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Antioxidative Profile and Histopathological Alterations of Brain Induced by Cadmium (Cd) and Chlorpyrifos (CPF) in Wistar Rats

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Abstract

Research was carried out to know the individual and synergistic effect of cadmium chloride and chlorpyrifas on brain in wistar rats for a study period of 28 days in rats. Group 1: Control. Group 2: CdCl2 @ 22.5mg/ kg b.wt / oral. Group 3: CPF @ 25 mg/ kg b.wt /per oral. Group 4: CdCl2@22.5 mg + CPF @ 25 mg/ kg b.wt /per oral. Lower mean values of brain GSH & SOD were observed in G-4, 2 and 3 on 15th and 29th day when compared with G-1. Brain section of group 2 on 15th day revealed mild perivascular cuffing, on 29th day degeneration of Purkinje cells. Group 3 revealed gliosis on 15th day, on 29th day moderate perivascular cuffing and shrinkage of Purkinje cells. Brain sections of group 4 on 15th day revealed moderate multifocal perivascular cuffing and mild degeneration of Purkinje cells, on 29th day, the lesions were same but severe. The adverse neurotoxic effects in combined group were severe than individual groups due to synergistic action of the combined pollutants.

Keywords: Antioxidative Profile, Brain, Cadmium, Chlorpyrifos, Histopathological Alterations, Wistar Rats

Introduction

There are several heavy metals found in our environment due to both natural and anthropogenic sources. 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. Increased concentration of Cd in agricultural soil comes from the application of phosphate fertilizers, sewage sludge and pesticides (Limei *et al*., 2008). The OP insecticides are extensively used for control of insects in home and agricultural practices. Chlorpyrifos (CPF) is one of the most heavily used organophosphate pesticide in domestic and agricultural operations by the farmers (Poulsen *et al.,* 2008). Cd and CPF intoxication may occur directly through drinking water, indirectly through irrigation water source 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 (Yadala *et al.,* 2019). Cd induces oxidative stress and apoptosis (Henson *et al.,* 2004), 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 (Idris *et al.,* 2012), thereby causing damage to various vital organs. Cd and CPF are known for causing organ toxicity in humans and experimental animals (Curcic *et al.,* 2012). The present work was aimed to study neurotoxicity induced by Cd, CPF and their combination in *Wistar* rats.

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 weighing 250-270g (48) 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. G-1 serves as control. G- 2 rats were administered $Cdcl_2$ @ 22.5mg/ kg b.wt /per oral / day. G-3 rats were administered CPF @ 25 mg/ kg b.wt /per oral / day. G-4 rats were administered $Cdcl_2$ @22.5 mg + CPF @ 25 mg/ kg b.wt /per oral / day for 28 days.

Tissue Antioxidative Parameters

Brain was quickly removed, washed with ice cold physiological saline solution and then homogenized 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 15^{th} and 29^{th} day of the experiment and gross changes were noticed, if any. Brains 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 Antioxidative Parameters

The mean values of reduced glutathione concentration (GSH) and superoxide dismutase activity (SOD) were significantly (P<0.05) lower in group 2, 3 and group 4 compared with group 1 on 15th and 29th day of the experiment. These values were significantly (P<0.05) lower in group 4 compared to groups 2 and 3 (Table 1).

Table 1: Brain reduced glutathione concentration (GSH) and Superoxide dismutase activity (SOD) in different groups

Groups	Reduced glutathione concentration in (GSH µg /mg of protein)		Superoxide dismutase activity (SOD-U/mg protein)	
	DAY 15	DAY 29	DAY 15	DAY 29
G1	8.64±0.11^{a}	8.82±0.12^{a}	10.05±0.11^{a}	9.82±0.12^{a}
G2	8.28±0.12^{b}	8.02±0.17^{b}	9.16±0.14^{b}	8.58±0.15^{b}
G3	7.30±0.08^{c}	6.40±0.06^{c}	8.52±0.18^{c}	7.27±0.21^{c}
G4	6.09±0.13^{d}	5.78±0.14^{d}	7.24±0.10^{d}	6.58±0.13^{d}

Values are Mean Standard error (n=6) One-way ANOVA; Mean ± SE with different alphabets as superscripts differ significantly (P<0.05)

Histopathological Findings in Brain

Brain sections in group 1 rats revealed normal histological architecture of cerebral cortex and cerebellum on 15th and 29th day of the experimental period (Fig. 1). Group 2 rats on 15th day of the experiment revealed congested vessels and mild perivascular cuffing (Fig. 2). On 29th day lesion noticed was mild to moderate degeneration of Purkinje cells (Fig. 3). The sections of brain from group 3 rats revealed gliosis (Fig. 4) on 15th day. On 29th day in addition to gliosis, moderate perivascular cuffing, congestion (Fig. 5) and shrinkage of Purkinje cells (Fig. 6) were noticed. Brain sections of group 4 rats on 15th day revealed marked congested vessels and moderate multifocal perivascular cuffing (PVC) (Fig. 7) and mild degeneration of Purkinje cells (Fig. 8). On 29th day, similar changes with increased severity were noticed (Fig. 9 and 10).

