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CHLORPYRIFOS IMPAIRS HAEMATOLOGICAL AND HEPATIC TISSUE FUNCTIONS BY PRODUCING OXIDATIVE STRESS

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ABSTRACT

Chlorpyrifos is a widely used organophosphate pesticide. The present study was undertaken to examine the effect of chlorpyrifos on haematological and blood biochemical variables in rat. Chlorpyrifos was administered orally to male rat in different doses (19.4, 38.8, 58.2mg/kg BW/day) for consecutive 10, 20, 30 days. We found that red blood cell (RBC) count and haemoglobin (Hb) level were decreased while total white blood cell (WBC) count was increased, along with significant increase in serum markers of hepatic damage like- aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), total bilirubin, in test groups. Further, chlorpyrifos resulted in a significant increase in lipid peroxidation of serum with decreased activities of antioxidant enzymes like superoxide dismutase (SOD) and catalase (CAT), suggesting that chlorpyrifos may cause damage of hepatic tissue due to oxidative stress. Thus, we conclude that chlorpyrifos impairs the homeostasis of haematological as well as hepatic tissue functions of rat presumably by inducing oxidative stress.

KEY WORDS: **Chlorpyrifos, transaminases, oxidative stress, blood.**

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INTRODUCTION

Chlorpyrifos is an organophosphate pesticide commonly known as Dursban. It is a broad spectrum insecticide used widely in agricultural aspect and to control pest (1, 2). It was first introduced in 1965; since then it is used globally to control crop and household pest and to reduce insect damage (3). Human beings are getting exposed to this chemical through ingestion of vegetables, inhalation, in addition to dermal exposure due to indiscriminate use of this chemical (4). After entering the body it is absorbed quickly from the alimentary and respiratory tract, then metabolized and excreted through urine and faeces. Various toxic effects of chlorpyrifos in different animal models and also on human workers have been reported (5). One study reported that chlorpyrifos causes significant reduction in total RBC and WBC count in the *Tilapia guineensis* (6). Whereas another study showed that chlorpyrifos decreases erythrocyte count, Hb level and total protein content in fresh water fish *Cyprinus carpio* with a significant elevation of leukocyte count (7). A study on young chickens stated that chlorpyrifos causes enhancement of different haematological variables and significant alteration in the activities of transaminases and serum protein level (8).There are several reports regarding the effect of chlorpyrifos on mammalian body. One such study reported significant reduction in the RBC count, Hb level and hematocrit value in chlorpyrifos treated rats (9). But another study reported opposite effect of chlorpyrifos on the same said variables (10). On the other hand, different study revealed that chlorpyrifos induces significant enhancement of activities of different hepatic enzymes (11). Several studies also showed that chlorpyrifos produces oxidative stress in the rat erythrocyte by decreasing the activities of antioxidant enzyme and increasing the degree of lipid peroxidation in the RBC biomembrane (12, 13). So the present study has been aimed to elucidate the toxic effect of different doses of chlorpyrifos on certain key haematological and blood biochemical parameters of rat after different duration of exposure.

MATERIALS AND METHODS

Animal and strain

Studies were carried out on adult male albino rats of Charles Foster strain with an initial body weight of 120-130gm.The animals were obtained from the local animal breeding center and maintained following the animal use protocols approved by the Kalyani University Animal Care Committee in accordance with national guidelines. The animals were kept under standard environmental conditions (12 hour artificially day-night cycle and normal room temperature) during the acclimatization and experimental period. Rats were provided with food and water *ad libitum*.

Animal exposure and grouping

After 2 weeks of acclimatization to the environment the rats were divided into nine experimental groups and three control groups. Different doses of chlorpyrifos were administered to animals by oral gavage.

Table 1
Experimental grouping of animals

Animal group	Dose	Duration
Control- a	Consumed distilled water	For 10 days
Control- b	Consumed distilled water	For 20 days
Control- c	Consumed distilled water	For 30 days
Group-Ia	Consumed 19.4mg/kg.body wt./day.(10%of LD_{50})	For 10 days
Group-Ib	Consumed 19.4mg/kg.body wt./day.(10%of LD_{50})	For 20 days
Group-Ic	Consumed 19.4mg/kg.body wt./day.(10%of LD_{50})	For 30 days
Group-IIa	Consumed 38.8mg/kg.body wt./day.(20% of LD_{50})	For 10 days
Group-IIb	Consumed 38.8mg/kg.body wt./day.(20% of LD_{50})	For 20 days
Group-IIc	Consumed 38.8mg/kg.body wt./day.(20% of LD_{50})	For 30 days
Group-IIIa	Consumed 58.2mg/kg.body wt./day.(30% of LD_{50})	For 10 days
Group-IIIb	Consumed 58.2mg/kg.body wt./day.(30% of LD_{50})	For 20 days
Group-IIIc	Consumed 58.2mg/kg.body wt./day.(30% of LD_{50})	For 30 days

Reagents and chemicals used

All the reagents were of analytical grade. Chlorpyrifos was purchased from Dow Agrosciences PVT. Ltd, India; thiobarbituric acid (TBA) was procured from Himedia, India; and pyrogallol and trichloroacetic acid (TCA) were procured from Merck, India. Besides, the commercial kits were procured from Span diagnostics and Merck, India.

