Biochemical alterations induced by the acute exposure to combination of chlorpyrifos and lead in Wistar rats

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Abstract
Chlorpyrifos, a well known organophosphorus insecticide and heavy metal lead, was challenged to Wistar rats to study their interactive effects on biochemical parameters (clinical pathology) after acute exposure via oral gavaging. Hematology and clinical chemistry parameters were estimated after 14 days of exposure. In addition, serum butryl and RBC cholinesterase was estimated on days 3rd and 15th of experimentation. The study was conducted using two different dose levels of chlorpyrifos and lead acetate and grouped into seven groups. The parameters of hematology and serum chemistry were analysed through automatic analyzers. No treatment related or interactive effects were noticed in hematology values except for the reduced RBC, Hb content and HCT values in lead treated animals at 1000mg/kg. A significant decrease in both serum and RBC cholinesterase enzymes were noticed in animals treated with chlorpyrifos at 50 mg/kg and in combination group (chlorpyrifos 50 + lead 1000 mg/kg), and increased inhibition along with delayed recovery was observed in the animals of combination group (i.e., chlorpyrifos plus lead). Chlorpyrifos in presence of lead increases the inhibition of both serum and RBC cholinesterase enzymes. The long lasting or persistence effects of CPF along with and lead may result in impaired cognitive functions of brain considering the role of cholinesterases in neuronal architecture of brain and other normal functioning of nervous system. Therefore, simultaneous exposure to a combination of chlorpyrifos and lead is considered to be more dangerous than to an exposure of either alone. In addition, serum chemistry revealed changes in concentrations of glucose and sodium owing to lead treatment.

Keywords: Chlorpyrifos, Lead, Cholinesterase, Combination effect.

Introduction
Acute exposure to pesticides and heavy metals at higher dose levels many times reveal altered physiological functions in blood and serum/plasma components. The presence or absence of biochemical changes in laboratory animals exposed to environmental chemicals/xenobiotics is an important tool in the overall assessment of the risk and hazards of potential human or animal exposure. The results of tests are used to identify general metabolic and pathological processes. The study of mixture of chemicals on clinical pathology parameters helps scientists and practitioners to understand the interaction or variability's. Hence, the present study was undertaken to evaluate the effect of a combination of chlorpyrifos (an OP pesticide) and lead (a heavy metal) on blood and serum biochemical parameters in the Wistar rats.

Materials and Methods
Chlorpyrifos Technical (CPF) of 98.0% purity and lead acetate (LA) of 99.1 % purity were used for the study.

Test Species and Husbandry
Healthy Wistar rats of 5- 6 weeks old were obtained from the Breeding Facility. Animals were acclimatized for a period of 5 days. The experimental room temperature was 22 ± 3°C and, 12 hours of artificial lighting and 12 hours of darkness were maintained. The relative humidity was 55 -65%. The rats were housed singly in solid floor polypropylene cages. A sterile rice husk was used as bedding material. The rats were provided with *ad libitum* laboratory rat feed and charcoal filtered, UV sterilized water. Individual animal was identified with picric acid marking over the body coat, and colored cage label having group details.

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Study Design

The animals were randomly allocated to 7 groups. Each group comprised of 5 male and 5 female rats. At the start of treatment, body weight variation among the animals was within the ± 20% of mean body weight range. All animals were treated only once during the course of experiment and were observed for a period of 14 days thereafter. The combination group animals (G4&G7) received chlorpyrifos and lead acetate simultaneously. The route of administration of test substance was through oral gavage and a dose volume of 5 mL/kg used. Chlorpyrifos was suspended in corn oil, and lead acetate was dissolved in distilled water.

On day 3rd and 15th of experimental period blood was collected from all animals. Around 0.5 mL of blood was collected in vials containing EDTA for hematology analysis. One drop of blood was taken on a clean glass slide, spread and stained with Leishman's stain for differential leucocyte count. For determination of clotting time, blood was allowed to flow in to a 7.5 cm capillary tube and the time required for clotting was recorded manually. Approximately 0.5-1.0mL for RBC acetylcholine esterase estimation and 2.0mL for clinical chemistry parameters were collected in respective labeled vials. Cholinesterase estimation was performed on day 3rd and 15th. The parameters included for the hematology and clinical chemistry were based on OECD N° 408 (1998); OECD N° 32 (2000); OPPTS 870.6200 (1996); OECD N° 424 (1997).

