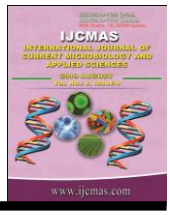
*Int.J.Curr.Microbiol.App.Sci* (2019) *8*(8): 480-485

*International Journal of Current Microbiology and Applied Sciences*
***ISSN: 2319-7706*** **Volume 8 Number 08 (2019)**
Journal homepage: http://www.ijcmas.com



**Original Research Article** https://doi.org/10.20546/ijcmas.2019.808.055

# Haematological Alterations Induced by Cadmium (Cd) and Chlorpyrifos (CPF) in Male *Wistar albino* Rats

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**Keywords**

Haematology, Cadmium, Chlorpyrifos and *Wistar albino* rats

**Article Info**

***Accepted:***
07 July 2019
***Available Online:***
10 August 2019

**A B S T R A C T**

The present study was aimed to know the haematological alteration due to individual toxicities of cadmium, chlorpyrifos and their combination in *albino wistar* rats. The experiment was carried out for 28 days. Group 1 - Control. Group 2 - Cadmium chloride (Cd) @ 22.5mg/ kg b.wt /per oral / day. Group 3 - Chlorpyrifos (CPF) @ 25 mg/ kg b.wt /per oral / day. Group 4 - Cadmium chloride (Cd) @22.5 mg + Chlorpyrifos (CPF) @ 25 mg/ kg b.wt /per oral / day. The weekly body weight gains were significantly ($P < 0.05$) decreased in groups 4, 3 and 2 when compare with control group. Haematological observations revealed a significant ($P < 0.05$) decrease in overall means of TEC, Hb, PCV in except TLC in groups 4, 2 and 3 when compare with control group. TLC values were increased 4, 2 and 3. These results revealed that combined toxicity of cadmium and chlorpyrifos is significant than exposure of individual components which resulted in alterations in haematological parameters.

## Introduction

Cadmium (Cd) and Chlorpyrifos (CPF) are the most abundant toxicants among all toxic compounds in the environment. The common sources of environmental contamination of Cd are industrial & mining activities, plastic stabilizers, batteries and also in use of pigments which may result in widespread into environment and agricultural fields (Cheng *et al.*, 2011). Organophosphorus (OP) 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 source and through feed ingredients of plant origin. Cd induces oxidative stress and apoptosis (Henson *et al.,* 2004), CPF causes deleterious effects through acetylcholinesterase 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 haemotoxicity, endocrine disruption and reproductive toxicity apart from damaging other organs *viz:* kidneys, liver, heart, lungs, retina and bones in humans and experimental animals (Abeer *et al.,* 2010 and Curcic *et al.,* 2012). In majority of animal studies, single pollutants were used at higher concentrations, however, population tend to receive combination of multiple intoxicants through environment contamination. Hence, there is a dire need for conducting induced toxicopathological studies to assess the impact of individual and combined environmental pollutants (Yuan *et al.,* 2014).

## Materials and Methods

Forty eight adult male *Wistar* albino rats were procured from Sanzyme Laboratories Ltd., Hyderabad, animals were devided into four groups, twelve animals in each group. The body weights were recorded and identification marks were allotted. The rats weree raised under controlled environmental conditions, fresh water, sterile feed to control group; toxin mixed water and sterile feed to experimental groups were provided at *ad libitum* regularly for four weeks. Rats were randomly divided into 4 groups consisting of 12 in each group. Group 1 - Control. Group 2 - Cadmium chloride (Cd) @ 22.5mg/ kg b.wt /per oral / day. Group 3 - Chlorpyrifos (CPF) @ 25 mg/ kg b.wt /per oral / day. Group 4 - Cadmium chloride (Cd) @22.5 mg + Chlorpyrifos (CPF) @ 25 mg/ kg b.wt /per oral / day. The experiment was carried out according to the guidelines and prior approval of the Institutional Animal Ethics Committee (IAEC).

### Drugs and chemicals

Cadmium chloride was procured from Thermo Fisher Scientific India Pvt. Ltd. Mumbai.

Chlorpyrifos was procured from Coromandel Fertilizers Pvt. Ltd. Vishakapatnam.

### Growth rate

Individual body weights of all the rats were recorded by using electronic balance on day zero and subsequently on $7^{th}$, $14^{th}$, $21^{st}$ and $28^{th}$ day of experiment to study the body weight gains.

