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Cadmium and Chlorpyrifos Induced Lipid Peroxidation in Brain, Liver and Kidney of Wistar Rats

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Abstract

The work was designed with 4 groups for 28 days. 1: Control. 2: CdCl2 @ 22.5 mg/kg b.wt/oral. 3: CPF @ 25 mg/kg b.wt/per oral. 4: CdCl2@22.5 mg + CPF @ 25 mg/kg b.wt/per oral. Higher mean values of liver, kidney and brain TBARS were observed in G-2, 3 and 4 on 15th and 29th day. Liver section in group 2 revealed mild degenerative changes in group 3; in group 4 mild to moderate peri portal fibrosis. Kidney section in group 2 showed shrunken glomeruli, necrosis; kidney section showed degeneration and necrosis of tubular epithelium with casts in the lumen in group 3 and necrosis with casts in the lumen in group 4. Brain section in group 2 showed mild perivascular cuffing, mild to moderate degeneration of Purkinji cells in group 3 and in group 4 degeneration of Purkinje cells. Effects in G-4 were severe than individual groups due to synergistic action of the combined pollutants than the individual effects.

1. Introduction

Cadmium (Cd) and Chlorpyrifos (CPF) are the most common components among all toxic compounds present in the environment. Cadmium (Cd) is a heavy metal and a major environmental toxicant. The general population is exposed to Cd via contaminants found in drinking water and food, while occupational exposure usually takes place during mining or manufacturing of batteries and pigments. Increased concentration of Cd in agricultural soil comes

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from the application of phosphate fertilizers, sewage sludge and pesticides[1]. Long term exposure of Cd leads to lipid peroxidation, induction of Reactive Oxygen Species (ROS) and DNA damage resulted by mitochondrial dysfunction of the cells[2]. Pesticides have played a vital role in controlling agricultural, industrial, home and public health pests worldwide[3]. Environmental pollution from pesticides is an important issue that attracts wide spread public concern. Among them, some organophosphate and organochlorine pesticides are routinely used in agriculture. However, their use poses animal and human health concerns because of their toxicity, widespread use and release into the environment. Chlorpyrifos (CPF) is one of the most heavily used organophosphate pesticide in domestic and agricultural operations by the farmers[3]. The principal mechanism of toxicity of CPF is due to its inhibition of Acetylcholinesterase (AChE) and accumulation of Acetyl Choline (ACh) at the nerve endings and the neuromuscular junctions[4]. CPF has been postulated to have multiple effects on the target cells including generation of reactive oxygen species and induction of intracellular oxidative stress thereby disrupting normal cellular development and differentiation[5]. Environment receives combination of multiple intoxicants through environment contamination. There is need for conducting induced toxicopathological studies to assess the impact of individual and combined environmental pollutants[6]. The present work was designed to evaluate the effects of combined cadmium and chlorpyrifos induced lipid peroxidation in rats.

2. Materials and Methods

2.1 Experimental Design

Forty-eight (48) Male Wistar albino rats were procured from Sanzyme Laboratories Ltd., Hyderabad, animals were divided into 4 groups, 12 animals in each group. Rats were randomly divided into 4 groups consisting of 12 in each group. Group 1 serves as control. Group 2 rats were administered Cadmium chloride ($CdCl_2$) @ 22.5mg/kg b.wt/per oral/day. Group 3 rats were administered Chlorpyrifos (CPF) @ 25 mgkg b.wt/per oral/day. Group 4 rats were administered Cadmium chloride (Cd) @22.5 mg + Chlorpyrifos (CPF) @ 25 mg/kg b.wt/per oral/day for 28 days of experiment.

2.2 Drugs and Chemicals

$CdCl_2$ was procured from Thermo Fisher Scientific India Pvt. Ltd. Mumbai. Chlorpyrifos was procured from Coromandel Fertilizers Pvt. Ltd. Vishakapatnam.

