Applied Biological Research 17 (1): 48-54; (2015)
DOI: 10.5958/0974-4517.2015.00007.5

HAEMATO-BIOCHEMICAL ALTERATIONS IN INDUCED CHLORPYRIFOS TOXICITY IN BROILER CHICKENS

Henna Wani*, Shafiqur Rahman, Shagufta Azmi, Mudasir Sultana[1], Navdeep Kour and Amit Khajuria

Division of Veterinary Pathology, [1]Division of Veterinary Pharmacology & Toxicology, Faculty of Veterinary Sciences and Animal Husbandry, SK University of Agricultural Sciences and Technology of Jammu, R.S. Pura, Jammu – 181102, Jammu & Kashmir (India)
**e-mail: dr.henna02@gmail.com*

(Received 8 November, 2014; accepted 1 February, 2015)

ABSTRACT

Effect of graded doses of chlorpyrifos on haemato-biochemical indices in broiler chickens was investigated. A total of 72 broiler chickens, 3 week old, were divided into four groups (I to IV) consisting of 18 birds each. Group I served as control, whereas groups II, III and IV received 3.2, 1.6 and 0.6 mg kg^{-1} B.W. chlorpyrifos in corn oil daily for 6 weeks. Blood samples were collected in sterile vials containing EDTA (@ 1 mg mL^{-1}) as anticoagulant for haematological studies and without anticoagulant for biochemical parameters. Mean values of Hb, PCV, TEC, MCV, MCH, MCHC, TLC and lymphocyte counts decreased significantly in all the treatment groups, whereas platelet count increased significantly. Serum total protein, albumin and A:G ratio showed decrease, whereas serum ALT, AST, RBC acetyl cholinesterase and butryl-cholinesterase activities exhibited increase. The study revealed that chlorpyriphos has haemotoxic, immune-toxic, hepatotoxic, nephrotoxic and neurotoxic effects in broiler chickens.

Key words: Broiler chicken, chlorpyrifos, hematology, serum biochemistry

INTRODUCTION

Organophosphate compounds are the most widely used insecticides accounting for 50% of global insecticidal use (Casida and Quinstad, 2004) and have widely been recognized as a health hazard due to environmental contamination (Al-Haj *et al.,* 2005). Exposures to high doses cause fatalities while low doses cause organ specific lesions in central nervous system (Lengyl *et al.,* 2005), liver (Gomes *et al.,* 1999) and kidney (Kossmann *et al.,* 1997), and generalized effects like immunosuppression, teratogenesis, carcinogenesis and metabolic disorders. Among organo-phosphates, chlorpyrifos has principally been used as a pesticide in agricultural sector, in home gardens as well as indoors including poultry houses to get rid of fleas, spiders and flies (Lemus and Abdelghani, 2000; Lengyl *et al.,* 2005). United States Environmental Protection Agency (USEPA) has restricted some of its domestic use due to toxicity. Also, USEPA has categorized chlorpyrifos as class II toxin (Eisler, 2000). Despite this, chlorpyrifos remains one of the most widely used organo-phosphate insecticides.

Chlorpyrifos is a sulphur containing organophosphate compound with P=S moiety having IUPAC name O, O-diethyl O-3, 5, 6-trichloro-2 pyridyl phosphorothioate, with molecular formula

$C_9H_{11}Cl_3NO_3PS$. Unlike other insecticides it remains in soil for long time. It has been detected in poultry egg, meat and cow milk and milk products (Rawat *et al.*, 2003). Toxicity has largely been associated with irreversible inhibition of acetylcholinesterase (AChE) resulting in the accumulation of acetylcholine in cholinergic receptors (Eaton *et al.*, 2008). Since chlorpyrifos is frequently used as insecticides by agriculturists in Jammu & Kashmir state, thereby it might be gaining an easy access to our environment. Chlorpyrifos remains a potential source of intoxication in animals and humans. The present study was, therefore, aimed to elucidate haemato-biochemical changes in birds given chlorpyrifos in different doses at various intervals.

