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Pathological alterations in the spleen induced by cadmium and chlorpyriphos in male Wistar rats

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Abstract
The main objective of the study was to know the pathological alterations of the spleen due to toxic effects of cadmium and chlorpyrifos. Present work was carried out for 28 days in rats. 1: Control. 2: $CdCl_2$ @ 22.5mg/ kg b.wt / oral. 3: CPF @ 25 mg/ kg b.wt /per oral. 4: $CdCl_2$@22.5 mg + CPF @ 25 mg/ kg b.wt /per oral. Sections of spleen of group 2 rats, on 15th day of experiment revealed mild to moderate vacuolation, thick trabeculi; on 29th day shrunken red pulp, hypoplasia of white pulp and dilated splenic vessel with thickening of the wall. Spleen sections of group 3 rats on 15th day showed hypoplasia of white pulp and thick trabeculi with haemorrhages and extension of trabeculi with focal areas of hypercellularity. On 29th day in addition to the above changes, shrunken red pulp and vacuolation were observed. Group 4 rats spleen on 15th and 29th day of the experiment showed similar lesions as seen in groups 2 and 3 but the lesions were moderate. The pathological changes in spleen in combined group were severe than individual groups due to synergistic action of the combined pollutants.

Keywords: Cadmium (Cd), chlorpyrifos (CPF), pathological alterations, spleen, Wistar rats

Introduction
Heavy metals are generally found in our environment due to both natural and anthropogenic sources [1]. Fact is that long-term exposure to lower levels of heavy metals [2] and pesticides causes toxicity worldwide [3]. Cadmium (Cd) and Chlorpyrifos (CPF) are very 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 [4]. Chlorpyrifos (CPF) is one of the most heavily used organophosphate pesticide in domestic and agricultural operations by the farmers [3]. Combined 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 [5]. 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 [6]. Cd induces oxidative stress and apoptosis [7], The principal mechanism of toxicity of CPF is due to its inhibition of acetyl cholinesterase (AChE) and accumulation of acetylcholine (ACh) at the nerve endings and the neuromuscular junctions [8] and also produces reactive oxygen species, thereby causing damage to various vital organs. Cd and CPF are known for damaging organs *viz:* liver, kidneys, brain, heart, lungs in humans and experimental animals [9]. The present work was aimed to study the toxic effects of Cd, CPF and their combination on spleen in Wistar rats.

Materials and Methods
The study was designed and conducted at Department of Veterinary Pharmacology and Toxicology & Department of Veterinary Pathology, College of Veterinary Science, Rajendranagar, Hyderabad. $CdCl_2$ was procured from Thermo Fisher Scientific India Pvt. Ltd. Mumbai. Chlorpyrifos was procured from Coromandel Fertilizers Pvt. Ltd. Vishakapatnam. Male Wistar albino rats (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 of experiment. Detailed necropsy was conducted on 15th and 29th day of the experiment and gross changes were noticed, if any. Spleen samples 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 given [10].

Results and Discussion

Group 1 rats on15th and 29th day of experiment showed normal architecture of spleen (Fig.1). Sections of spleen of group 2 rats, on 15th day of experiment revealed mild to moderate vacuolation (Fig.2), thick trabeculi (Fig.3); on 29th day shrunken red pulp (Fig.4), hypoplasia of white pulp (Fig.5) and dilated splenic vessel with thickening of the wall (Fig.6). These alterations were observed due to the deteriorative action of oxygen free radical released due to action of cadmium and these observations are similar with the other findings [11, 12] in $CdCl_2$ treated rats. Spleen sections of group 3 rats on 15th day showed hypoplasia of white pulp and thick trabeculi (Fig.7) with haemorrhages and extension of trabecula with focal areas of hypercellularity (Fig.8). On 29th day in addition to the above changes, shrunken red pulp (Fig.9) and vacuolation (Fig.10) were observed. These changes were mediated by oxidative stress by chlorpyrifos and these observations are similar to those of researchers [13, 14] in CPF treated rats. Group 4 rats spleen on 15thand 29th day of the experiment showed similar lesions as seen in groups 2 and 3 (Fig.11 and 12) but the lesions were moderate. These changes in spleen could be attributed to excess generation of ROS and decreased antioxidant levels [5].

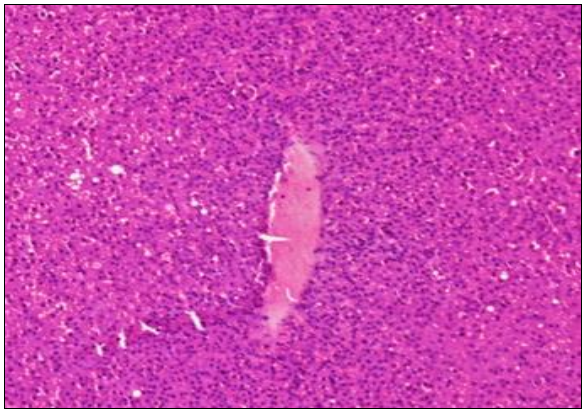

Fig 1: Photomicrograph of spleen showing normal architecture (Group 1, Day 29): H&E x 100.