2.3 Estimation of Lipid Peroxidation

Liver, kidneys and brain were removed, trimmed and washed with ice cold physiological saline solution and then weighed. Tissue homogenate (10%) was prepared in ice cold phosphate buffered saline for estimation of Thiobarbituric Acid Reacting Substances (TBARS)[7].

2.4 Histopathology

Detailed necropsy was conducted at the end of the experiment and gross changes were noticed, if any. Pieces ofliver, kidneys and brain were collected in 10% Neutral Buffer Formalin (NBF). Samples were processed, sectioned (5 µm), stained with Hematoxylin and Eosin (H and E) as per the standard protocol[8].

2.5 Statistical Analysis

Data obtained were subjected to statistical analysis by applying one way ANOVA using Statistical Package for Social Sciences (SPSS) version 16.0. Differences between means were tested by using Duncan's multiple comparison tests and significance level was set at $P<0.05$[9].

3. Results

3.1 Lipid Peroxidation Marker

There was a significant ($P<0.05$) increase in mean values of liver, kidney and brain Thiobarbituric Acid Reacting Substances–Malondialdehyde (TBARS-MDA) in groups 2, 3 and 4 compared with group 1 on 15th and 29th day of the experiment respectively. The TBARS-MDA levels were significantly higher in group 4 than the groups 2 and 3 on 15th and 29th day of the experiment (Table 1).

3.2 Histopathological Observation in Liver, Kidney and Brain

Section of liver in group 2 revealed severe congestion, dilation of sinusoids, haemorrhages and mild degenerative changes in hepatocytes (Figure 1A); severe congestion, vacuolar degeneration and loss of architecture in group 3 (Figure 1B); in group 4 liver revealed severe congestion with mild to moderate peri portal fibrosis (Figure 1C). Section of kidney in group 2 revealed swollen to shrunken

Table 1. Liver, kidneys and brain thiobarbituric acid reacting substances concentration **(TBARS- n moles of MDA/mg of tissue) in different groups**

GROUP	Liver TBARS		Kidney TBARS		Brain TBARS	
	DAY 15	DAY 29	DAY 15	DAY 29	DAY 15	DAY 29
G1	3.51±0.09^{c}	3.77±0.05^{d}	3.72±0.16^{c}	4.15±0.24^{d}	2.38±0.12^{d}	2.54±0.11^{d}
G2	7.43±0.07^{b}	9.07±0.08^{b}	7.71±0.18^{b}	9.14±0.15^{b}	5.39±0.17^{c}	6.58±0.14^{c}
G3	7.25±0.06^{b}	8.22±0.12^{c}	7.41±0.17^{b}	8.85±0.21^{c}	7.27±0.15^{b}	8.32±0.11^{b}
G4	9.06±0.07^{a}	10.33±0.09^{a}	10.13±0.21^{a}	11.49±0.24^{a}	8.34±0.11^{a}	9.50±0.16^{a}