MATERIALS AND METHODS

The experiment protocol used in this study was approved by Animal Ethical Committee (IAEC - 862/ac/04/CPCSEA-16-12-2004). The study was conducted on seventy two (72) broiler birds of 21 day old of either sex at the Divisional animal house in the year 2013. These birds were procured from a private hatchery in Jammu and maintained on commercially available feed for birds obtained from Shalimar Feeds Pvt. Ltd., Bari Brahmana, Jammu (India). On arrival, all the birds were examined for any abnormality and overt ill health and weighed. The birds were given a series of vaccines and all sanitary and hygienic measures strictly observed. All the experimental birds were acclimatized to the experimental conditions for 3 weeks with proper identical housing, feeding and watering (*ad libitum*). After acclimatization for 21 days, the birds were randomly divided into four groups of 18 birds each. Group I served as control (no chlorpyriphos treatment) while group II, III and IV birds were given chlorpyrifos (commercially obtained from Tata Rallis India Ltd., Mumbai as Tafaban) @ 3.2, 1.6 and 0.64 mg kg^{-1} body weight, respectively, orally in corn oil for a period of 6 weeks and 6 animals from each group were sacrificed at 2, 4 and 6 week's interval.

The blood samples were collected using EDTA (ethylene diamine-tetra-acetic acid, (@ 1mg mL^{-1} blood as anticoagulant at all the three sacrificing intervals and used for the estimation of Hb, PCV, TEC, TLC and DLC as per standard procedures (Jain, 1986) and the mean values for corpuscular volume and corpuscular haemoglobin concentration were derived from the values of Hb, PCV and TEC by using standard formulae (Jain, 1986). For biochemical studies, serum was collected for total serum protein analysis by Biuret method (Fenk *et al.*, 2007) and albumin by BCG dye binding method (Doumas and Peters, 2009). The A:G ratio was calculated by dividing albumin with globulin values. The AST and ALT was assayed by IFCC method (Ceriotti *et al.*, 2010) by using standard kits obtained from Erba Mannheim Transasia Bio-Chemicals Ltd, India. RBC acetyl-cholinesterase and butryl-cholinesterase was estimated by DGKC method (Burtis and Ashwood, 1994) using standard kits obtained from Agappe Diagnostics Ltd. The data were analyzed using two-way analysis of variance (Snedecor and Cochran, 1994).

RESULTS AND DISCUSSION

The analysis of haemoglobin (Hb), packed cell volume (PCV), total erythrocyte (TEC), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) in birds revealed a dose and duration dependent decrease in the values of above parameters in treatment group birds as compared to control group birds (Table 1), thereby indicating microcytic hypochromic anemia and erythrocytopaenia. A significant decrease in Hb, PCV and TEC in chicken has also been reported by Gayal *et al*. (2012). The reason for anemia

Table 1: Effect of chlorpyrifos on haematological parameters in birds of various treatment groups*

