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CLINICO-HAEMATO-BIOCHEMICAL CHANGES DUE TO THE INDUCED ACUTE TOXICITY OF CHLORPYRIFOS IN RABBITS (*Oryctolagus Cuniculus)*

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ABSTRACT

The present study was aimed to assess the acute toxic effects of chlorpyrifos using rabbits as mammalian model. Chlorpyrifos @ 1000 mg kg^{-1} body weight as single oral dose (Lara-909 containing 50% chlorpyrifos) caused marked clinical changes with 50% mortality over 24 hr. Death was associated with muscular spasms and unconsciousness. Haematology showed a significant increase in mean lymphocyte and basophil counts. The blood glucose, total protein, albumin, globulin, alanine transaminase, aspartate aminotransferase, blood urea nitrogen and creatinine were not altered significantly. It was concluded that acute chlorpyrifos toxicity was characterized by marked clinical changes and mortality without any marked alterations in the haemato-biochemical indices.

Key words: Chlorpyrifos, pathology, rabbit, toxicity

INTRODUCTION

Organophosphorus insecticides have widely been recognized as health hazard due to their wide-spread use in agriculture and subsequent release into the environment (Al-Haj *et al*., 2005). Besides fatalities caused by high dose, the exposure of animals to low dose organophosphorus insecticides has been found to widely affect the body including organ-specific lesions in central nervous system, liver, kidney and generalized effects like immune-suppression, teratogenesis, carcinogenesis and metabolic disorders (Lengyl *et al.*, 2005). Chlorpyrifos (O,O-diethyl 0-[3,5,6-] trichloro-2-pyridinol phosphorothionate is widely used in agriculture and also for domestic pest control (Gralewicz *et al*., 2002). Experimental studies have revealed that chlorpyrifos has dose-related effects on liver, testes, spermatozoa and nervous system, besides causing wide ranges of clinicopathological alterations (Akhtar *et al*., 2009; Solati *et al.*, 2011). Since chlorpyrifos is widely used in agriculture so it remains a potential health hazard to animals and humans. Hence, a study was conducted to assess the patho-logical effects of acute chlorpyrifos toxicity on clinico-haemato-biochemical parameters in rabbits.

MATERIALS AND METHODS

A total of 12 Gray giant rabbits of either sex, each 1.0-1.5 kg in weight, were used in the present study. They were maintained in cage system under standard laboratory conditions. They were

acclimatized to rearing conditions for one week before the start of experiment. The experimental protocol was approved by the Institutional Animal Ethics Committee, Faculty of Veterinary Sciences and Animal Husbandry, SKUAST-K vide No. AU/FVS/C-9/10507-12 dated 27-2-2012 and conformed to the guidelines for the Care and Use of Laboratory Animals. The rabbits were divided into 2 groups of 6 rabbits each by equal random allocation. Group-I served as control. Group-II rabbits were given single oral dose of Lara-909 (containing 50% chlorpyrifos) @ 1000 mg chlorpyrifos kg^{-1} body weight ($\approx LD_{50}$ dose) [Fikes, 1992]. The rabbits were monitored continuously for clinical signs. Respiration rate, pulse, body weight, body temperature were noted.

Haematological studies were recorded on day 0 and 24 hr after intoxication. Blood samples were collected using heparin @ 20 units mL^{-1} as blood anticoagulant. Sodium fluoride (2.5 mg mL^{-1} blood) was added as glucose preservative. The investigations were carried using MS_4 multi-species haematological analyzer (Melet Schloesing Laboratoires, France). The parameters studied included total erythrocyte count (TEC), haemoglobin (Hb) concentration, packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular haemoglobulin (MCH), mean corpuscular haemoglobin concentration (MCHC), total leukocyte count (TLC) and differential leucocyte count (DLC).

