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Changes in Biochemical Parameters, Antioxidative Enzymes and Histopathology of Liver Induced by Cadmium (Cd) and Chlorpyrifos (CPF) in *Wistar* Rats

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ABSTRACT

Background: Cd and CPF intoxication may occur directly through drinking water. Since the population tend to receive combination of multiple intoxicants through environment contamination, there is need for conducting studies to assess the impact of individual and combined environmental pollutants. The present research work was designed to study hepatotoxicity induced by Cd, CPF and their combination.

Methods: The experiment was carried out for 28 days in *Wistar* rats. G1: Control. G-2:$CdCl_2$ @ 22.5mg/ kg b.wt / oral. G3: CPF @ 25 mg/ kg b.wt /per oral. G4:$CdCl_2$@22.5 mg + CPF @ 25 mg/ kg b.wt /per oral. Biochemical parameters ware estimated from serum and liver samples were processed for tissue antioxidative parameters and histopathological examination.

Result: Higher mean values of AST, ALT, ALP and lower liver GSH and SOD were observed in G2, 3 and 4 on 15th and 29th day when compared with G1. Liver in G2 and 3 showed mild degenerative changes, areas of necrosis and loss of architecture. In G4, lesions were moderate in severity. In addition, moderate perivascular fibrosis of portal triad was observed. The effects in combined group were severe than individual groups due to synergistic action of the combined pollutants.

Key words: Cadmium, Chlorpyrifos, Hepatotoxicity, *Wistar* albino rats.

INTRODUCTION

There is growing evidence that long-term exposure to lower levels of heavy metals (Calderoni *et al.* 2005) and pesticides causes toxicity worldwide (Poulsen *et al.* 2008). Cadmium (Cd) and Chlorpyrifos (CPF) are the most common toxicants among all toxic compounds in the environment. The common sources of environmental Cadmium contamination are industrial, mining activities, plastic stabilizers and batteries which may result in widespread into environment and agricultural fields (Cheng *et al.* 2011). The Organo--phosphorus insecticides are extensively used for control of insects in home and agricultural practices. Chlorpyrifos (CPF) is one of the most commonly used organophosphate pesticides in domestic and agricultural applications throughout the world (Asperlin, 1994). Cd and CPF intoxication may occur directly through drinking water, indirectly through irrigation water and through feed ingredients of plant origin and also through inhalation of polluted air. Since the population tend to receive 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 (Ravikumar *et al.* 2019). Cadmium induces oxidative stress (Rajender *et al.* 2011) and apoptosis (Henson *et al.* 2004). CPF causes deleterious effects through acetyl cholinesterase inhibition at synapse of central and peripheral nervous system (Gordon *et al.* 1997), thereby causing damage to various vital organs. Cd and CPF are known for damaging organs *viz.* liver, kidneys, heart, lungs, retina and bones in humans and experimental animals (Curcic *et al.* 2012). The present research work was designed to study hepatotoxicity induced by Cd, CPF and their combination in *Wistar* rats.

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MATERIALS AND METHODS

Drugs and chemicals

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

Experimental design

Male *Wistar* albino rats (48) were procured from Sanzyme Laboratories Ltd., Hyderabad. Rats were randomly divided into 4 groups consisting of 12 in each group. G-1 serves as control. G- 2 rats were administered with $Cdcl_2$ @ 22.5mg/ kg b.wt /per oral / day, G-3 rats were administered with CPF @ 25 mg/ kg b.wt /per oral / day and G-4 rats were administered $Cdcl_2$ @22.5 mg + CPF @ 25 mg/ kg b.wt /per oral / day for 28 days of experiment.

Biochemical parameters

Blood was collected from *retro-orbital plexus* of rats, with the help of a capillary tube in to serum vaccutainers on 15th day and 29th day and serum was separated for estimation of biochemical parameters. Biochemical parameters *viz,* aspartate transaminase (AST), alanine transaminase (ALT) and alkaline phosphatase (ALP) were estimated in auto-biochemical analyzer by using Erba kits supplied by M/s Perala agencies, Hyderabad.

Tissue antioxidative parameters

Liver was quickly removed after sacrifice, trimmed of extraneous tissue and washed with ice cold physiological saline solution. After that liver tissue was divided into different parts. Tissue homogenate (10%) was prepared in ice cold phosphate buffered saline for estimation of GSH (Moron *et al.* 1979) and SOD (Madesh and Balasubramanian, 1998).