Parameters	Weeks post-exposure	Group-I	Group-II	Group-III	Group-IV
Hb (g dL^{-1})	2nd	10.30 ± 0.15^{aA}	7.80 ± 0.07^{dA}	8.56 ± 0.02^{cA}	$8.94 \pm 0 .07^{bA}$
	4th	10.39 ± 0.17^{aA}	7.28 ± 0.07^{dB}	8.22 ± 0.09^{cA}	8.98 ± 0.02^{bA}
	6th	11.24 ± 0.18^{aB}	6.86 ± 0.07^{dC}	7.48 ± 0.01^{cB}	8.14 ± 0.12^{bB}
PCV (%)	2nd	30.32 ± 0.18^{aA}	23.48 ± 0.15^{dA}	25.72 ± 0.05^{cA}	27.72 ± 0.22^{bA}
	4th	30.33 ± 0.13^{aA}	20.78 ± 0.26^{dB}	25.42 ± 0.19^{cA}	27.76 ± 0.16^{bA}
	6th	33.90 ± 0.39^{aB}	19.20 ± 0.37^{dC}	22.68 ± 0.07^{cB}	24.96 ± 0.13^{bB}
TEC (10^6 μL^{-1})	2nd	3.58 ± 0.01^{aA}	3.12 ± 0.02^{dA}	3.31 ± 0.01^{cA}	3.44 ± 0.04^{bA}
	4th	3.60 ± 0.00^{aA}	3.06 ± 0.00^{dB}	3.30 ± 0.00^{cA}	3.41 ± 0.04^{bA}
	6th	3.82 ± 0.07^{aB}	3.00 ± 0.00^{dC}	3.18 ± 0.02^{cB}	3.35 ± 0.06^{bB}
MCV (fl)	2nd	85.77 ± 0.36^{aA}	82.71 ± 0.07^{cA}	83.28 ± 0.08^{cA}	84.48 ± 0.02^{bA}
	4th	86.58 ± 0.15^{aB}	82.09 ± 0.05^{dA}	83.88 ± 0.11^{cA}	84.33 ± 0.07^{bA}
	6th	86.76 ± 0.06^{aB}	81.60 ± 0.24^{dB}	82.36 ± 0.03^{cB}	83.38 ± 0.11^{bB}
MCH (pg)	2nd	28.30 ± 0.19^{aA}	24.90 ± 0.20^{cA}	25.32 ± 0.24^{cA}	25.20 ± 1.06^{bA}
	4th	29.85 ± 0.15^{aB}	25.00 ± 0.20^{dA}	25.95 ± 0.00^{cA}	27.80 ± 0.20^{bB}
	6th	31.88 ± 0.23^{aB}	28.00 ± 0.20^{dB}	29.04 ± 0.05^{cB}	30.00 ± 0.00^{bB}
MCHC (g %)	2nd	32.08 ± 0.67^{aA}	31.02 ± 0.40^{cA}	31.72 ± 0.11^{cA}	31.96 ± 0.43^{bA}
	4th	32.15 ± 0.47^{aB}	31.00 ± 0.60^{dA}	31.51 ± 0.67^{cA}	31.87 ± 0.16^{bA}
	6th	33.85 ± 0.24^{aC}	30.78 ± 0.86^{dB}	32.08 ± 0.59^{cB}	32.79 ± 0.09^{bB}
Platelet count (10^3 μL^{-1})	2nd	20.30 ± 0.11^{aA}	24.84 ± 0.00^{dA}	23.16 ± 0.24^{cA}	$21.04 \pm 0 .41^{bA}$
	4th	20.47 ± 0.19^{aA}	24.48 ± 0.72^{dB}	23.32 ± 0.09^{cA}	21.78 ± 0.00^{bA}
	6th	22.00 ± 0.15^{aB}	25.66 ± 0.07^{dC}	24.18 ± 0.11^{cB}	22.94 ± 0.04^{bB}

Mean±SE bearing at least one common lower case superscript across columns and upper case superscript across rows do not differ significantly between groups and weeks ($P<0.05$), respectively;

*Group I = control; Group II, III and IV birds treated with chlorpyrifos @ 3.2, 1.6 and 0.64 mg kg^{-1} body weight, respectively.

in chlorpyrifos poisoning has been associated with its ability to decrease serum iron concentration, thereby interfering in hemoglobin synthesis (Goel *et al.*, 2006). The observed decrease in PCV, MCV, MCH and MCHC correlates with the earlier studies on chlorpyrifos intoxication in birds and may be attributed to the changes in RBC destruction (size and shape) and decrease in Hb synthesis and Hb content, thereby implying microcytic hypochromic anemia (Savithri *et al.*, 2010). An increase in platelet counts in all the toxin-treated birds at all sacrifice-intervals in the present study correlated with the previous reports (Ambali *et al.*, 2010). The reason for discrepancy is unclear but might be related to the duration of exposure. However, thrombocytosis is associated with coagulopathic disorders characterized by wide spread clotting in tissue, resulting in embolism, tissue ischemia and tissue/organ damage. ROS reportedly facilitates platelet activation (Krotz *et al.*, 2004; Essex, 2009).