Dose Selection

Based on the findings of preliminary dose-range finding study, the main study was conducted using the following doses; control (group 1), chlorpyrifos-5mg/kg (group 2), lead acetate- 100mg/kg (group 3), chlorpyrifos-5mg/kg + lead acetate- 100mg/kg (group 4), chlorpyrifos-50mg/kg (group 5), lead acetate-1000mg/kg (group 6) and chlorpyrifos-50mg/kg + lead acetate-1000mg/kg (group 7).

Hematology

Erythrocytes, hemoglobin, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, platelets, clotting time, total WBC count and differential leucocyte count (lymphocyte, neutrophil, monocyte, eosinophil and basophil). Hematological parameters were analysed in hematology analyzer, Sysmex K-1000.

Clinical Chemistry

Hitachi 902, a fully automated instrument, was used for analysis of clinical chemistry parameters except sodium, potassium and chloride. The later parameters were analysed in Rapid Chem 744. The studied clinical chemistry parameters were include: glucose, protein, albumin, globulin, cholesterol, blood urea nitrogen, urea, creatinine, calcium, phosphorus, ALT, AST, ALP, ChE (serum), ChE (RBC), sodium, potassium and chloride.

Around 1.5 mL of blood was collected from each animal in clean centrifuge tubes for serum preparation. The blood was allowed to clot at room temperature and the serum was separated by centrifugation at low speed. RBC acetylcholine esterase activity was estimated through the procedure of Ellman *et al.,*(1961).

Evaluation of Data

All the parameters characterized by continuous data were subjected to Bartlett's test to meet the homogeneity of variance before conducting Analysis of Variance (ANOVA) and Dunnett' s t-test. Where the data did not meet the homogeneity of variance, Student's t-test was used.

Results

Haematology

No statistically significant differences were observed in hematological analysis of treatment group animals as compared to control group animals. A slight parallel reduction in values of RBC, Hb and HCT was observed in group 6 males (Table 1). In females, hematological parameters of treatment group females were comparable to control group females.

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Table 1: Hematology (RBC, Hb and HCT) – Group Mean Values.

Sex : Male **Period : 15th Day**

Parameter/Group		G1	G2	G3	G4	G5	G6	G7
RBC (10^6/µL)	**Mean**	6.6	6.5	6.2	6.2	6.5	6.2	6.2
	SD	0.35	0.46	0.22	0.34	0.32	0.24	0.26
Hb (g/dL)	**Mean**	14.2	13.2	13.6	13.1	13.4	12.6	13.2
	SD	0.77	0.89	0.46	1.15	0.95	0.92	0.47
HCT (%)	**Mean**	41.1	38.9	41.7	38.2	39.2	36.8	38.2
	SD	3.65	1.54	2.02	2.68	2.96	2.75	1.66

Table 2: Alterations in Clinical Chemistry – Group Mean Values.

Sex : Male **Period : 15th Day**

				Male				
Parameter/Group		**G1**	**G2**	**G3**	**G4**	**G5**	**G6**	**G7**
Glucose (mg/dL)	**Mean**	74.4	78.4	72.1	84.1	86.6	120.5**	102.0*
	SD	9.13	10.23	6.70	10.46	11.60	11.70	10.29
Sodium (mEq/L)	**Mean**	147.1	148.2	150.0*	151.8**	149.5	150.6**	151.1**
	SD	1.23	1.28	0.91	0.58	1.14	1.0	0.69
				Female				
Sodium (mEq/L)	**Mean**	142.5	143.6	143.7	146.0**	144.5	146.2**	146.2**
	SD	0.96	1.02	1.15	1.23	1.27	1.26	1.12
Chloride (mEq/L)	**Mean**	104.5	105.1	105.8	107.1	107.0	107.1	107.4*
	SD	1.25	1.97	0.95	2.50	2.15	2.28	2.33

Key : * = significant at 5% level ($p \le 0.05$); **= significant at 1% level ($p \le 0.01$)

Clinical Chemistry

Males : On day 3, the serum and RBC cholinesterase activity of groups 5 and 7 and serum cholinesterase in group 4 animals were significantly decreased as compared to control group animals (Figure 1). On day 15, animals treated with CPF at 50mg/kg and animals treated with combination of CPF (50mg/kg) and LA (1000 mg/kg) revealed reduction in RBC cholinesterase activity of 20.7% and 25.5% respectively (Figure 1).

Other findings include significant increase in the concentration of glucose in groups 6 and 7 animals, sodium in groups 3, 4, 6 and 7 as compared to control animals (Table 2). Other parameters such as total protein, albumin, globulin, cholesterol, bilirubin, creatinine, BUN, urea, calcium, potassium, ALT, AST, ALP of treatment group animals were very much comparable to control group animals.