For biochemical estimations plasma was collected following centrifugation of blood and stored in multiple aliquots at -20°C until use. To avoid freezing and thawing effects during multiple analyses, the samples were divided into sufficient number of aliquots for laboratory investigations. Biochemical parameters were recorded on day 0 and 24 hr after intoxication. The biochemical investigations included blood glucose (glucometer method); plasma proteins *viz.*, total protein (Biuret method), albumin (BCG dye binding method), globulin (difference method); plasma enzymology *viz*., asparatate transaminase (AST) and alanine transaminase (ALT) (IFCC method/ Reitman and Frankel's method); kidney function tests (KFT) *viz.*, blood urea nitrogen (BUN) (Berthelot method) and creatinine (modified Jaffe's method) using diagnostic kits (Aspen Laboratories, New Delhi, India) and semi-automatic blood chemistry analyzer (model ERBA CHEM-PRO). The data was analyzed by t-test using SPSS statistical software.

RESULTS AND DISCUSSION

Chlorpyrifos treated rabbits appeared dull and depressed with decreased activity and partial to complete anorexia. They started lying down and lacrimating within one hour of intoxication. Bradypnea, mydriasis, muscle contractions and staggering were observed. Rabbits fell down suddenly and revealed uneasiness and paddling of legs followed by unconsciousness before death. Three out of six chlorpyrifos treated rabbits died within 24 hr. In intoxicated rabbits body weight showed no significant change. The mean body temperature at 24 hr was significantly lower than that of control (Table 1). The possible mechanism of toxicity has explained by Klassen (2008). Signs and symptoms developed within minutes to hours after an acute exposure to chlorpyrifos (Thompson and Richardson, 2004). Acute death due to organophosphates was ascribed to respiratory failure caused by the inhibition of respiratory centers in the brain stem, broncho-constriction and increased

Table 1: Effect of acute chlorpyrifos toxicity on body weight and temperature in rabbits

Parameter	Time period (hrs)	Groups	
		Chlorpyrifos	Control
Body weight (kg)	0	1.26 ± 0.05^{aA}	1.38 ± 0.06^{aA}
	24	1.25 ± 0.07^{aA}	1.38 ± 0.06^{aA}
Temperature (^oC)	0	38.35 ± 0.22^{aA}	38.47 ± 0.27^{aA}
	24	37.68 ± 0.22^{aA}	38.64 ± 0.14^{aB}

Means for a parameter in different rows with common 'lowercase alphabet' superscript and in different columns with at least one common 'uppercase alphabet' superscript do not differ significantly ($P \geq 0.05$).

Table 2: Effect of acute chlorpyrifos toxicity on haemato-logical indices in rabbits (mean ± SE)

Parameters	Time period (hr)	Groups	
		Chlorpyrifos	Control
Haemoglobulin (g dL^{-1})	0	12.51 ± 0.61^{aA}	12.00 ± 0.33^{aA}
	24	11.93 ± 0.24^{aA}	12.10 ± 0.28^{aA}
PCV (%)	0	42.03 ± 1.20^{aA}	41.86 ± 1.03^{aA}
	24	42.00 ± 2.01^{aA}	41.63 ± 0.54^{aA}
TEC (10^6 mm^{-3})	0	6.74 ± 0.30^{aA}	6.40 ± 0.30^{aA}
	24	5.96 ± 0.43^{aA}	6.28 ± 0.21^{aA}
MCV (fL)	0	62.76 ± 4.25^{aA}	65.82 ± 2.02^{aA}
	24	71.74 ± 1.41^{aA}	66.68 ± 2.42^{aA}
MCH (Pg)	0	18.70 ± 1.55^{aA}	18.86 ± 0.60^{aA}
	24	20.22 ± 1.48^{aA}	19.35 ± 0.63^{aA}
TLC (10^3 mm^{-3})	0	8.58 ± 0.80^{aA}	9.18 ± 0.45^{aA}
	24	9.30 ± 0.27^{aA}	9.24 ± 0.24^{aA}
Neutrophils (%)	0	32.30 ± 4.74^{aA}	0.00 ± 1.71^{aA}
	24	32.33 ± 2.02^{aA}	29.66 ± 2.36^{aA}
Lymphocytes (%)	0	31.05 ± 3.34^{aA}	18.67 ± 0.66^{aB}
	24	61.00 ± 1.00^{bA}	18.31 ± 1.03^{aB}
Monocytes (%)	0	10.50 ± 1.14^{aA}	3.57 ± 0.49^{aB}
	24	4.33 ± 0.88^{aA}	3.45 ± 0.60^{aA}
Eosinophils (%)	0	3.66 ± 1.14^{aA}	0.10 ± 0.02^{aB}
	24	1.66 ± 0.33^{aA}	0.10 ± 0.02^{aA}
Basophils (%)	0	0.56 ± 0.20^{aA}	0.01 ± 0.00^{aA}
	24	0.66 ± 0.33^{aA}	0.01 ± 0.00^{aB}