Histopathology

Detailed necropsy was conducted on 15th and 29th day of the experiment and gross changes were noticed, if any. Pieces of liver were collected in 10 % neutral buffer formalin (NBF). Samples were processed, sectioned (5µm), stained with Hematoxylin and Eosin (H&E) as per the standard protocol (Luna, 1968).

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 (Snedecor and Cochran, 1994).

RESULTS AND DISCUSSION

Effect on biochemical parameters

Significantly (P<0.05) higher in aspartate transaminase (AST), alanine transaminase (ALT) and alkaline phosphatase (ALP) mean values (IU/L) were observed in rats of group 2, 3 and group 4 on 15th and 29th day respectively when compared with group 1. However, there was a significant (P<0.05) difference in mean values in the rats of combined toxic dose (*i.e.* group 4) than the rats of individual treated groups 2 and 3 (Table 1).

Effect on antioxidative parameters

The mean values of reduced glutathione concentration (GSH) and superoxide dismutase activity (SOD) were

Table 1: Values (Mean ± S.E.) of serum biochemical parameters and oxidative enzymes in experimental rats of different groups.

Group	Aspartate transaminase activity (AST-IU/L)		Alanine transaminase (ALT-IU/L)		Alkaline phosphatase (ALP-IU/L)		GSH (µg /mg of protein)		SOD (U/mg protein)	
	DAY 15	DAY 29	DAY 15	DAY 29	DAY 15	DAY 29	DAY 15	DAY 29	DAY 15	DAY 29
G1	51.08±4.35^{d}	58.62±5.38^{d}	35.47±3.35^{d}	39.67±4.02^{d}	86.13±3.27^{d}	92.68±4.38^{c}	9.97±0.08^{a}	10.42±0.10^{a}	11.48±0.10^{a}	11.83±0.14^{a}
G2	123.95±8.34^{b}	175.20±7.64^{b}	111.95±4.35^{b}	163.20±5.34^{b}	159.38±8.37^{b}	243.28±8.66^{b}	8.60±0.02^{c}	7.64±0.05^{c}	9.16±0.18^{c}	7.77±0.16^{c}
G3	100.80±7.55^{c}	134.92±6.33^{c}	88.80±3.03^{c}	122.92±3.42^{c}	138.43±5.67^{c}	218.78±4.91^{c}	9.23±0.07^{b}	8.35±0.09^{b}	10.17±0.08^{b}	8.86±0.09^{b}
G4	151.93±4.35^{a}	194.55±3.67^{a}	139.93±5.34^{a}	182.55±5.66^{a}	192.20±4.58^{a}	272.88±4.12^{a}	7.61±0.09^{d}	6.53±0.07^{d}	8.80±0.15^{d}	6.72±0.11^{d}

*Changes in superscript in the same column and row indicates significant (p<0.01) changes.

