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LETTERS

Monocrotophos induced Biochemical and Histopathological alterations in the Kidney tissues of Mice

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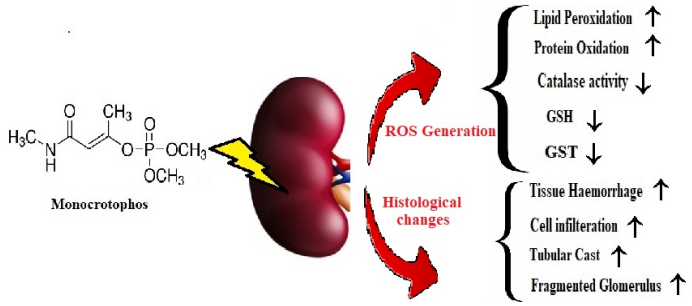
ABSTRACT



The present study investigated the effect of monocrotophos, a commonly used organophosphate pesticide exposure in the kidney tissues of the swiss albino mice. Monocrotophos was administered at the sub-lethal doses of 1.25mg/kg, 2.5 mg/kg and 5.0 mg/kg body weight for 24 hr. Monocrotophos toxicity generated oxidative stress in the mice as evidenced by significant decrease in the activities of glutathione, superoxide dismutase and catalase enzymes. The exposure increased the lipid peroxidation and protein oxidation in a dose dependent manner. Oxidative stress generation also elicited cytotoxic effects on the mice kidney which were supported by the histopathological changes like degeneration in glomerulus, bowmen's capsule and tubules, hemorrhage, mononuclear cell infiltration, tubular cast and congested blood vessels in a dose-dependent manner. In conclusion, the study indicated that monocrotophos exposure at various doses induces significant deleterious health effects in mice kidney tissues via oxidative stress generation.

Keywords: Monocrotophos, histopathology, oxidative stress, kidney, lipid peroxidation.

INTRODUCTION

Pesticides are the agrochemicals used to control the insects and weeds population associated with variety of crops. The usage of pesticides is aimed to improve the crop yields to meet the food requirements of drastically increasing population of the world.[1] Despite advanced scientific efforts to obviate pesticide use, agriculture is still indispensably dependent on these chemicals.[2] The situation becomes worse by the indiscriminate use of these toxic chemicals, resulting in the environmental pollution and toxicity of the various non target organisms.[3] Continuous use of pesticides has the adverse effects on health of target as well as non target organisms through their carcinogenic, mutagenic and teratogenic properties.[4-7] Organophosphate pesticides are used extensively both in developing and developed countries resulting annual exposure of 2–3 million people. These adversely affect almost all the organs including liver, kidney, intestine, lungs, gonads and brain.[8,9] These pesticides induce oxidative stress and imbalance antioxidant enzymes level by directly inhibiting their activity.[10,11]

Monocrotophos (MCP) (Figure 1) is a systemic organophosphate pesticides used widely on variety of crops and in animal husbandry.[12] It is a highly hazardous pesticide resulting in serious consequence of accidental or intentional fatal poisoning. Monocrotophos has been shown to cause the histopathological, genotoxic, hyperglycemic and stressogenic effects by inhibiting the cholinesterase activity.[13-15] It may cross the cell membrane easily due to its lipophilic nature and induces oxidative stress by the production of reactive oxygen species resulting in lipid peroxidation and DNA damage.[16] Progressive

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studies on MCP toxicity in different organisms have shown the morphological and cellular alterations and even genetic toxicity.[17,18] A study by Yaduvanshi et al. (2010) has shown a dose-dependent increase in lipid peroxidation in the liver, kidney, spleen and brain of MCP exposed rats indicating that it is toxic to almost all the body organs.[19]

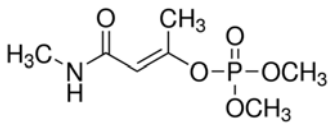

Figure 1. Chemical structure of Monocrotophos

It has also been shown to induce somatic and germ cells mutation through structural and numerical chromosomal aberrations in bone marrow, spleen and spermatocytes cells in mouse.[20,21] Various organophosphate pesticides including MCP have been studied extensively for their toxic effects leading to oxidative stress and DNA damage in different tissues of human and other organisms. However, constraints in toxicity data collection and variability in action and activity of individual pesticides with different manifestations in different living systems makes it necessary to screen pesticides repeatedly for their harmful effects. Moreover there are limited acute studies published on mechanistic toxicity studies of MCP exposure on kidney tissues. Therefore, the present study investigated the mechanistic aspects of MCP induced nephrotoxicity in the swiss albino mice at various sub-lethal doses.