Sample collection

After the completion of treatment rats were sacrificed by cervical dislocation on the 24th hour after the last dose. Blood sample was obtained by puncturing the heart using a syringe. The blood sample was collected in small glass vials containing EDTA as an anticoagulant for haematological studies. The serum sample was obtained by centrifugation of blood sample at 3,000 rpm for 15min at 4^0C. The serum was preserved in minus 20^0C for further biochemical studies.

Haematological study

Haemoglobin concentration was estimated by the acid hematin method according to the Sahli's method (14). Both RBC and WBC count were measured by Neaubaur Hemocytometer according to the method of Paul and Paul (15).The total bilirubin was measured by using ERBA assay kit, produced by Transasia Bio-Medicas, India following by Diazo method.

Biochemical assay

Both serum AST and ALT were measured by using assay kits (code no -2506, Lot no -4000006215 and code no 2506, Lot no 40000i0095 respectively supplied by Span Diagnostics Ltd, India) according to the Reitman and Frankel method. Serum ALP was measured by using an assay kit (Merck, India, Catalog no of assay kit was 1.10937) followed by DGKC method. Total protein content of serum was measured by the method of Lowry et al., 1951 (16) with bovine serum albumin as the standard.

Assay of oxidative stress induced parameters

Serum superoxide dismutase (SOD) activity was measured as per the protocol of Marklund and Marklund, 1974 (17).One unit of SOD was defined as the enzyme activity that inhibits the auto-oxidation of pyrogallol by 50%. Serum catalase (CAT) activity was measured following the protocol of Beers and Sizer method, 1952 (18). The amount of malondialdehyde (MDA) in serum was estimated according to the protocol of Bug and Aust, 1978 (19).

Statistical Analysis

All results were presented as mean±SEM. Statistical comparisons between the values obtained for control and in treated rat were carried out by using a Student's t test for paired values and $p \leq 0.05$ was considered as significant.

RESULTS

Haematological Study

From the haematological study it has been seen that all the variables become changed in the

test groups. The level of blood haemoglobin (Hb) and red blood cell (RBC) count were decreased significantly in higher exposure dose; and total white blood cell (WBC) count and total bilirubin level were also increased significantly in high chlorpyrifos treated groups compared to the respective control group in a duration dependant manner (Table-2).

Table 2

Haematological parameters of chlorpyrifos exposed groups, compared to respective control groups.

BLOOD PARA - METER	DURATION OF EXPOSURE											
	10Day				20Day				30Day			
	Control group a	TREATED GROUP			Control group b	TREATED GROUP			Control group c	TREATED GROUP		
		Ia	Ib	Ic		IIa	IIb	IIIc		IIIa	IIb	IIIc
		10%	20%	30%		10%	20%	30%		10%	20%	30%
Hb(gm/dl)	14.40± 0.18	14.20± 0.72	14.0± 0.50	13.80± 0.39	14.20± 0.48	12.40±0. 18*	12.20±0. 54*	12.00± 0.41*	14.60±0. 71	14.00±. 48	12.80± 0.84*	12.20± 0.48*
RBC(cu mm)	7.20 ±0.13	7.10 ±0.16	7.10 ±0.11	7.00 ±0.09	7.30 ±0.13	7.10 ±0.09	7.00 ±0.18	7.10± 0.09	7.20± 0.13	7.00± 0.09	6.70± 0.20	6.04± 0.21*
WBC(cu mm)	4900.00 ±135.40	5000.0 0±158. 11	5200.0 0±122. 47	5400.0 0±91.2 9	4800.00± 158.11	4900.00± 91.29	5300.00± 129.10	5900.00 ±158.11 *	5000.00± 91.29	5600.00 ±91.29	6200.00±1 47.20*	7800.00±1 08.01*
Total bilirubin (mg/dl)	0.42± 0.02	0.42± 0.02	0.44± 0.03	0.46± 0.03	0.42± 0.02	0.44± 0.03	0.50± 0.04*	0.62± 0.03*	0.44± 0.02	0.48± 0.02	0.60± 0.03*	0.80± 0.04*

Values are represented as Mean±SEM (n=6), *p<0.05 vs. control group.

Biochemical study

Serum biochemical variables were seen to be altered in all the test groups (Table -3). The activities of transaminases like aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP) were significantly increased in higher dose groups; and total serum protein level was significantly decreased in all treated groups than the control group.

Table 3

Biochemical parameters of chlorpyrifos exposed groups, compared to respective control groups.

