIR findings that indicate possible change to lipid and proteins structural backbone. Reduction in band area and shift in band position of 3297 cm^{-1} , 3080 cm^{-1} , 1652 cm^{-1} and 1540 cm^{-1} are indicative of structural rearrangement of proteins inside cell. It is well known that CH_2 stretching band at 2854 cm^{-1} is indicator of “state of order” of biological membrane(Kartheek and David 2018). Decrease in band area at 2854 cm^{-1} confirmed MCP mediated alteration in biological membrane and mobility of fatty acids. Further, changes in CH_2 asymmetric and CH_2 symmetric stretching of lipids also depicts conformational disorder and lipid acyl chain flexibility. Alteration in olefinic $=\text{CH}$ band area at 3014 cm^{-1} indicates change in quantity of unsaturated lipids. The study shows that the decrease in peak of the amide I and II were consistent with the decline in amide A band area. Any change in the position of amide bands or decrease in peak area of amide bands may quantitatively reflect alterations in composition of protein secondary structure. This may be attributed to the increased protein oxidation and peroxidation of lipids indicating reduction in free radical scavengers due to MCP exposure in rat liver as evident by biochemical and histological studies also. The administration of NAC along with MCP effectively ameliorated these structural and molecular alterations in lipids and proteins. The results further support the various previous *in vitro* studies showing that NAC can directly scavenge free radicals(Custódio et al. 2002; Sadegh Soltan-Sharifi et al. 2007; Kamboj et al. 2008).

Oxidative stress due to toxic insults is also attributed to alteration of cellular antioxidants such as SOD and CAT(Kaur and Sandhir 2006; Lasram et al. 2014). SOD and CAT are the main enzymes that assist the elimination of oxidants formed during the bio-activation of pesticides and function as a first-line of defense against oxidative stress(Turkmen et al. 2019). These enzymes convert superoxide radicals and peroxides radicals into water and oxygen, thus removes ROS(Bhattacharjee and Sil 2006). In relation to this, we found decrease in SOD and CAT activities in rat liver suggesting decreased scavenging of free radicals. The depletion of cellular antioxidants following MCP exposure has also been reported by various studies in liver tissue(Rao 2006; Yaduvanshi et al. 2010; Begum and Rajini 2011). The depletion of antioxidant enzyme activity reported in this study could be tracked to their overutilization in quenching free radicals produced during bio-activation of MCP. N-acetylcysteine-induced augmentation of CAT and SOD activities in the MCP exposed group could be a result of reduced lipid peroxidation and protein oxidation. The nucleophilic and oxidant quenching properties of NAC could have played a role in the positive concomitant loop manner by reducing ROS generation. Being an antioxidant, NAC treatment might have prevented the overutilization of CAT and SOD and could have restored their activity.

Intracellular GSH is a thiol-containing non-enzymatic antioxidant and plays pivotal role in detoxification and metabolism of both endogenous and exogenous substances(Eraslan et al. 2009). It is an important index of cellular antioxidant status and also a biomarker to detect oxidative stress(Yurumez et al. 2007). The present study shows that MCP exposure was associated with severe depletion of cellular GSH levels.

The decline of GSH content is in concordance with various *in vivo* and *in vitro* studies that have implicated GSH depletion in MCP-induced toxicity(Siddiqui et al. 1990; Rao 2006; Dwivedi et al. 2010). Further, NAC has been reported to increase cellular GSH levels both *in vivo* and *in vitro* studies against pesticides-induced oxidative stress in rat tissues(Mostafalou et al. 2012; El-Bini Dhouib et al. 2015; Galal et al. 2019). We also observed significant augmentation of GSH content by prior oral administration of NAC to the MCP exposed group. In fact, NAC is a source of cysteine that is a precursor of de novo GSH synthesis(Sadowska et al. 2007). After the incorporation of NAC into cells, NAC is readily deacylated to deliver intact cysteine for GSH generation. Further NAC has direct antioxidant properties that might have reduced free radicals and have protected GSH from oxidation.

The histopathological and electron microscopy assessment of liver tissue further confirmed the biochemical and structural changes following MCP exposure for 28 days. The findings of the present study showed noticeable degenerative and ultra structural changes in liver sections which are direct shreds of evidence to oxidative damage and apoptosis reported in the study. Ultra structural changes like nuclear membrane breakage, shrinking of nucleus and constriction of chromatids as seen in our study are testimony to MCP-induced apoptosis and lipid damages. Further, the intact nuclear membrane and proper shape and size of nucleus in NAC pre-treatment group marked its anti-apoptotic and antioxidant potential against MCP toxicity. Histopathological findings of the present study are in agreement with previous studies that depicted oxidative stress as the main vehicle for MCP-mediated toxicity in liver tissue(Karami-Mohajeri et al. 2017). Administration of NAC prior to MCP exposure reduced histopathological alternations. Histological restoration of liver architecture by NAC administration to MCP treated group indicates its anti-inflammatory and antioxidant potential. The ameliorative potential can be mainly traced to its antioxidant properties(Abdel-Daim et al. 2019).