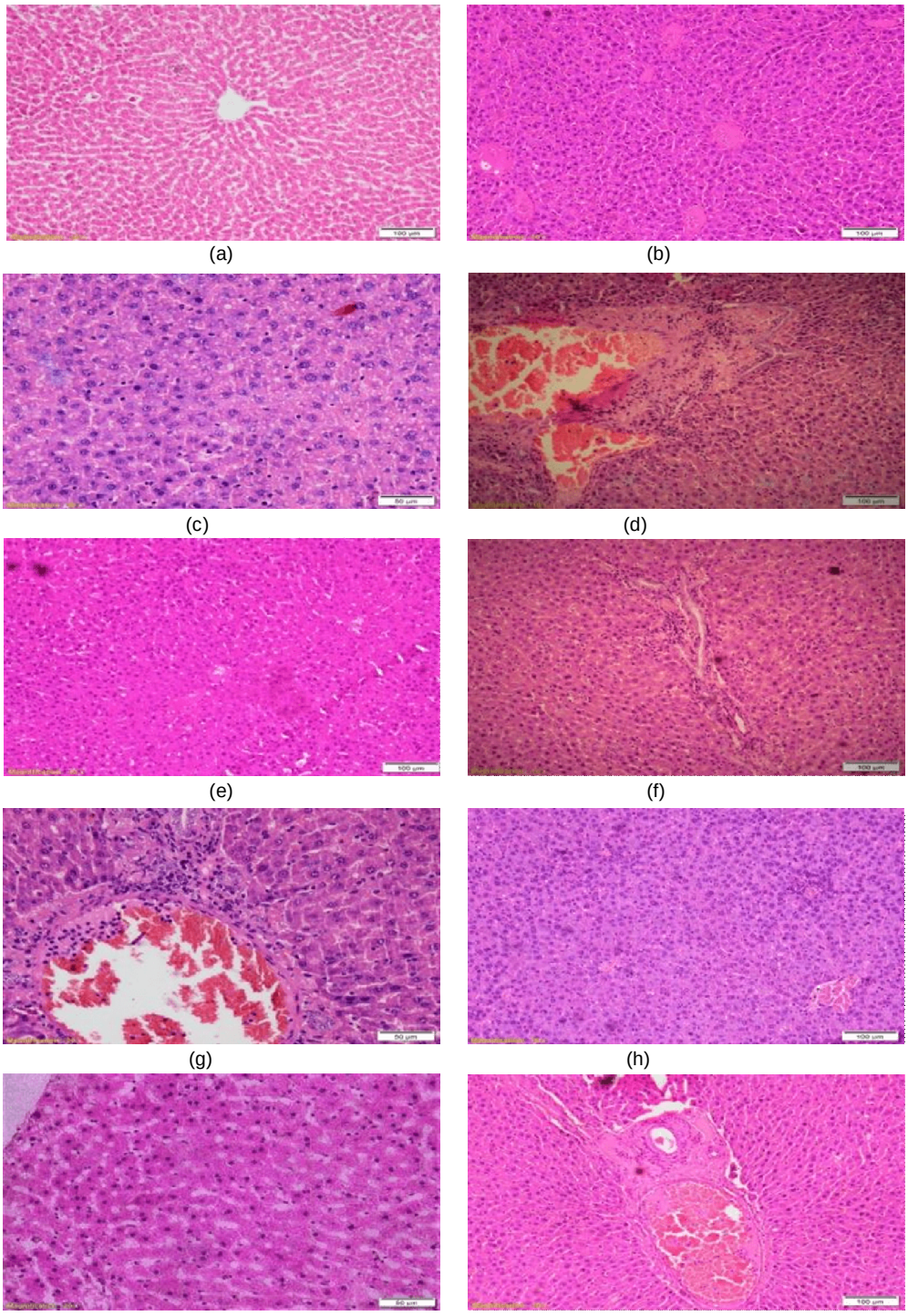

Fig 1: Photomicrograph of liver showing (a) Normal architecture in control group (G1) x100. (b) Dilation of sinusoids, haemorrhages and mild degenerative changes in hepatocytes (G-2, D-15). H&E x100. (c) Sinusoidal congestion, vacuolar degeneration, necrosis and loss of architecture (G-2, D-29). H&E x 200. (d) Severe congestion with infiltration of lymphocytes, disrupted cords with swollen nuclei and shrunken sinusoids (G-3, D-15). H&E x100. (e) Disrupted hepatic cord, pyknotic to swollen nuclei, shrunken sinusoids and coagulative necrosis of hepatocytes (G-3, D-29). H&E x100. (f) Coagulative necrosis of hepatic cords with infiltration of lymphocytes (G-4, D-15). H&E x100. (g) Severe congestion and dilation with perivascular infiltration of lymphocytes and pyknotic nuclei in perivascular hepatocytes (G-4, D-29). H&E x200. (h) Congestion, disorganisation of hepatic cords (G-4, D-29). H&E x100. (i) Marked dilatation of sinusoidal spaces, cloudy swelling and areas of necrosis (G-4, D-29). H&E x200. (j) Severe congestion with moderate perivascular fibrosis of portal triad and shrunken, irregular hepatic cords (G-4, D-29). H&E x100.

significantly (P<0.05) lower in group 2, 3 and group 4 compared with group 1 on 15th and 29th day of the experiment. Again, these values were significantly (P<0.05) lower in rats of group 4 compared to groups 2 and 3 (Table 1).