Material and Methods

Chemicals

Monocrotophos, 5,5-dithiobis-2-nitrobenzoic acid (DTNB), bovine serum albumin (BSA), thiobarbituric acid (TBA), and nitro blue tetrazolium (NBT) were obtained from Sigma-aldrich, St Louis, USA. 2,4-dinitrophenylhydrazine (DNPH), Tris (hydroxymethyl) aminomethane, folin-ciocalteau reagent, ethylenediamine tetracetic acid (EDTA) were from Sisco Research Laboratoty, Mumbai, India; rest of all the chemicals were of analytical grade. Glass redistilled water was used throughout the study.

Experimental animals

Swiss albino male mice (*Mus musculus*) of 8-12 weeks old weighing 20-25 g were procured from the approved small animal house source. The mice were housed in polypropylene cages and kept in well ventilated rooms. Mice were provided standard rat pellet diet and water *ad libitum*. Ethical clearance for killing of mice was duly obtained from the Institute Animal Ethical Committee.

Experimental design

A total of 20 mice were used and randomly divided into four groups containing 5 animals in each. The group I of mice was given a constant volume of 0.2 ml distilled water and served as control. Mice of II, III and IV groups were given 1.25mg, 2.5mg, and 5mg of MCP/kg body weight orally; equivalent to 12.5%, 25% and 50% of the reported LD_{50} (10 mg/kg body weight) of MCP.[22] All the mice were sacrificed after 24 hr of administration of doses. Kidney tissues were removed, rinsed in physiological saline and processed for histopathological and biochemical studies. Kidney tissues were homogenized (10% w/v) in ice-cold sodium phosphate buffer (pH 7.4) in a glass dounce homogenizer. The homogenate was centrifuged at 5000 rpm for 10 min at 4°C, and the resultant supernatant was used for different biochemical assays.

Lipid peroxidation

The lipid peroxidation was assessed by the method of Wills (1966) .[23] Briefly, kidney tissue homogenate (0.5 ml) was mixed with TCA (Trichloroacetic acid) (10%, w/v) and reaction mixture was centrifuged at 1000 rpm for 10 minutes. To 1.5 ml supernatant, 1.5 ml TBA (0.67% w/v) was added and colour was developed by placing the tubes in a boiling water bath. The amount of TBA reactive substance was determined and values were then expressed as equivalent nmol of malondialdehyde (MDA)/mg protein.

Protein oxidation

Quantization of protein carbonyl as an index of protein oxidation in the kidney tissues were determined after derivatization with DNPH according to the procedure of Stadtman and Levine (2003) .[24] Briefly, kidney homogenate was mixed with10 mM DNPH in 2.5 M HCl and incubated in dark for 60 minutes at room temperature. The mixture was vortexed and 20% TCA was added to it followed by washing with ethanol/ethyl acetate (1:1 v/v) mixture. Precipitated proteins were then re-dissolved in 6 M guanidine-HCl and absorbance of the supernatant was read at 370 nm. The amount of protein carbonyl was calculated on the basis of molar extinction coefficient of DNPH (0.022 μM^{-1} cm^{-1}). The results were expressed as nmol carbonyl/mg protein.

Catalase activity assay

Catalase activity was assayed by enzyme-catalyzed decomposition of H_2O_2 at 240 nm. The decrease in absorption was recorded at 240 nm and the amount of H_2O_2 decomposed was calculated on the basis of the molar extinction coefficient of H_2O_2 (43.1×10^{-9} nM^{-1} cm^{-1}). The results were expressed as µmol H_2O_2 oxidized/min/mg protein.

Superoxide dismutase activity assay

Superoxide dismutase (SOD) activity was measured according to the method as described by Kono (1978). The method is based on the inhibitory effect of SOD on the reduction of NBT by the superoxide anions generated by the photo-oxidation of hydroxylamine hydrochloride. Decrease in the absorbance at 560 nm was recorded. The amount of enzyme required to produce 50% inhibition was considered as 1 unit of SOD activity, and results were expressed as U/mg protein.

Glutathione (GSH) estimation

Glutathione was estimated by the method of Ellman (1978) using DTNB as a substrate.[25] The yellow color development was read immediately at 412 nm and the results were expressed as mg GSH/g tissue.