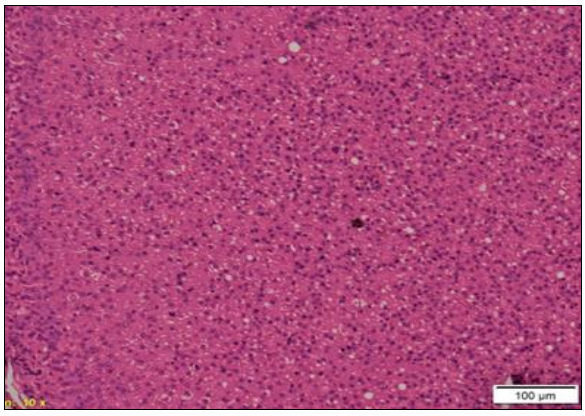

Fig 2: Photomicrograph of spleen showing mild to moderate vacuolation (Group 2, Day 15): H&E x 100.

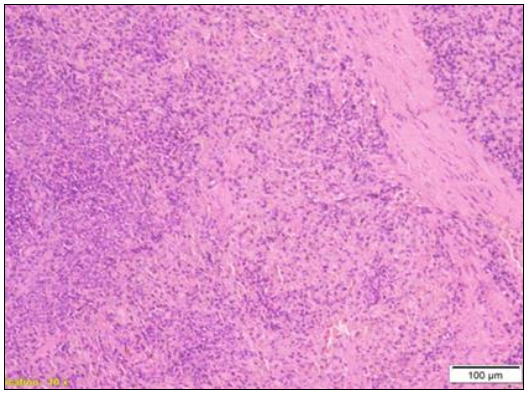

Fig 3: Photomicrograph of spleen showing thick trabeculi (Group 2, Day 15): H&E x 100.

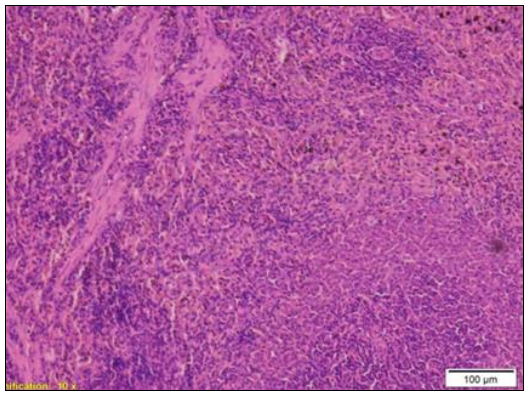

Fig 4: Photomicrograph of spleen showing shrunken red pulp (Group 2, Day 29): H&E x 100.

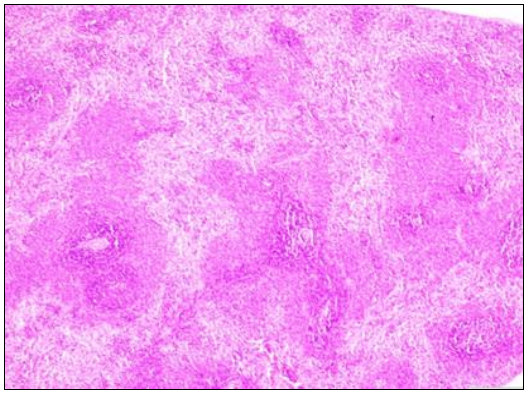

Fig 5: Photomicrograph of spleen showing hypoplasia of white pulp (Group 2, Day 29). H&E x 40.

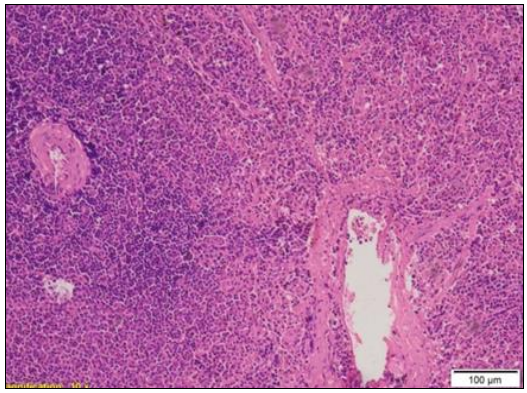

Fig 6: Photomicrograph of spleen showing dilated splenic vessel with thickenned wall (Group 2, Day 29): H&E x 100.