BIOCHEMICAL VARIABLES	DURATION OF EXPOSURE											
	10days				20days				30days			
	Control group a	TREATED GROUP			Control group b	TREATED GROUP			Control group c	TREATED GROUP		
		Ia	Ib	Ic		IIa	IIb	IIc		IIIa	IIIb	IIIc
		10%	20%	30%		10%	20%	30%		10%	20%	30%
AST (U/L)	120.20±4.0 8	122.00± 1.83	124.00± 3.56	126.00±2 .58	118.00±.58	120.00±3 .37	126.00±3 .46	132.00±2 .58	116.00±7. 07	118.00± 3.37	130.00 ±4.55*	148.00±4 .97*
ALT (U/L)	90.00± 1.83	92.00±. 16	94.00± 4.97	102.00±3 .16	122.00±0.47	124.00±1 .41	140.40±2 .64	156.60±1 .68*	96.00±3.1 6	98.00±4 .40	102.00 ±5.48	130.00±6 .32*
ALP (U/L)	170.00±3.3 7	170.00± 1.83	172.00± 2.58	176.00±1 .83	172.00±2.45	174.00±3 .16	177.00±1 .29	198.00±9 .83*	170.00±1. 83	174.00± 3.65	180.00 ±3.16	194.00±2 .16*
TOTAL PROTEIN(m g /ml)	8.94±.375	9.14±0. 172	8.4±0.53 8	8.1±0.29 5	8.73±.114	9.073±0. 144	7.46 ±.344*	6.76±0.1 78*	8.84±0.14 2	9.22±0. 109	6.88±0. 231*	6.36±0.1 33*

Values are represented as Mean±SEM (n=6), *p<0.05 vs. control group.

Oxidative stress related study

The activities of serum antioxidant enzymes like SOD and CAT were decreased significantly in all test groups compared to the control group. The production of MDA, a biomarker of lipid peroxidation was increased significantly in all treated groups (Table -4).

Table-4

Oxidative stress induced parameters of chlorpyrifos exposed groups, compared to respective control groups.

OXIDATIVE STRESS RELATED VARIABLES	DURATION OF EXPOSURE											
	10day				20day					30day		
	Control group a	Treated group			Control group b	Treated group			Control group c	Treated group		
		Ia	Ib	Ic		IIa	IIb	IIc		IIIa	IIIb	IIIc
		10%	20%	30%		10%	20%	30%		10%	20%	30%
SOD (U/mg protein)	6.32±0.613	5.27±0.479	3.95±0.671	3.84±0.350*	7.69±0.987	5.23±0.509	5.00±0.564	3.96±0.553*	6.70±0.654	4.78±0.412	3.41±0.549*	2.48±0.660*
CAT (U/mg protein)	28.42±0.561	24.46±0.583*	16.96±0.726*	23.46±0.533*	27.30±1.011	25.10±1.746	17.60±1.340*	14.70±0.629*	26.86±0.705	10.86±0.508*	8.46±0.635*	6.90±1.137*
MDA (nmol /mg protein)	8.69±0.45	8.67±0.40	12.24±0.49*	13.67±0.54*	8.70±0.61	10.22±0.99	11.85±0.84*	14.32±0.58*	8.82±1.05	10.86±0.87	13.93±0.87*	16.23±1.62*

Values are represented as Mean±SEM (n=6), *p<0.05 vs. control group.

DISCUSSION

We found that chlorpyrifos decreases RBC count in all treated groups. From this resultwe suggest that chlorpyrifos might inhibit the production of RBC from the bone marrow pluripotent RBC precursor cell, presumably by suppressing erythropoiesis. This finding is corroborated by our result that chlorpyrifos decreases blood haemoglobin level in rats. Therefore, the decrease in RBC count in the blood is one of the probable factors for the decrease in whole blood haemoglobin level. We also observed increase in total blood WBC count in chlorpyrifos exposed rats. This suggests that chlorpyrifos may suppress WBC related immunity as a result of the activation of the negative feedback loop of the servo mechanism related to xenobiotic induced defense mechanism. In this study we also found increase in activities of serum AST, ALT, ALP and decrease in total serum protein level in chlorpyrifos exposed rats. These results suggest that chlorpyrifos might induce the damage of cellular architecture of liver tissue, because the serum enzyme activities measured are the specific marker of hepatic tissue injury. We found the activities of serum antioxidant enzymes have been decreased and the amount of MDA marker of lipid peroxidation, has been increased in chlorpyrifos treated rats. These results indicate that chlorpyrifos induces oxidative stress in the blood and liver tissues by inhibiting antioxidant activity and inducing degree of lipid peroxidation. So we may conclude that the release of hepatic injury marker enzymes into the blood is due to oxidative stress induced damage of the hepatic tissue cells.

CONCLUSION

From the present investigation we may conclude that chlorpyrifos impairs haematological and hepatic tissue functions by producing oxidative stress.

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