Histopathological studies

Kidney tissues were removed after killing of mice, washed with normal saline and preserved in 10% neutral formalin for 24 hr. After fixation, tissues were washed under tap water for 2 hr to remove fixative followed by dehydration in ascending grades of alcohol. Tissues were cleared in xylene and embedded in paraffin wax. The sections of kidney tissues were cut with the thickness of 5-7µm by using a semiautomatic microtome. Deparaffinization of sections were done with xylene then stained with Hematoxylin (nuclear stain) and Eosin (cytoplasmic stain) and mounted with Dibutylphthalate Polystyrene Xylene (DPX). Slides were observed under Nikon light microscope at 40X and microphotographed.

Total Protein content estimation

Total protein content in each tested sample was measured by following the method of Lowry et al. (1951) using BSA as standard.[26]

Statistical analysis

The results are expressed as mean ± SD. To measure statistical significance, data was analyzed through one-way ANOVA. Results were considered significant at p-value ≤ 0.05. Statistical analysis was done by using "IBM SPSS statistics 20" version of SPSS.

Results and Discussion

Monocrotophos induced morphological changes

The acute MCP exposure for 24 hr produced various morphological symptoms in mice such as skin itching, redness of skin, lacrymation, reduced physical activities followed by lethargic behaviour within a few minutes (30-40 minutes) of exposure to monocrotophos.

Lipid peroxidation

Lipid peroxidation indicates the effect of free radicals on lipids. Significant increase in lipid peroxidation was observed in dose dependent manner as evident by high MDA levels in kidney homogenate of mice after 24 hr of MCP exposure. The levels of MDA increased by 25%, 60% and 80% in mice treated with 1.25 mg/kg, 2.5 mg/kg and 5.0 mg/kg doses of MCP respectively (Figure 2).

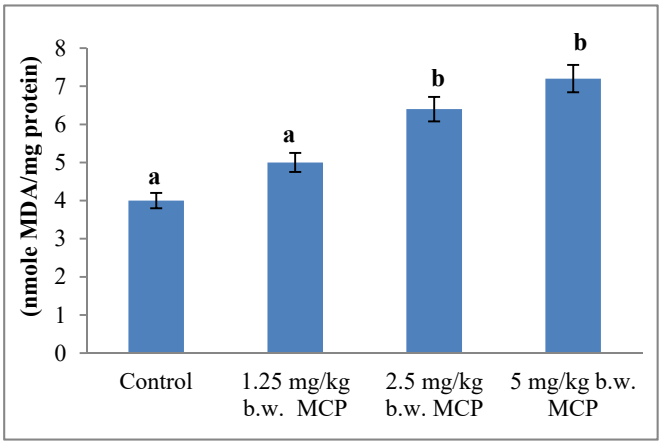

Figure 2. Effect of MCP exposure on lipid peroxidation in mice kidney homogenate. The values represented are mean ± S.D. (N=5). The bars labeled with 'b' only are significantly different from control.

Protein oxidation

Protein carbonyls are the markers of protein oxidation, so we measured the protein carbonyls in kidney tissues. Similar to lipid peroxidation, a significant increase in protein carbonyls content was observed in kidney homogenate of mice after 24 hr MCP exposure. Protein carbonyls level increased by 29.41%, 52.94% and 76.47% in mice treated with 1.25 mg/kg, 2.5 mg/kg and 5.0 mg/kg doses of MCP respectively (Figure 3).

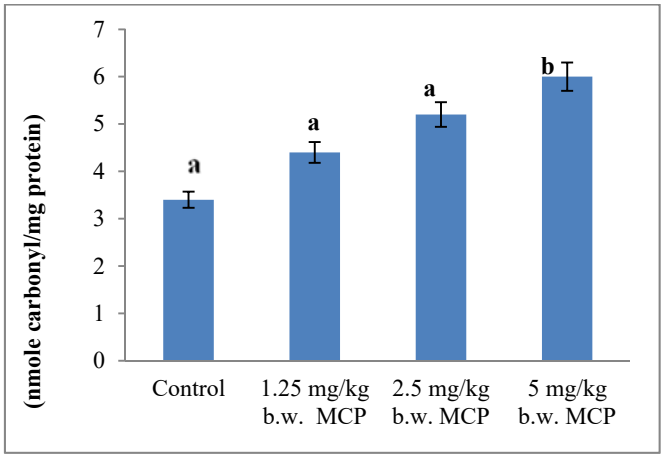

Figure 3. Effect of MCP exposure on protein oxidation in mice kidney homogenate. The values represented are mean ± S.D. (N=5). The bars labeled with 'b' only are significantly different from control.