### Haematology

Blood was collected on $15^{th}$ day and $29^{th}$ day and estimated for haematological parameters 2-3 ml of blood was collected from *retro-orbital plexus* of rats, with the help of a capillary tube in to an anticoagulant coated vaccutainers {(K2-EDTA tube, 13mm x 75mm, 4ml (Rapid Diagnostics Pvt Ltd., Delhi)} to carry out all hematological parameters. Prior to blood collection, the selected experimental rats were put to fast for 12 hours. The blood samples were used for estimation of total erythrocyte count (TEC), total leukocyte count (TLC), hemoglobin (Hb) concentration, and packed cell volume (PCV) by using an automatic whole blood analyzer (BC-2800Vet), Veterinary Biological Research Institute (VBRI) Hyderabad.

The results obtained were tabulated for 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

### Growth rate (Body Weights) (g)

Significantly (P<0.05) higher mean values (264.31±2.13, 265.64±2.37, 267.27±2.34 and 268.5±2.71) were recorded in group 1 on 7th, 15th, 21st and 29th day respectively than the group 2 (261.28±3.57, 260.27±3.60, 259.34±3.11and 257.22±3.43), group 3 (259.31±4.25, 258.15±4.16, 257.12±4.35 and 255.34±4.61) and group 4 (253.24±4.60, 252.37±5.21, 251.37±4.38 and 249.88±5.08) on 7th, 15th, 21st and 29th days of experiment respectively (Table 1).

There was a significant (P<0.05) reduction in mean body weight gain in the last week of the experiment in Group 2, Group 3 and Group 4 when compared to that of Group 1. However, no significant difference noticed in the mean values of Group 2 & 3.

### Haematology

The TEC (millions/µl) mean values were significantly (P<0.05) low in Group 2 (7.34±0.13, 6.53±0.24), Group 3(8.65±0.16, 8.17±0.25) and Group 4 (8.27±0.14, 8.04±0.11) on 15th and 29th day respectively when compared with Group 1 (8.86±0.16, 8.73±0.10). There was no significant difference in mean values between Group 2 and Group 1 on 15th but differ significantly on 29th day respectively. The values were significantly (P<0.05) low in Group 4 when compared with Group 2 and Group 3. (Table 2).

The haemoglobin (g %) values were significantly (P<0.05) low in Group 2 (14.52±0.18, 13.48±0.26), Group 3 (15.47±0.16, 15.21±0.22) and Group 4 (13.84±0.24, 13.14±0.41) on 15th and 29th day respectively when compared with Group 1 (16.04±0.14, 16.48±0.23). The values were significantly (P<0.05) low in Group 4 when compared with Group 2 and Group 3 (Table 2).

The packed cell volume (%) mean values were significantly (P<0.05) low in Group 2 (34.42±0.52, 32.53±0.61), Group 3 (38.37±0.34, 35.54±0.38) and Group 4 (31.54±0.68, 28.21±0.71) on 15th and 29th day respectively when compared with Group 1 (41.37±0.28, 46.62±0.31). The values were significantly (P<0.05) low in Group 4 when compared with Group 2 and Group 3 (Table 2).

**Table.1** Weekly body weight gain (g) in different groups

| GROUP | 7th DAY | 14th DAY | 21th DAY | 28th DAY |
|---|---|---|---|---|
| G1(CONTROL) | 264.31±2.13$^{c}$ | 265.64±2.37$^{d}$ | 267.27±2.34$^{d}$ | 268.5±2.71$^{d}$ |
| G2($CdCl_2$) | 261.28±3.57$^{b}$ | 260.27±3.60$^{c}$ | 259.34±3.11$^{c}$ | 257.22±3.43$^{c}$ |
| G3(CPF) | 259.31±4.25$^{a}$ | 258.15±4.16$^{b}$ | 257.12±4.35$^{b}$ | 255.34±4.61$^{b}$ |
| G4($CdCl_2$+ CPF) | 253.24±4.60$^{a}$ | 252.37±5.21$^{a}$ | 251.37±4.38$^{a}$ | 249.88±5.08$^{a}$ |