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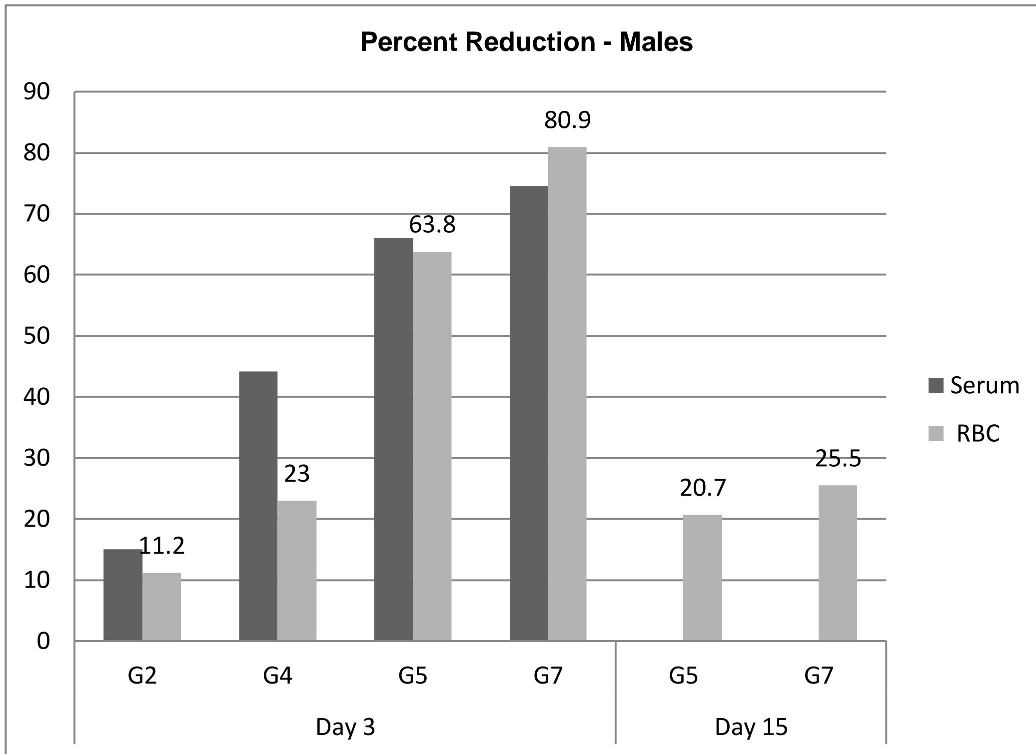

Figure 1: Percent Reduction in Cholinesterase Enzymes (Serum and RBC) – Males.

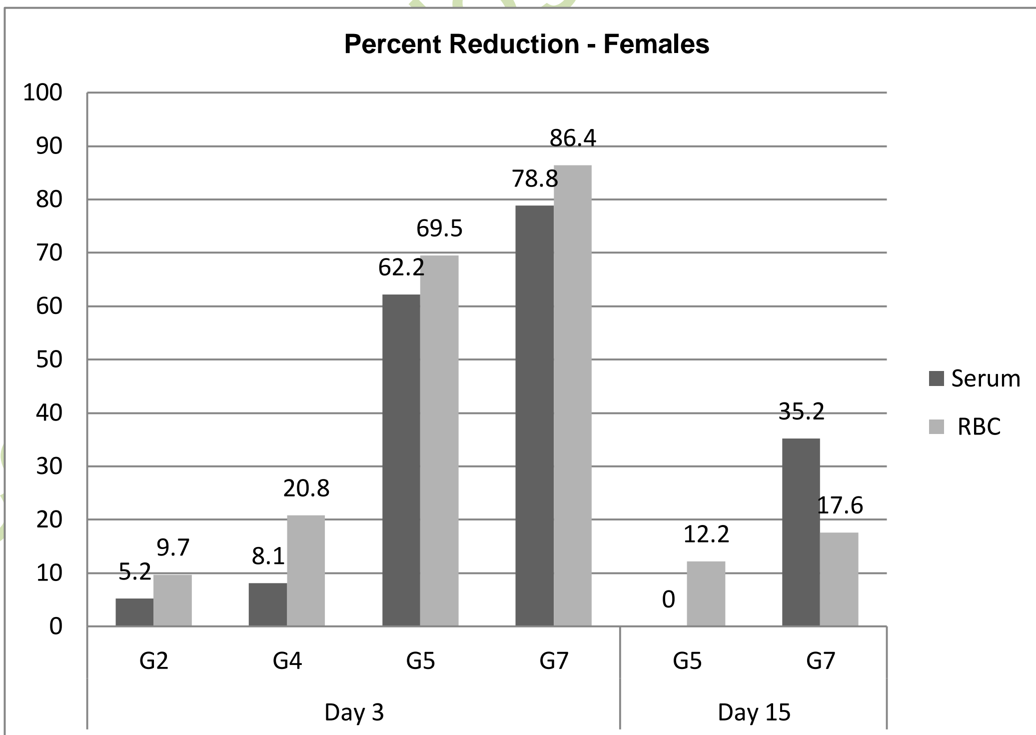

Figure 2: Percent Reduction in Cholinesterase Enzymes (Serum and RBC) – Females.