The findings of the present study suggest that NAC significantly ameliorates oxidative stress-mediated MCP hepatotoxicity in rats. Reduction of lipid peroxidation, protein oxidation and augmentation of antioxidant enzymes is a major protective evidence of NAC against MCP exposure. In addition, the results depicted that NAC also attenuates pathogenesis, apoptosis and ultra structural anomalies of the liver. The ameliorative potential of NAC and other potent antioxidants is still obscure and more scientific approach via *in vivo* and *in vitro* investigation is warranted to identify the molecular mechanism and their effectiveness.

Declarations

Ethics approval: The study was duly approved by Institutional Animal Ethical Committee (Approval No. 210-19).

Consent to participate: Not applicable

Consent for publication: Not applicable.

Availability of data and materials: Not applicable.

Competing interest: Not applicable.

Funding: Not applicable.

Authors' contribution: All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Jagjeet Singh, Annu Phogat and Vijay Kumar. The first draft of the manuscript was written by Jagjeet Singh. Visualization and validation were completed by Chandra Prakash and Vijay Kumar. Vinay Malik and Vijay Kumar reviewed and edited the final draft. All authors read and approved the final manuscript.

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Tables

Table 1 Effects of NAC on MCP exposure induced alterations in hepatic antioxidant status of rats

Group	SOD (U/mg protein)	Catalase (μ mole H ₂ O ₂ decomposed /min/mg protein)	GSH (nmole/mg protein)
Control	8.73 $\pm$ 0.35	50.80 $\pm$ 4.82	41.66 $\pm$ 4.08
NAC	8.41 $\pm$ 0.60	52.17 $\pm$ 4.60	43.73 $\pm$ 2.77
MCP	5.44 $\pm$ 0.51 ^{a ***}	28.20 $\pm$ 3.56 ^{a ***}	25.61 $\pm$ 2.03 ^{a ***}
NAC+MCP	7.03 $\pm$ 0.37 ^{b ***}	40.20 $\pm$ 5.07 ^{b ***}	36.94 $\pm$ 2.01 ^{b ***}

The values represented are mean $\pm$ S.D. (N=5). ^a as compared to control group; ^b as compared to MCP treated group. *** p<0.001, significant different

Table 2 Description of frequency assignment of FTIR spectra in rat liver

Wave number cm ⁻¹				Peak assignment
Control	NAC	MCP	NAC+MCP	
3297	3297	3294	3296	N-H stretch of proteins: mainly Amide A
3080	3081	3077	3080	N-H stretch of proteins: mainly Amide B
3014	3014	3012	3014	Olefinic=C-H stretch: unsaturated lipids
2959	2961	2952	2957	CH ₃ asymmetric stretching: mainly lipids
2925	2926	2921	2923	CH ₂ asymmetrical stretching: mainly lipids
2854	2854	2852	2854	CH ₂ symmetric stretch: lipids
1745	1744	1738	1742	Ester C=O stretch: lipids
1652	1653	1649	1652	C=O stretch of proteins: Amide I
1541	1541	1537	1540	N-H bend, C-N stretch of proteins: Amide II
1456	1458	1451	1456	CH ₂ bend: lipid and protein
1397	1398	1393	1396	COO ⁻ symmetric stretch: fatty acids

Table 3 Effect of NAC on peak area values of FTIR spectra in hepatic tissue of rat exposed to MCP for 28 days

Peak position	Experiment groups			
	Control	NAC	MCP	NAC+MCP
3297 cm ⁻¹	170.33 ± 3.42	169.95 ± 2.96	108.00 ± 4.07 ^{a ***}	163.05 ± 3.17 ^{b ***}
3080 cm ⁻¹	17.97 ± 1.35	18.44 ± 1.73	12.46 ± 0.93 ^{a ***}	16.73 ± 1.12 ^{b ***}
3014 cm ⁻¹	2.53 ± 0.35	2.69 ± 0.41	1.77 ± 0.14 ^{a ***}	2.44 ± 0.27 ^{b ***}
2959 cm ⁻¹	9.49 ± 0.86	9.53 ± 0.94	07.70 ± 1.01 ^{a *}	08.72 ± 0.78 ^{b *}
2925 cm ⁻¹	14.08 ± 0.61	14.14 ± 0.53	11.25 ± 0.76 ^{a **}	12.58 ± 0.62 ^{b **}
2854 cm ⁻¹	20.70 ± 0.77	21.07 ± 0.87	14.67 ± 1.12 ^{a ***}	18.88 ± 0.68 ^{b ***}
1745 cm ⁻¹	01.53 ± 0.15	01.62 ± 0.12	0.93 ± 0.12 ^{a ***}	01.37 ± 0.08 ^{b ***}
1652 cm ⁻¹	29.96 ± 1.73	29.12 ± 1.23	14.68 ± 1.52 ^{a ***}	25.28 ± 1.28 ^{b ***}
1540 cm ⁻¹	17.41 ± 1.27	16.77 ± 1.02	09.01 ± 1.36 ^{a ***}	14.89 ± 1.11 ^{b ***}
1456 cm ⁻¹	04.39 ± 0.38	04.58 ± 0.59	02.38 ± 0.37 ^{a ***}	03.49 ± 0.45 ^{b ***}
1397 cm ⁻¹	05.69 ± 0.72	05.87 ± 0.44	03.23 ± 0.60 ^{a ***}	04.48 ± 0.19 ^{b ***}