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) and Pari and Shagirtha (2012). In the present study, our results indicated that CPF exposure inhibited SOD and GPx activities. This depletion might be due to the decreased synthesis of enzymes or oxidative inactivation of enzyme protein. The changes in CPF group were similar to those in the reports of Hassani *et al.* (2014) and Yongfeng Deng *et al.* (2016). In group 4, a marked reduction in GSH levels compared to groups 2 and 3 indicate synergistic action of $CdCl_2$ and CPF leading to higher oxidative damage. In the present study, a significant (P< 0.05) decrease in SOD in brain was observed in toxic groups 2, 3 and 4 when compared to control group 1 on 15th and 29th day of the experiment. There was a significant (P< 0.05) decrease in the combined group than in the individual toxicity groups. Co exposure of rats to $CdCl_2$ and CPF might have generated excessive free radicals resulting in the depletion of antioxidants. The present observations and interpretations in $CdCl_2$ treated group are in accordance with Messaoudia *et al.* (2010) and Christian Eseigbe Imafidon *et al.* (2016). Observations and interpretations in CPF treated group are in accordance with Aly *et al.* (2010), Hassani *et al.* (2014) and Yongfeng Deng *et al.* (2016).

Grossly mild to moderate congestion in brain on 15th day and severe congestion on 29th day of the experiment was noticed in all the toxic groups. In group 2, brain sections on 15th day of the experiment revealed congested vessels and mild perivascular cuffing; on 29th day lesion noticed was mild to moderate degeneration of Purkinje cells. Similar changes were noticed by Yoshida (2001) and Gupta (2012). The changes observed might be due to the toxic effects of cadmium and high production of free radicals in the brain of adult rats that interfere with the antioxidant defence system, which in turn leads to alteration of the structural integrity of membrane lipids and secondarily affect the membrane bound enzymes such as Na^{+} K^{+}-ATPase (Yoshida, 2001 and Kumar *et al.*, 2012). The sections of brain from group 3 rats revealed gliosis, moderate perivascular cuffing (PVC), congestion and shrinkage of Purkinje

cells.

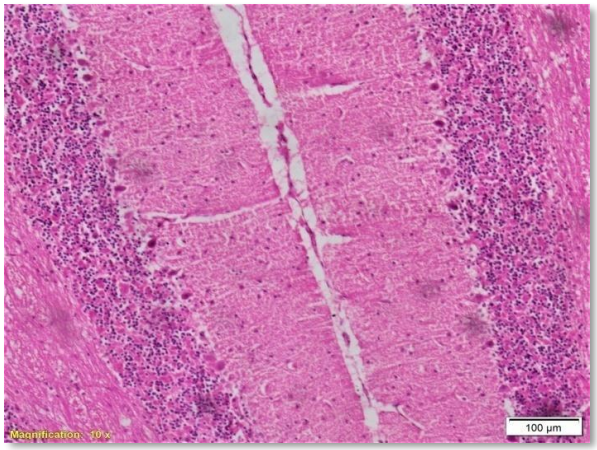

Figure 1: Photomicrograph of brain showing normal cerebellum (Group 1, Day 29): H&E×100.

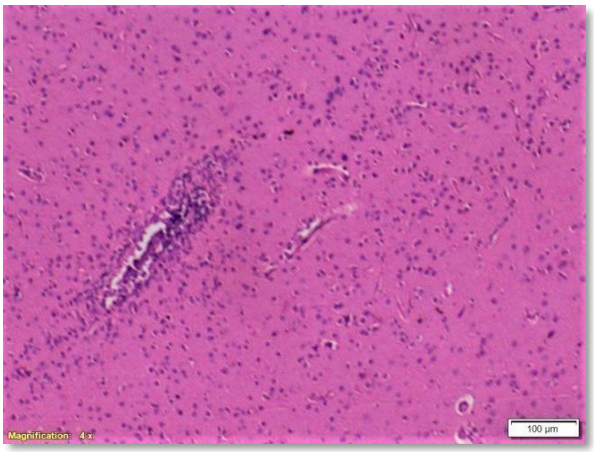

Figure 2: Photomicrograph of brain (Cerebrum) section showing mild perivascular cuffing (Group 2, Day 15): H&E x 40.