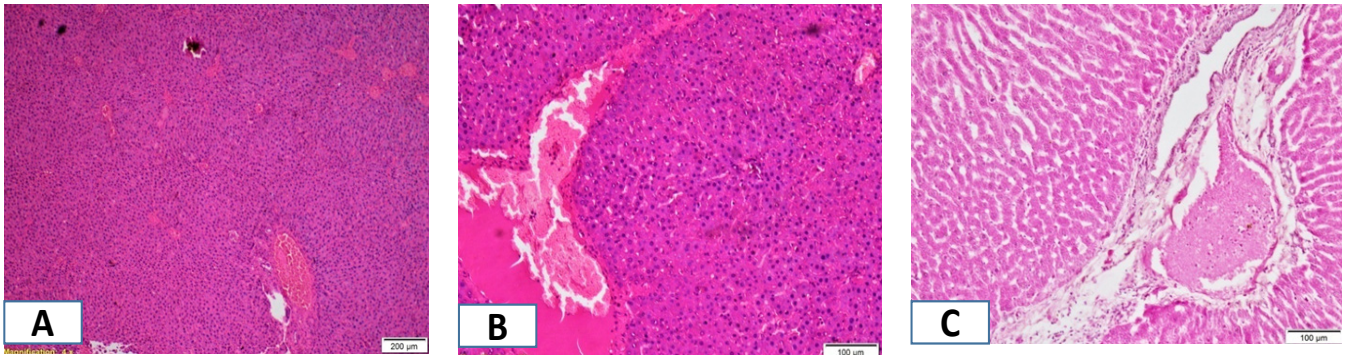


Figure 1. - **(A)** Photomicrograph of G-2 liver showing severe congestion, dilation of sinusoids, haemorrhages and mild degenerative changes in hepatocytes (H and E; 40X). **(B)** Photomicrograph of G-3 liver showing severe congestion, vacuolar degeneration and loss of architecture (H and E;100X). **(C)** Photomicrograph of G-4 liver showing severe congestion with mild to moderate peri portal fibrosis (H and E; 100X).

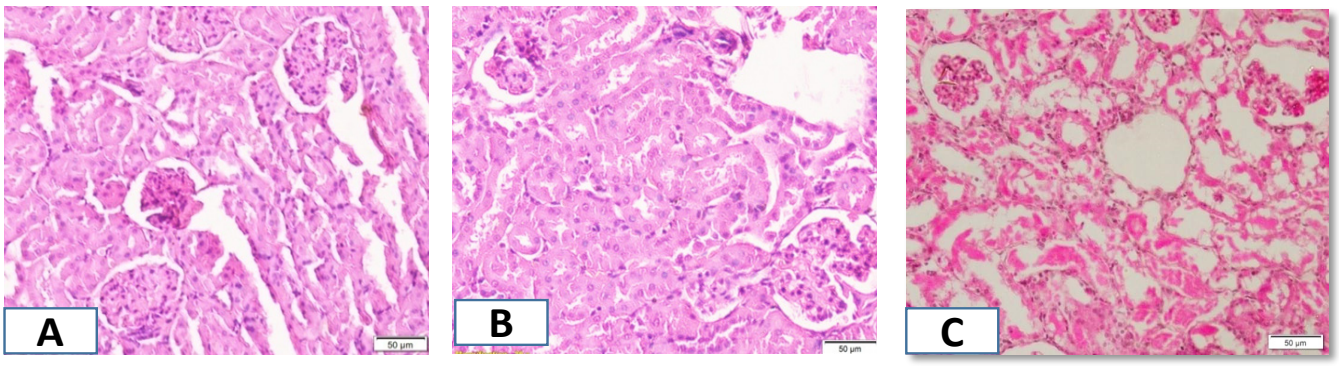


Figure 2. - **(A)** Photomicrograph of G-2 kidney showing swollen to shrunken glomeruli, necrosis of epithelial cells and increased glomerular space, necrosis and disintegrated tubules and glomeruli (H and E;100X). **(B)** Photomicrograph of G-3 kidney section showing shrunken and swollen glomeruli, degeneration and necrosis of tubular epithelium with casts in the lumen (H and E; 200X). **(C)** Photomicrograph of G-4 showing atrophy of glomeruli, severe degeneration and necrosis with casts in the lumen (H and E; 100X).