Females: Like males, females also revealed similar trend on days 3 and 15. On day 15, reduced activity of RBC cholinesterase i.e., 12.2 % in group 5 and 17.7 % in group 7 was observed as compared to control group animals. The group 7 animals also revealed reduction in seum cholinesterase activity (35.2%). A slight reduction was also observed in the activities of serum and RBC cholinesterases at lower dose levels of CPF, on day 3 (Figure 2). In addition, concentrations of sodium in groups 4, 6 and 7 and chloride in group 7 animals were significantly increased as compared to control group animals (Table 2). Remaining parameters of treatment group animals were comparable to control group animals.

Discussion

The present study has evaluated the effects of an acute combination of chlorpyrifos and lead on hematology and clinical chemistry parameters of blood. There seems to be no effect of chlorpyrifos or lead either individually or in combination on hematological parameters except for a slight decrease in RBC count, haemoglobin count and HCT value of high lead group (group 6). This non-significant decrease is understandable in the light of the known anaemic effect of lead[6]. No interactive effect was manifested.

On day 3, the serum and RBC cholinesterases in groups 5 and 7 and serum cholinesterase in group 4 animals (males) were significantly decreased. It is well known that OP compounds acts by inhibiting enzyme cholinesterase. In combination groups of males i.e., groups 4 and 7, reduction in both serum and RBC cholinesterase was comparatively higher as compared to groups 2 and 5 respectively, i.e., treated with chlorpyrifos alone. The reduction in RBC cholinesterase observed in groups 5 and 7 animals on day 15 suggest that the enzyme activity has not returned to normalcy completely after 14 days of exposure. The recovery of enzyme activity to normal level depends on the dose of chlorpyrifos administered (Extoxnet, 1993).

Local activation of CPF to CPF oxon and availability of oxon in general circulation contributes to cholinergic toxicity. Higher inhibition of both types of cholinesterase enzyme in animals treated with lead and chlorpyrifos combination group possibly due to higher availability of chlorpyrifos at the site of activation. Owing to formation of chelating complex between metal (lead) and OP (Tomlin, 1997) bypassing or escaping of parent chlorpyrifos from the detoxification mechanism of liver may be the probable reason for higher availability in general circulation. It is known that maximum amount of lead accumulates in RBC after absorption from the digestive system. This unique property of lead also may contribute to higher amount of parent CPF in circulation with their covalent association. Hence, these conditions might be providing extrahepatic activation of chlorpyrifos to its oxon and thereby providing additional scope for inhibition of its target enzymes.

Other findings such as, increase in the concentrations of glucose in groups 6 and 7 males and sodium in lead treated males and females observed (Table 2) could be correlated to lead treatment. Stevenson *et al.,* (1976) have reported effects of subacute and chronic lead treatment on glucose homeostasis and renal cyclic AMP metabolism in rats. Rats treated for short term revealed marked decrease in the insulinogenic index in 15 minutes after the administration of a glucose load. They concluded that increased glucose synthesis as well as suppressed pancreatic function may be responsible for lead-induced disturbances in glucose homeostasis.The variation observed in the sodium concentration might be associated with functional alteration in the proximal tubules of the nephron after acute exposure. Dysfunction of the proximal tubules after acute exposure is a common finding at higher level of acute lead exposure (ATSDR, 1999).

Conclusion

The severity and persistent inhibition of cholinesterases for longer duration observed in lead plus chlorpyrifos treated animals suggest increased cholinergic toxicity of chlorpyrifos in presence of lead. Since cholinesterase plays an important role in neuronal architecture of brain and other normal functioning of nervous system, and hence, the long lasting or persistence effects of CPF along with and lead may result in impaired cognitive functions of brain. Hence, exposure to combination of chlorpyrifos and lead simultaneously is more dangerous than to an exposure of either alone.

Acknowledgement

Authors would like to thank Jai Research Foundation, Valvada, Gujarat, for providing the necessary facility to conduct the experiment.

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