Catalase activity

Monocrotophos exposure significantly decreased the activity of catalase by 20.38%, 52.86% and 72.58% in kidney homogenate of mice treated with 1.25 mg/kg, 2.5 mg/kg and 5.0 mg/kg doses of MCP respectively for 24 hr (Figure 4).

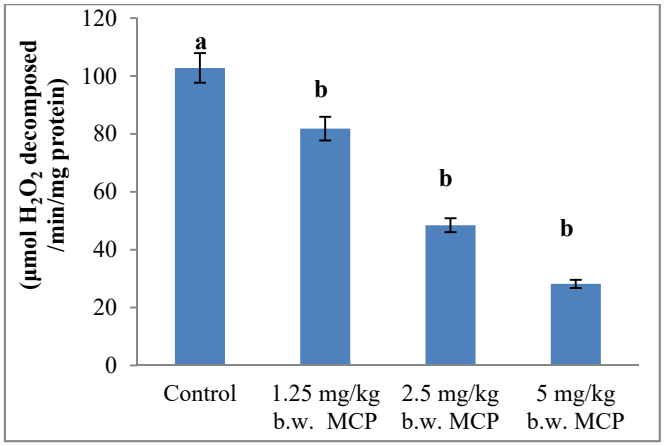

Figure 4. Effect of MCP exposure on catalase activity in mice kidney homogenate. The values represented are mean ± S.D. (N=5). The bars labeled with 'b' only are significantly different from control.

Superoxide dismutase activity

Superoxide dismutase activity was also decreased significantly by 16.58%, 37.52% and 55.56% in kidney homogenate of mice treated with 1.25 mg/kg, 2.5 mg/kg and 5.0 mg/kg doses of MCP respectively (Figure 5).

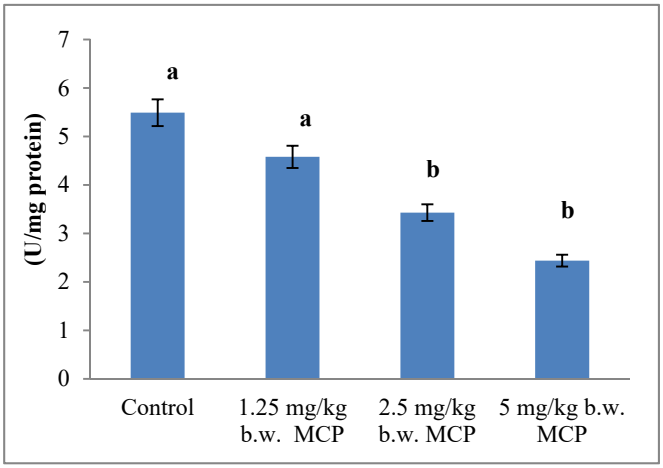

Figure 5. Effect of MCP exposure on SOD activity in mice kidney homogenate. The values represented are mean ± S.D. (N=5). The bars labeled with 'b' only are significantly different from control.

Glutathione (GSH) level

Glutathione level was decreased in dose dependent manner in MCP exposure groups. There was significant decrease of GSH by 17.73%, 48.29% and 65.57% in kidney homogenate of mice treated with 1.25 mg/kg, 2.5 mg/kg and 5.0 mg/kg doses of MCP respectively (Figure 6).

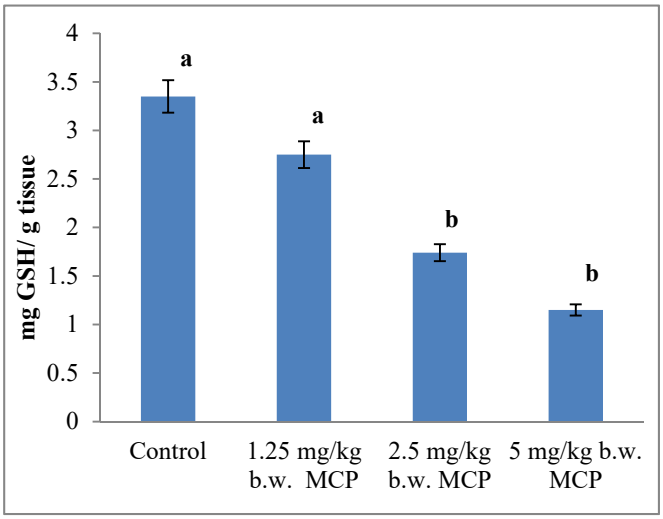

Figure 6. Effect of MCP exposure on GSH content in mice kidney homogenate. The values represented are mean ± S.D. (N=5). The bars labeled with 'b' only are significantly different from control.