The repeated exposure of birds to chlorpyrifos resulted in a significant reduction in TLC and lymphocyte count with increase in heterophil, basophil and monocyte count in group II, III and IV from 2nd week post-exposure. These observations are in agreement with Gayal *et al.* (2012). Heterophils are the first line of defence against infectious agents, tissue injury, parasites and inflammatory or foreign materials and exert their activity by eliminating foreign material through phagocytosis (Kobayashi *et al.*, 2003). So, the observed decrease in leukocyte counts following intoxication with chlorpyrifos could be attributed either to the slower rate of leukocytes production or due to their inhibited release into the blood circulation (Goel *et al.*, 2006). Heterophils were higher in treated groups, which might be due to the relative reduction in lymphocyte count or as a result of reparation processes of haemopoiesis in birds following chlorpyrifos exposure. Increase in basophil and monocyte count was observed but eosinophil values in chlorpyrifos group did not vary significantly. Contrary to our finding, increase in these values has been reported (Savithri *et al.*, 2010). The increase might be attributed to the chlorpyrifos-induced tissue damage and severe

Table 2: Effect of chlorpyrifos on total leucocyte count (10^{-3} mm^{-3}) and differential leucocyte count (%) in birds of different treatment groups (n = 6)

Parameters	Weeks post-exposure	Group-I	Group-II	Group-III	Group-IV
TLC (10^{-3} µL)	2nd	28.34 ± 0.73^{aA}	23.04 ± 0.41^{dA}	25.45 ± 0.58^{cA}	26.23 ± 1.23^{bA}
	4th	28.78 ± 0.44^{aA}	21.58 ± 0.52^{dB}	24.89 ± 0.44^{cB}	25.45 ± 0.44^{bB}
	6th	29.53 ± 0.52^{aB}	20.45 ± 0.46^{dC}	23.26 ± 0.47^{cC}	24.69 ± 0.73^{bC}
Heterophils (%)	2nd	28.24 ± 1.14^{dA}	31.23 ± 1.20^{aA}	30.86 ± 1.02^{bA}	29.33 ± 1.20^{cA}
	4th	29.47 ± 0.66^{dB}	36.47 ± 0.66^{aB}	33.20 ± 0.47^{bB}	30.67 ± 0.66^{cB}
	6th	29.72 ± 0.85^{dB}	38.00 ± 1.45^{aC}	35.00 ± 1.52^{bC}	33.33 ± 1.65^{cC}
Lymphocytes (%)	2nd	64.73± 1.88^{aA}	62.43 ± 0.48^{dA}	63.21 ± 0.84^{cA}	63.33 ± 0.88^{bA}
	4th	65.07 ± 0.43^{aB}	55.87 ± 0.13^{dB}	57.37 ± 0.56^{cB}	59.67 ± 0.43^{bB}
	6th	65.31 ± 1.24^{aB}	51.66 ± 1.10^{dC}	56.00 ± 1.67^{cC}	59.33 ± 1.20^{bB}
Monocytes (%)	2nd	4.83 ± 0.88^{cA}	5.33 ± 0.18^{aA}	5.00 ± 0.58^{bA}	4.33 ± 0.80^{cA}
	4th	4.71 ± 1.44^{cA}	5.80 ± 0.64^{aA}	5.33 ± 0.68^{bA}	4.75 ± 0.54^{cA}
	6th	5.24 ± 0.20^{cB}	7.00 ± 1.70^{aB}	6.33 ± 0.57^{bB}	5.37 ± 1.50^{cB}
Basophils (%)	2nd	0.30 ± 0.33^{dA}	0.50 ± 0.47^{aA}	0.45 ± 0.33^{bA}	0.40 ± 0.57^{cA}
	4th	0.33 ± 0.20^{dA}	0.60 ± 0.10^{aB}	0.50 ± 0.34^{bA}	0.43 ± 0.20^{cA}
	6th	0.55 ± 0.37^{dB}	0.67 ± 0.13^{aB}	0.65 ± 0.33^{bB}	0.60 ± 0.33^{cB}
Eosinophils (%)	2nd	1.00 ± 0.69^{aA}	1.00 ± 0.66^{aA}	1.00 ± 0.66^{aA}	1.00 ± 0.57^{aA}
	4th	1.13 ± 0.30^{aA}	1.58 ± 0.33^{aA}	1.54 ± 0.33^{aA}	1.33 ± 0.33^{aA}
	6th	1.32 ± 0.33^{aA}	1.60 ± 0.44^{aA}	1.58 ± 0.88^{aA}	1.33 ± 0.23^{aA}