The values represented are mean ± S.D. (N=3). ^a as compared to control; ^b as compared to MCP exposed group. * p<0.05; ** p<0.01; *** p<0.001, significantly different

Table 4 Description of histopathological alterations in different groups of the study (N=3)

Parameters	Control	NAC	MCP	NAC+MCP
Central vein degeneration	-	-	++++	+
Sinusoidal space broadening	-	-	++++	+
Macrophage Infiltration	-	-	+++	+
Focal Hepatocellular necrosis	-	-	++	+
Edema	-	-	++++	+
Hepatocyte apoptosis	-	-	+++++	+
Portal triad dilation	-	-	+++	-

Table 5 Description of connective tissue alterations in different groups of the study (N=3)

Parameters	Control	NAC	MCP	NAC+MCP
Focal necrosis plaques	-	-	+++++	++
Replication of collagen fibers	-	-	+++	+
Fibrotic generation	-	-	+++++	++
Degeneration of smooth muscles in the central vein	-	-	++++	+

Table 6 Ultra structural assessment of hepatic cells in different groups of the study (N=3)

Parameters	Control	NAC	MCP	NAC+MCP
Ovulated Nucleus	-	-	+++++	++
Breaks in Nuclear Envelop	-	-	+++++	+
Hyperchromatic Chromatids	-	-	++++	++
Glycogen Particles	-	+	++++	++

Figures

Figure 1

Effect of NAC on MCP exposure induced alteration of AChE activity in serum of the different experimental groups. The values represented are mean $\pm$ S.D. (N=5). a as compared to control group; b as compared to MCP treated group. *** $p < 0.001$, significant different

Figure 2

Effect of NAC on MCP exposure induced alteration of AChE activity in liver homogenate of liver homogenate of the different experimental groups. The values represented are mean $\pm$ S.D. (N=5). a as compared to control group; b as compared to MCP treated group. *** $p < 0.001$, significant different

Figure 3

Effect of NAC on MCP exposure induced lipid peroxidation in rat liver tissue homogenate. The values represented are mean $\pm$ S.D. (N=5). a as compared to control group; b as compared to MCP treated group.

*** $p < 0.001$, significant different

Figure 4

Effect of NAC on MCP exposure induced protein oxidation in rat liver tissue homogenate. The values represented are mean $\pm$ S.D. (N=5). a as compared to control group; b as compared to MCP treated group.

*** $p < 0.001$, significant different

Figure 5

Effect of NAC on MCP exposure induced structural alterations as represented by FTIR spectra of rat liver in region 3700 to 2750 cm^{-1} . The spectra are representative of $N = 3$

Figure 6

Effect of NAC on MCP exposure induced structural alterations as represented by FTIR spectra of rat liver in region 1800 to 1200 cm^{-1} . The spectra are representative of N = 3

Figure 7

Histopathological examination of liver tissues stained with H & E (200X) (a) Control group; (b) NAC group; (c) MCP group; (d) NAC+MCP group; depicting Apoptosis (narrow arrow), Edema (filled triangle), Sinusoidal broadening (bold arrow) and central vein degeneration (rhombus). CV - Central Vein; BD - Bile Duct; HA - Hepatic Artery; Mif - Macrophage Infiltration

Figure 8

Photomicrographs of liver tissue of experimental groups stained with Van-Geison stain (200X) (a) Control group; (b) NAC group; (c) MCP group; (d) NAC+MCP group; depicting the fibrotic generation (narrow arrow), collagen fibre replication (bold arrow), smooth muscle degeneration (star), focal necrosis plaques (asterside). CV - Central Vein, PT - Portal Triad, HA - Hepatic Artery

Figure 9

Transmission Electron Microscopy images to represent ultra structure of hepatocytes nuclei in rat (a) Control group; (b) NAC group; (c) MCP group; (d) NAC+MCP group