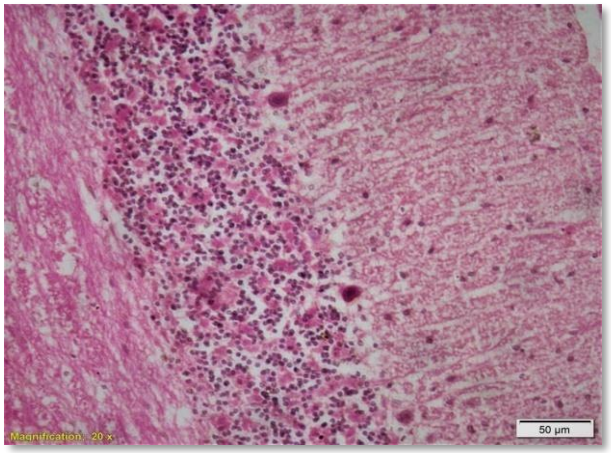

Figure 3: Photomicrograph section of brain (Cerebellum) showing mild to moderate degeneration of Purkinje cells (Group 2, Day 29): H&E x 200.

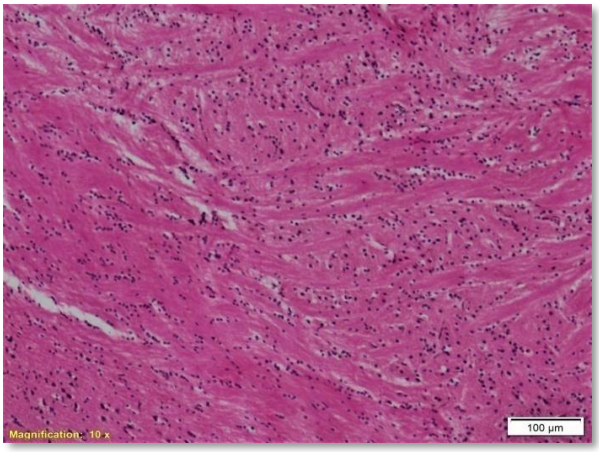

Figure 4: Photomicrograph brain (Cerebrum) section showing gliosis (Group 3, Day 15): H&E x 100.

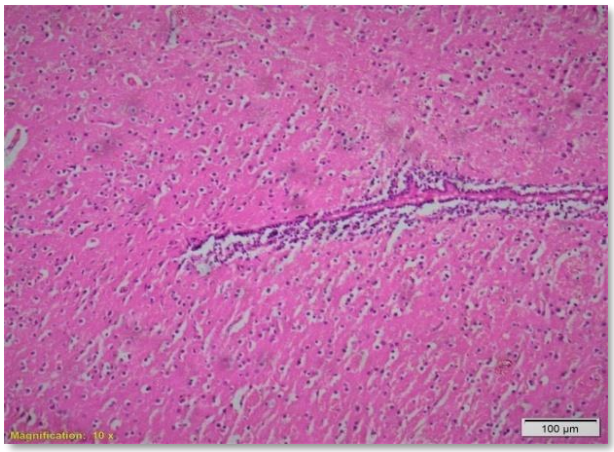

Figure 5: Photomicrograph brain (Cerebrum) section showing moderate perivascular cuffing and congestion (Group 3, Day 29): H&E×100.

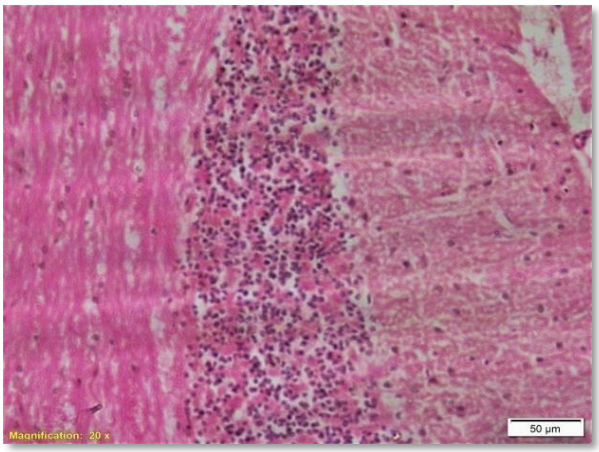

Figure 6: Photomicrograph section of brain (Cerebellum) showing progressive degeneration of Purkinje cells (Group 3, Day 29): H&E×200.