Histopathological findings in liver

At the end of the experiment animals were sacrificed and thoroughly examined for gross changes if any. Hepatomegaly with round edges was noticed on 15th and 29th day of experiment. The liver showed mild to moderate congestion in rats of groups 2 and3 and moderate to severe in group 4 on 15th and 29th day of the experiment. Liver sections of control group showed normal architecture of hepatic parenchyma with central vein and portal triad (Fig 1a). The sections of liver in group 2 rats on 15th day showed increased sinusoidal spaces and mild degenerative changes (Fig 1b). On 29th day, vacuolar degeneration and areas of necrosis were observed (Fig 1c). The sections of liver on 15th day in rats of group 3 showed perivascular lymphocytic infiltration and disrupted cords with swollen hepatocytes (Fig 1d). On 29th day, the changes were more pronounced as well as focal areas of necrosis was evident (Fig 1e). In the rats of group 4, the liver sections showed similar type of lesion as noticed in groups 2 and 3 on 15th and 29th day but the intensity was moderate to severe (Fig 1f to 1i). In addition, moderate perivascular fibrosis of portal triad and shrunken, irregular hepatic cords were observed (Fig 1j).

Biochemical parameters revealed a significant (P<0.05) increase in the activity of AST, ALT and ALP in groups 2, 3 and 4 on 15th and 29th day of the experiment. An increased activity of AST, ALT and ALP in cadmium and CPF treated rats separately indicated liver dysfunction, which was accompanied by elevated levels of these hepatic marker enzymes in the blood stream. Elevated levels of ALP suggested biliary damage, which disrupts flow of blood to the liver. Similar findings were reported by Tomaszewska *et al*. (2015) and Nasim Babaknejad *et al.* (2015) in Cd treated rats. In the present study, these results are in accordance with the findings of Ambali *et al.,* (2010) and Barski and Spodniewska (2018). Co-administration of both the toxicants produced further increase in these enzyme levels as compared to individual administration of either toxicant. The increased levels could be due to severe degeneration and necrosis of hepatocytes that result in release of transaminases in the blood. These findings can be correlated with the histological changes in the present study and are in agreement with the reports of Prabu *et al*. (2012) and Singh *et al.* (2016).

Significant (P< 0.05) reduction in GSH and SOD levels in rats liver of combined group than those in the individual toxicity groups is suggestive of oxidative stress in the present study. El-Sharaky *et al.* (2007) observed that the increase in lipid peroxidation might be attributed to alterations in the antioxidant defence system. This defence system includes the glutathione peroxidase, thioredoxin reductase as well as the reduced glutathione (GSH), which normally protect the biological system against free radical toxicity. Sarkar *et al.* (1998) demonstrated that Cd modulates toxic effects through oxidative stress mechanisms. The changes in $CdCl_2$ treated rats are in agreement with those of Renugadevi and Prabu (2010), Messaoudia *et al.* (2010), Pari and Shagirtha (2012) and Christian *et al.* (2016). The results in Group 3 indicated that CPF exposure inhibited GSH and SOD. These depletion might be due to the decreased synthesis of enzymes or oxidative inactivation of enzyme protein. The changes in CPF group were similar to the reports of Aly *et al*. (2010), Hassani *et al.* (2014) and Deng *et al.* (2016). In rats of group 4, a marked reduction in GSH levels compared to groups 2 and 3 indicated synergistic action of $CdCl_2$ and CPF leading to higher oxidative damage.

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 (Dudley *et al*. 1985). 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. Similar lesions were also noticed by Jihen *et al*. (2008), Renugadevi and Prabu (2010) and Prabu *et al*. (2012). The histopathological changes are further corroborated by the decreased levels of GSH and SOD in rats exposed to CPF that might have caused membrane damage of cells resulting into degenerative to necrotic changes in liver. Similar changes were also reported by Savithri *et al.* (2010) and Singh *et al.* (2016). Irregular hepatic cords were observed. Similar observations were also noticed by Singh *et al.* (2016) and might be due to synergistic action of $CdCl_2$ and CPF.

CONCLUSION

In conclusion, the adverse effects of combined $CdCl_2$ and CPF group (Group 4) were more severe than the individual groups (Group 2 and 3) due to synergistic action of the combined pollutants.

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Declaration of conflict of interest

Authors declare that there are no conflicts of interest to report.

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