Histopathological alterations in mice kidney

Histopathological changes were observed in mice kidney of MCP treated groups in a dose dependant manner (Table 1).

Table 1: Summary of histopathological alterations induced after monocrotophos exposure in the kidney of swiss albino mice control mice.

Sr. no.	Concentration of Dose (mg/kg b.w.)	Control (0.2 ml DW)	1.25 mg/kg b.w.	2.5 mg/kg b.w.	5.0 mg/kg b.w.
1.	Glomerular degeneration	–	+	++	+++
2.	Tubular degeneration	–	+	++	+++
3.	Haemorrhage	–	+	++	+++
4.	Infilteration	–	+	++	+++
5.	Hydropic changes	–	–	+	++
6.	Widening of capsular space	–	+	++	+++
7.	Tubular cast	–	–	++	+++
8.	Necrosis	–	–	++	+++
9.	Fibrosis	–	–	+	++

(–) = none, (+) = mild, (++) = moderate, (+++) = severe

Histological studies of kidney from control group of mice showed the normal arrangement of bowmen's capsule with glomerulus and renal corpuscles, renal tubules including proximal convoluted tubules and distal convoluted tubules as shown in Figure 7.

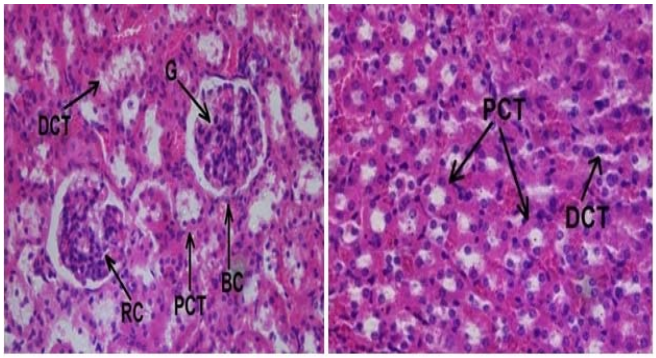

Figure 7: Photomicrographs of a section of kidney (H and E, ×400) of control mice. Section depicts normal arrangement of bowmen's capsule (BC) with glomerulus (G) and renal corpuscles (RC), proximal convoluted tubules (PCT) and distal convoluted tubules (DCT).

The histopathological studies of the mice kidney showed dose dependent effect on the different tissues sections as indicated by observed images. The mice treated with 1.25 mg/kg of MCP showed mild damage in kidney tissues including glomerular degeneration with slightly fragmented glomerulus, tubular degeneration (widening of tubular lumen), haemorrhage, cell infilteration (Figure 8).

The mice treated with 2.5 mg/kg of MCP showed moderate damage in kidney tissues including glomerular degeneration with more fragmented glomerulus and widening of capsular space, mononuclear cell infilteration, more haemorrhage, tubular cast (sloughed off cells in tubular lumen) showing necrosis, tubular degeneration with more dilated tubules as shown (Figure 9).

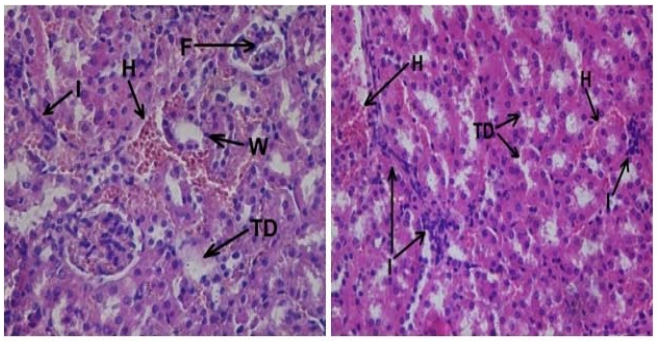

Figure 8: Photomicrographs of a section of kidney (H and E, ×400) treated with 1.25 mg/kg body weight MCP. Section shows glomerular degeneration with fragmentation (F), tubular degeneration (TD), widening of tubular lumen (W), haemorrhage (H), infilteration (I).