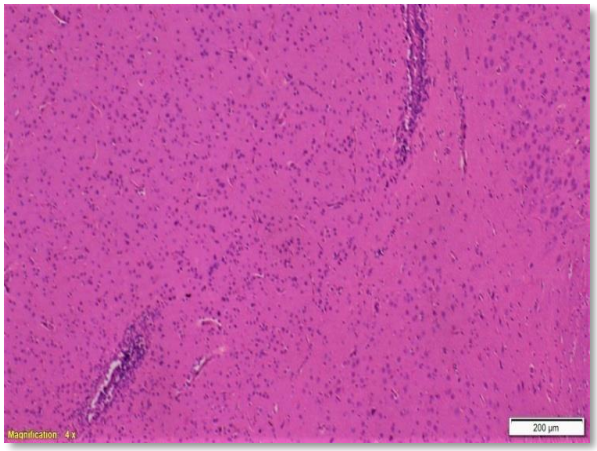

Figure 7: Photomicrograph brain (Cerebrum) section showing moderate multifocal perivascular cuffing (Group 4, Day 15): H&E×40.

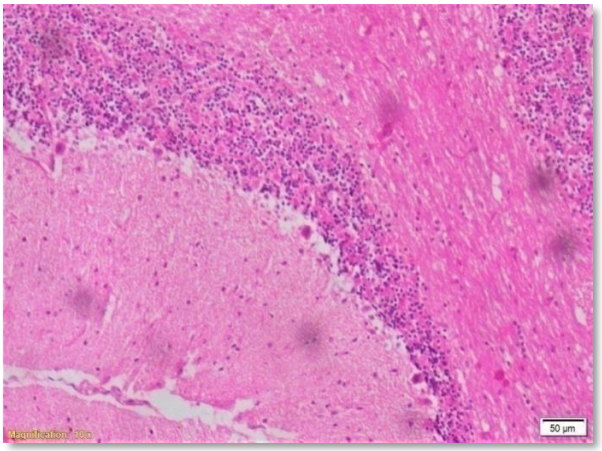

Figure 8: Photomicrograph of Group 4 section of brain (Cerebellum) showing initial mild degeneration of Purkinje cells (Group 4, Day 15): H&E×100.

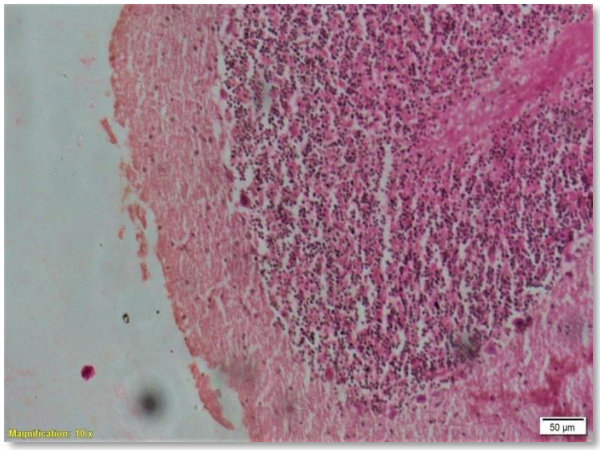

Figure 9: Photomicrograph of Group 4 section of brain (Cerebellum) showing progressive degeneration of Purkinje cells (Group 4, Day 29): H&Ex100.

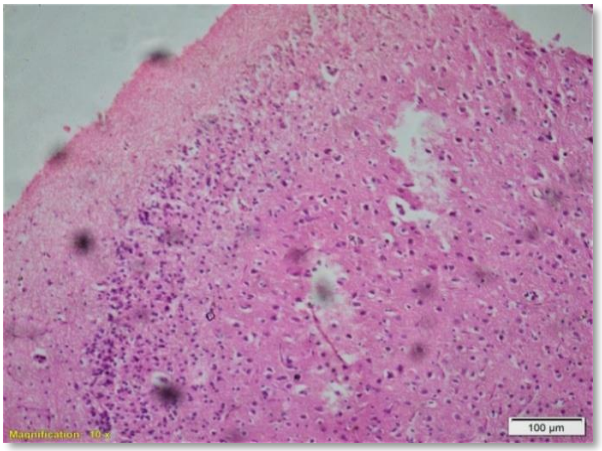

Figure 10: Photomicrograph brain (Cerebellum) section showing loss of Purkinje cells (Group 4, Day 29): H&E x 100.

These findings are in close agreement with the results of Nahid Akhtar *et al.* (2009) and Barski and Spodniewska (2018). Brain sections of group 4 on 15th day revealed marked congested vessels, moderate multifocal perivascular cuffing (PVC) and mild degeneration of Purkinje cells; on 29th day, similar changes with increased severity were noticed. The increased intensity of lesions in the group 4 might be due to the synergistic effects of $CdCl_2$ and CPF.

Conclusion

It is concluded that the adverse neurotoxin effects of combined $CdCl_2$ and CPF group (Group 4) were more severe than the individual groups (Group 2 & 3) due to synergistic action of the $CdCl_2$ and CPF.

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Conflict of Interests

There is no conflict of interest.

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