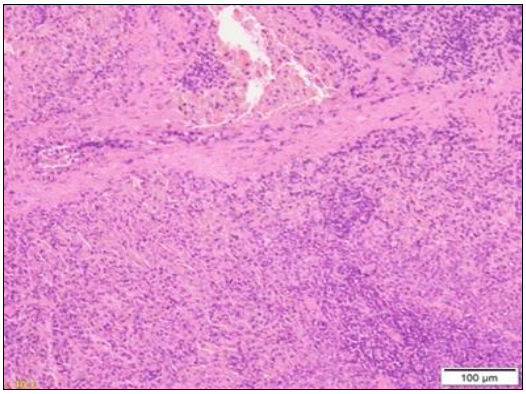

Fig 7: Photomicrograph of spleen showing hypoplasia of white pulp with thick trabeculi (Group 3, Day 15): H&E x 100

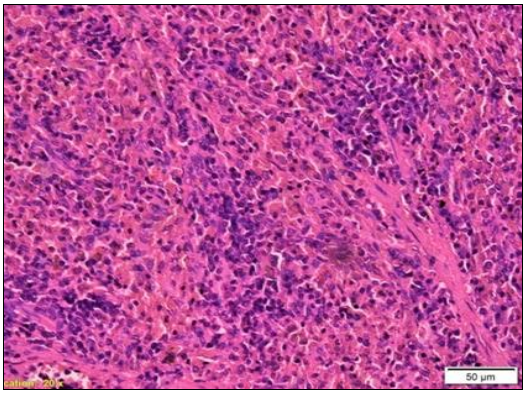

Fig 8: Photomicrograph of spleen showing haemorrhages and trabecula extension with focal areas of hyper cellularity (Group 3, Day 15): H&E x 200.

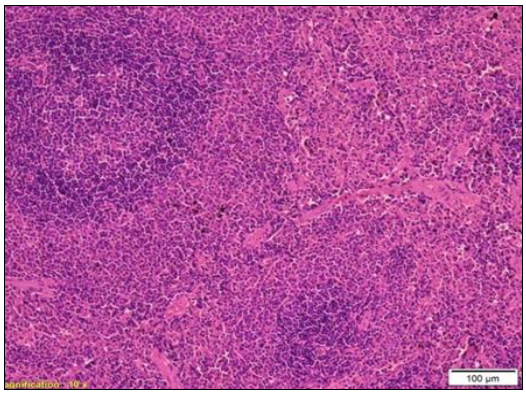

Fig 9: Photomicrograph of spleen showing shrunken red pulp (Group 3, Day 29): H&E x 100.

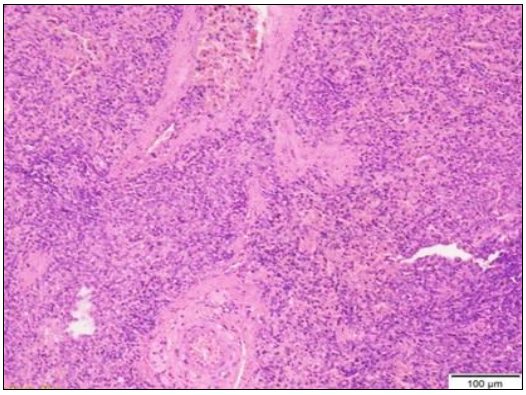

Fig 10: Photomicrograph of spleen showing congestion and vacuolation (Group 3, Day 29): H&E x 100

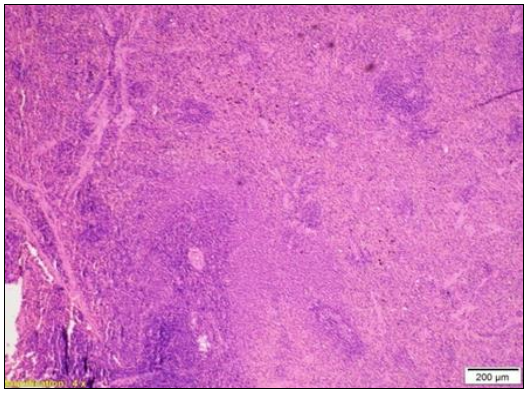

Fig 11: Photomicrograph of spleen showing shrinkage of red pulp (Group 4, Day 15): H&E x 100.

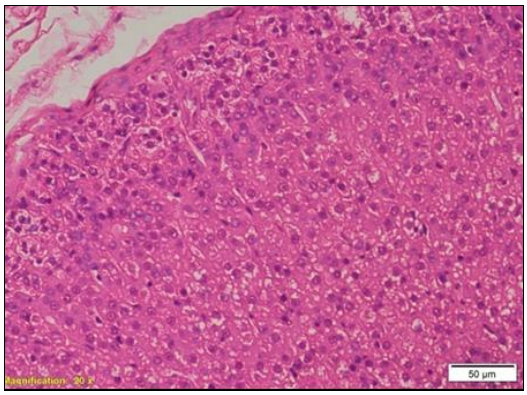

Fig 12: Photomicrograph of spleen showing moderate vacuolation in the pulp (Group 4, Day 29): H&E x 200.

Conclusion

The present study suggests that the $CdCl_2$ and CPF either alone and in combination could be responsible for significant pathological alterations in spleen. These components caused dysfunctioning of spleen and the toxicity was more pronounced in combined group than individually exposed groups. The toxicity could be due to lipid peroxidation by oxidative stress, impairment of antioxidant defences that lead to apoptosis of spleen. It is concluded that the adverse effects of combined $CdCl_2$ and CPF in the spleen of 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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