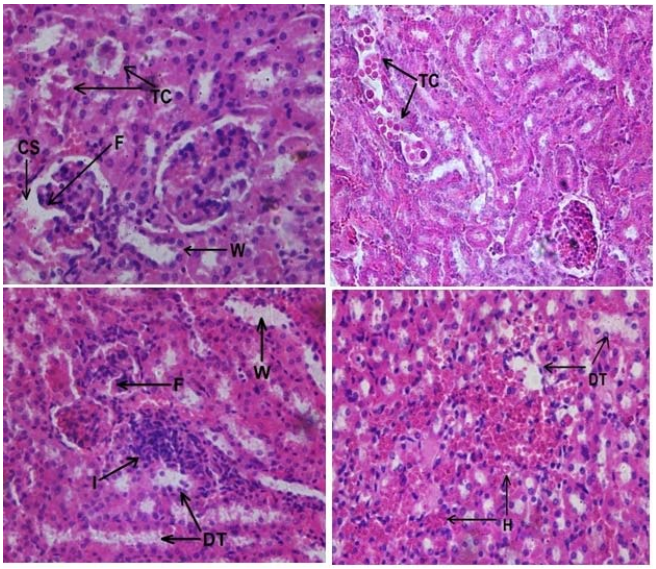

Figure 9: Photomicrographs of a section of kidney (H and E, ×400) treated with 2.5 mg/kg body weight MCP. Section shows more glomerulated degeneration with fragmentation (F), widening of capsular space (CS), infilteration (I), more intertubular haemorrhage (IH), tubular cast (TC) showing necrosis, degenerated tubules (DT) with widening of tubular lumen (W).

The mice treated with 5.0 mg/kg body weight of MCP showed severe damage in kidney tissues including fragmented and shrinked glomerulus, more widening of capsular space, more haemorrhage, tubular cast indicated necrosis, highly degenerated tubules, infiltration and fibrosis as shown in (Figure 10).

Pesticides serve as an economic tool to control various pests and were used intentionally as a strategic planning in the green revolution in India to increase agricultural yield. But because of regular and extensive use these chemicals contaminate environment and are highly toxic to non target organisms. Organophosphates are group of pesticides being used extensively globally. The toxicity of organophosphate results in enhanced oxygen free radicals which causes detrimental effects on the cellular bio molecules leading to manifestation of DNA damage and oxidative stress. Monocrotophos is one of the most widely used organophosphate pesticide used against pests throughout the world. It is classified as extensively hazardous pesticide in India. Progressive research studies have proven MCP as an extremely toxic pesticide that affects target as well as non-target organisms. Various studies have been done on toxicity of MCP in lower organisms (invertebrates) and some vertebrates such as aquatic organisms and birds which depicts toxic effects of MCP exposure. However, limited literature is present on organ specific toxicity of MCP in mammalian system. Kidney tissues are active and strong detoxifying organ in mammalian system which plays crucial role in detoxification and biotransformation processes i.e. metabolism and excretion of xenobiotics.[17,27] The present study thus executed to evaluate the toxic effects of MCP exposure on mice kidney.

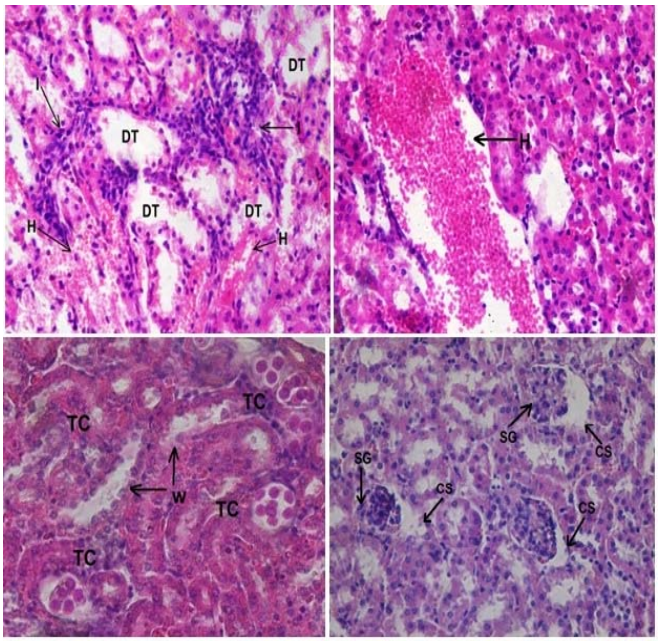

Figure 10: Photomicrographs of a section of kidney (H and E, ×400) treated with 5 mg/kg body weight MCP. Section depicts highest damage with shrunken glomerulus (SG) with more widening of capsular space (CS), more haemorrhage (H), tubular cast (TC) showing necrosis, highly degenerated tubules (DT) with more widening of tubular lumen (W) and, infiltration (I).