Means for a parameter in different rows with at least one common 'lowercase alphabet' superscript and in different columns with at least one common 'uppercase alphabet' superscript do not differ significantly.

bronchial secretion and flaccid paralysis of respiratory muscles (Lotti, 2001). Similar findings have been reported by Wilmer *et al*. (1992), except for body weight. The non-significant variation in body weight in present study might be due to the short study period. Hypothermia may be attributed to agonal vascular disturbances or CNS mediated altered thermo-regulation. However, further tailored investigations are warranted to elucidate the mechanism. In chlorpyrifos treated rabbits the mean values of Hb, PCV, TEC, MCV, MCH, MCHC, TLC and differential counts of neutrophil, monocyte and eosinophil at 24 hr did not differ significantly from the baseline values or that of control rabbits. However, the mean lymphocyte and basophil counts increased significantly (Table 2) which

Table 3: Effect of acute chlorpyrifos toxicity on biochemical parameters in rabbits (Mean ± SE)

Parameters	Time period (hr)	Groups	
		Chlorpyrifos	Control
Glucose (mg dL^{-1})	0	114.53 ± 4.78^{aA}	111.00 ± 4.2^{aA}
	24	107.70 ± 1.47^{aA}	113.66 ± 5.32^{aA}
Total Protein (gmdL^{-1})	0	5.93 ± 0.42^{aA}	6.00 ± 0.34^{aA}
	24	7.08 ± 0.46^{aA}	5.81 ± 0.40^{aA}
Albumin (g dL^{-1})	0	3.77 ± 0.25^{aA}	3.88 ± 0.38^{aA}
	24	4.24 ± 0.48^{aA}	3.79 ± 0.38^{aA}
Globulin (gm dL^{-1})	0	2.16 ± 0.33^{aA}	2.12 ± 0.11^{aA}
	24	2.84 ± 0.44^{aA}	2.01 ± 0.14^{aA}
AST (IU L^{-1})	0	23.42 ± 2.18^{aA}	14.85 ± 3.41^{aA}
	24	26.26 ± 3.19^{aA}	15.49 ± 3.96^{aA}
ALT (IU L^{-1})	0	25.93 ± 2.80^{aA}	17.98 ± 3.83^{aA}
	24	26.89 ± 2.66^{aA}	16.54 ± 4.24^{aA}
BUN (mg dL^{-1})	0	32.57 ± 3.62^{aA}	29.96 ± 3.06^{aA}
	24	34.57 ± 7.60^{aA}	30.78 ± 3.41^{aA}
Creatinine (mg dL^{-1})	0	0.82 ± 0.10^{aA}	0.88 ± 0.05^{aA}
	24	0.89 ± 0.12^{aA}	0.97 ± 0.05^{aA}

Means for a parameter in different rows with at least one common 'lowercase alphabet' superscript and in different columns with at least one common 'uppercase alphabet' superscript do not differ significantly.

is in accordance with reports of Krishna and Ramachandran (2009).

The mean values of blood glucose, total protein, albumin, globulin, SGOT, SGPT, BUN and creatinine at 24hr in chlorpyrifos treated rabbits were comparable to the base line and control values (Table 3). The changes observed in these biochemical indices are in accordance with earlier report in rats (Krishna and Ramachandran, 2009). However earlier studies carried for longer periods had shown marked alterations in plasma protein levels and kidney functions due to chlorpyrifos (Ambali *et al*., 2010; Mansour and Mossa, 2010) intoxications which has been attributed to marked hepatotoxic and nephrotoxic effects produced by chlorpyrifos. It was concluded that acute chlorpyrifos toxicity was characterized by marked clinical changes and mortality without any marked alterations in the haemato-biochemical indices.

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