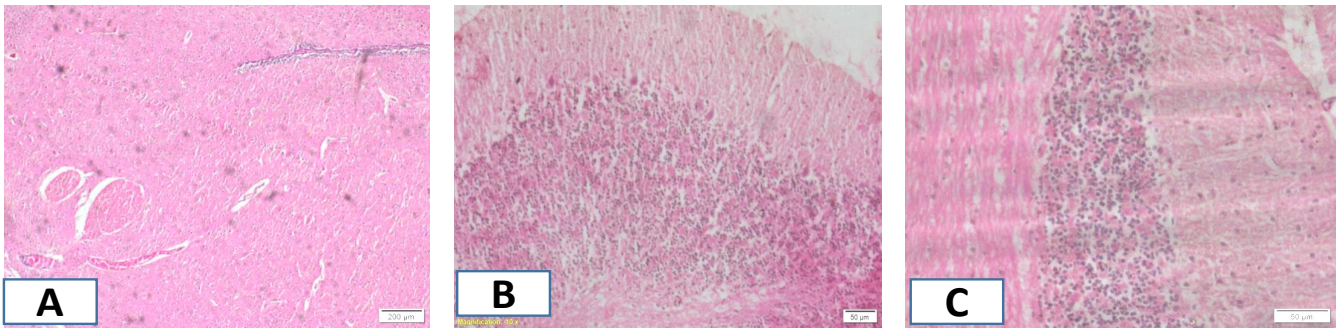


Figure 2. **(A)** Photomicrograph of G-2 brain (Cerebrum) section showing congestion and mild perivascular cuffing (H and E; 40X). **(B)** Photomicrograph section of G-3 brain (Cerebellum) showing mild to moderate degeneration of Purkinje cells (H and E; 200X). **(C)** Photomicrograph of G-4 section of brain (Cerebellum) showing progressive degeneration of Purkinje cells (H and E; 100X).

glomeruli, necrosis of epithelial cells and increased glomerular space, necrosis and disintegrated tubules and glomeruli (Figure 2A); kidney section showed shrunken and swollen glomeruli, degeneration and necrosis of tubular epithelium with casts in the lumen in group 3 (Figure 2B) and atrophy of glomeruli, severe degeneration and necrosis with casts in the lumen in group 4 (Figure 2C). Section of group 2 brain (Cerebrum) showed congestion and mild perivascular cuffing (Figure 3A); brain (Cerebellum) showed mild to moderate degeneration of Purkinje cells in group 3 (Figure 3B) and in group 4 section of brain (Cerebellum) revealed progressive degeneration of Purkinje cells (Figure 3C).

4. Discussion

TBARS levels in liver, kidneys and brain were significantly ($P<0.05$) increased in groups 2, 3 and 4 when compared to those in group 1 on 15th and 29th day of experiment. However, the rise was significantly ($P<0.05$) higher in the combined group 4 than in the individual toxicity groups 2 and 3. The increased TBARS levels might be an index of enhanced lipid peroxidation by $CdCl_2$ and CPF which lead to the excessive formation of free radicals that caused the Increases peroxidation of membrane lipids. The intensity of these components was high in the combined group due to their synergistic action. The increase in TBARS in liver, kidneys and brain might be attributed to the organ damage. The present observations and interpretations in $CdCl_2$ treated group are in accordance with opinion of other workers[10–12]. Observations and interpretations in CPF treated group are in accordance with opinion of many previous interventions[13–15].

The changes in the liver, kidney and brain were mainly due to increased lipid peroxidation and interaction of Cd with membrane components due to its long retention in tissues. The Cd induced hepatotoxicity was thought to be mediated through the cadmium metallothionein (Cd-Mt) complex, which is synthesized in the liver, released into circulation and taken up by renal proximal tubule cells[16]. In fact, when the synthesis of Mt becomes insufficient for binding all Cd ions in the liver, Cd not bound to Mt produce hepatocytes injury and caused different histopathological lesions. Cd-induced nephrotoxicity might be due to accumulation of cadmium metallothionein (Cd-Mt) complex, which enters into lysosome where metallothione gets degraded and free Cd is released leading to damage to tubules and glomeruli[17]. Moreover, these changes may be due to the accumulation of free radicals as the consequence of increased lipid peroxidation by free Cd ions in the renal tissues of Cd-treated rats[18]. The histopathological changes in group 3 were due to increased levels of MDA in rats exposed to CPF that might have caused membrane damage of cells resulting into degenerative to necrotic changes in liver, kidney and brain. Similar changes were also reported by other researchers[19,20]. Similar observations were also noticed in group 4 might be due to synergistic action of $CdCl_2$ and CPF.

5. Conclusion

Increased lipid peroxidation indicated oxidative damage in liver, kidney and brain of rats following repeated exposure of toxicants. Increasing oxidative damage is more pronounced in co-exposed group. These observations are

further corroborated with the histopathological alterations in these tissues.

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