There is paucity of literature that explains relation between MCP exposure and oxidative stress in kidney tissues. Lipid peroxidation and protein oxidation are the strong biomarker of oxidative stress. Lipid peroxidation was measured by increase in the level of MDA and protein oxidation was measured in terms of protein carbonyl levels in kidney. The increased lipid peroxidation and protein carbonylation in present study demonstrate generation of oxidative stress in mice kidney after MCP exposure. Monocrotophos induced lipid peroxidation has also been demonstrated in liver, spleen, kidney and brain tissues of rats.[7] The antioxidant enzymes like catalase and SOD provide the first line of defense against oxidative stress. There was decrease in the activity of catalase and SOD enzymes in a dose dependent manner following MCP exposure for 24 hr in mice.

The decrease in activity of these enzymes clearly indicated the increased oxidative stress in kidney of mice. Although many studies have shown antioxidant imbalance in various tissues following MCP exposure;[28, 29] few more studies have provided the evidence of alteration of antioxidant enzymes in mammalian kidney tissues. Reduced GSH is the principle antioxidant responsible for detoxification of free radicals and prevention of oxidative damage. The present study also showed reduced GSH following MCP exposure in a dose dependent manner indicating oxidation of GHS due to free radicals generated by oxidative stress. Earlier, MCP exposure has been shown to markedly enhance the lipid peroxidation in kidney tissues as well as alteration of antioxidant enzymes in both liver and kidney of diabetic rats.[30]

Cytotoxicity in kidney tissues was measured through histopathology which is a sensitive biomarker and cost effective tool to detect the chemical effects in targeted tissues of organisms and provide additional information to physicochemical analysis.[31] Results showed glomerular degeneration with fragmentation, tubular degeneration, haemorrhage, infilteration, widening of tubular lumen of mice kidney treated with 1.25 mg/kg body weight of MCP for 24 hr. Kidney of mice treated with 2.5 mg/kg body weight of MCP for 24 hr showed more glomerulated degeneration with fragmentation, widening of capsular space, infiltration, more intertubular congestion and haemorrhage, tubular cast (necrosis), degenerated tubules with widening of tubular lumen. The mice kidney treated with 5 mg/kg body weight of MCP for 24 hr showed highest damage with fragmented glomerulus, more widening of capsular space, more congestion and haemorrhage, tubular cast (necrosis), highly degenerated tubules, infiltration and fibrosis. All the histological alterations were induced in a dose dependant manner in treated groups in comparison to control group in which intact tissues were observed. The results of present study are in concordance with various previous studies conducted with various organophosphates pesticides. A similar study has shown the infiltration in mononuclear cells and dilation in renal tubules at low dose of MCP exposure while vascular dilation, dilated Bowman's space and glomerular atrophy, edema, necrosis and calcification was noticed in kidney of rats after acute MCP exposure.[16] Similarly genotoxicity and cytotoxicity in different tissues of rats and mice was observed after organophosphates exposure.[32,33] Malathion has been shown to induce nephrotoxicity and hepatotoxicity generating oxidative stress and histopathological changes in rat tissues.[34] Significant damage was observed in liver and kidney tissues of rat after Fenthion treatment.[35,36] Lesions within proximal and distal tubules were observed in the kidneys of female wistar rats exposed to deltamethrin.[37]

Conclusion

The findings of present study indicate that an acute exposure to MCP can induce oxidative stress and histopatological alterations in kidney tissues of mice in a dose dependent manner. On the basis of results, it is suggested that monocrotophos exposure might cause hazardous and deleterious effect on mammalian system including man. It might also affect environment adversely. The present study will provide the more mechanistic understanding of acute toxicity of MCP exposure on kidney tissues; that will help in remodelling safety guidelines of this pesticide and help in creating awareness among the farmers about toxicity of MCP.

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Conflict of Interest

The authors declare no conflict of interest.

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