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

**Table.2** Hematological parameters (TEC, Hb, PCV and TLC) in different groups at different time Intervals

| GROUP | TEC (millions/µl) | | Hb (g %) | | PCV (%) | | TLC (Thousands/µl) | |
|---|---|---|---|---|---|---|---|---|
| | DAY 15 | DAY 29 | DAY 15 | DAY 29 | DAY 15 | DAY 29 | DAY 15 | DAY 29 |
| **G1-Control** | 8.93±0.15$^{d}$ | 9.15±0.21$^{d}$ | 16.04±0.14$^{d}$ | 16.48±0.23$^{c}$ | 41.37±0.28$^{d}$ | 46.62±0.31$^{d}$ | 12.53±0.39$^{a}$ | 13.61±0.44$^{a}$ |
| **G2-$CdCl_2$** | 7.34±0.13$^{b}$ | 6.53±0.24$^{b}$ | 14.52±0.18$^{b}$ | 13.48±0.26$^{a}$ | 34.42±0.52$^{b}$ | 32.53±0.61$^{b}$ | 16.10±0.94$^{b}$ | 16.84±1.02$^{b}$ |
| **G3-CPF** | 8.65±0.16$^{c}$ | 8.17±0.25$^{c}$ | 15.47±0.16$^{c}$ | 15.21±0.22$^{b}$ | 38.37±0.34$^{c}$ | 35.54±0.38$^{c}$ | 12.81±0.34$^{a}$ | 13.75±0.51$^{a}$ |
| **G4-$CdCl_2$+ CPF** | 6.67±0.18$^{a}$ | 6.04±0.43$^{a}$ | 13.84±0.24$^{a}$ | 13.14±0.41$^{a}$ | 31.54±0.68$^{a}$ | 28.21±0.71$^{a}$ | 16.42±0.97$^{b}$ | 17.12±1.09$^{c}$ |

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

The TLC (thousands/µl) mean values were significantly ($P<0.05$) higher in Group 2 (16.10±0.94, 16.84±1.02) and Group 4 (16.42±0.97, 17.12±1.09) on 15th and 29th day respectively when compared with Group 1 (12.53±0.39, 13.61±0.44). There was no significant difference in mean values between Group 3 (12.81±0.34, 13.75±0.51) and Group 1 on 15th and 29th day respectively.

The values were significantly ($P<0.05$) high in Group 4 when compared with Group 2 and Group 3 (Table 2).

There was a significant reduction in mean body weight gain in Group 4, Group 3 and Group 2 when compared to that of Group 1. Reduced body weights were significant in combined toxicity group than individual toxicities. This decrease in body weight gain is due to decreased feed and water intake as a result of hepato, renal toxicity. Reduction in body weight in cadmium intoxicated rats was in agreement with Ibraheem *et al.,* (2016); Dwivedi (2015); Kour *et al.,* (2014) and chlorpyrifos intoxicated was in agreement with Farag *et al.,* (2003) and Banan (2014).

There was a significant decrease in hematological parameters like TEC, Hb, and PCV whereas TLC values were increased in Group 2, 4 and 3 when compared to control group. Cadmium treated (Group 2) showed decreased values of TEC, Hb and PCV which was in agreement with El-Demerdash *et al.,* (2004) and AI-Asgah *et al.,* (2015) who showed $CdCl_2$ caused changes in blood indices of rats. These reduction in TEC, Hb and PCV; increased concentration of TLC in the present results were also in accordance with findings of Nahid Akhtar *et al.,* (2009) Ambali *et al.,* (2011). The reduction in TEC, Hb and PCV might be due to the increased rate of destruction or reduction in the rate of formation of erythrocytes. Increased TLC levels might indicate an activation of the rat immune system.

In conclusion, these body weight gains and haematological values were significantly differing in combined toxicity group (Group 4) when compare with individual groups (Group 2 & 3).

## Acknowledgement

The authors are thankful to the PV Narsimharao Telangana Veterinary University for providing support and necessary facilities to carry out the research work.

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**How to cite this article:**

Yadala Ravikumar, D. Madhuri, M. Lakshman, A. Gopala Reddy and Kalakumar, B. 2019. Haematological Alterations Induced by Cadmium (Cd) and Chlorpyrifos (CPF) in Male *Wistar albino* Rats. *Int.J.Curr.Microbiol.App.Sci*. 8(08): 480-485.
doi: https://doi.org/10.20546/ijcmas.2019.808.055