Mean±SE bearing at least one common lower case superscript across columns and upper case superscript across rows do not differ significantly between groups and weeks (P<0.05), respectively.
*Group I = control; Group II, III and IV birds treated with chlorpyrifos @ 3.2, 1.6 and 0.64 mg kg^{-1} body weight, respectively.

disturbance of non-specific immune system leading to relative reduction in lymphocyte count or as a result of reparation processes of haemopoiesis in birds.

All the treatment group birds showed significant decline in total protein concentration, albumin, globulin and A:G ratio amongst each other and than those of control birds at all sacrifice intervals in dose- and duration-dependent manner (Table 3). Decrease in protein in chlorpyrifos-treated groups might be due to increased catabolism of proteins and their decreased synthesis (David *et al.* 2004). Attia and Nasir (2009) attributed the lower albumin values to the changes in protein and free amino acid metabolism and their synthesis in liver. Significant decrease in albumin and globulin levels suggesting hepatotoxicity has previously been reported by Salih (2010). The decreased levels of these biochemical parameters might be attributed to decreased feed consumption, increased protein catabolism and serum protein leakage through urine. Besides liver being the main organ of protein synthesis especially albumin (Kaneko *et al*., 2008), the hepatic damage observed in toxin- fed groups in this study may be the other contributing factor for hypoproteinemia, hypoalbumi-nemia and hypoglobulinemia. The increase in the levels of serum AST and ALT in treated birds in dose- and duration-dependent manner suggested damage to heart and skeletal muscles, liver and kidneys. Our findings are in agreement with Ambali (2009) and Mansour and Mossa, (2009).

In present study, the activity of erythrocyte acetylcholinesterase (RBC AchE) and pseudo-cholinesterase/ butyryl cholinesterase (BuChE) significantly declined in all toxin-treated birds. The results are in agreement with Karanth and Pope (2000). AChE concentration is low in plasma and plasma BuChE is used as an indicator of AchE in myoneuronal junction. Alterations in this enzyme level indicate impairment of cholinergic function. Thus, in the present study, inhibition of acetyl cholinesterase enzyme by chlorpyrifos resulted in the accumulation of acetylcholine at myo-neuronal junctions causing cholinergic hyperactivity, with cognitive dysfunctions observed in experimental animals. The study revealed that chlorpyrifos has haemotoxic, immunotoxic, hepatotoxic, nephrotoxic and neurotoxic effects in broiler chickens.

Table 3: Effect of chlorpyrifos on serum biochemical parameters in birds of various treatment groups (n = 6)

Parameters	Weeks post-exposure	Group-I	Group-II	Group-III	Group-IV
Total protein (g dL^{-1})	2nd	2.90 ± 0.01^{aA}	2.23 ± 0.01^{dA}	2.56 ± 0.01^{cA}	2.75 ± 0.01^{bA}
	4th	3.50 ± 0.04^{aB}	2.27 ± 0.00^{dB}	2.70 ± 0.01^{cB}	2.89 ± 0.01^{bB}
	6th	3.84 ± 0.03^{aC}	2.43 ± 0.00^{dC}	2.79 ± 0.01^{cC}	2.97 ± 0.00^{bC}
Albumin (g dL^{-1})	2nd	1.30 ± 0.09^{aA}	0.91 ± 0.05^{dA}	1.10 ± 0.08^{cA}	1.20 ± 0.07^{bA}
	4th	1.63 ± 0.01^{aB}	0.96 ± 0.01^{dB}	1.19 ± 0.07^{cB}	1.30 ± 0.05^{bB}
	6th	1.84 ± 0.05^{aC}	1.05 ± 0.00^{dC}	1.25 ± 0.08^{cC}	1.50 ± 0.04^{bC}
Globulin (g dL^{-1})	2nd	1.59 ± 0.01^{aA}	1.31 ± 0.07^{dA}	1.45 ± 0.10^{cA}	1.54 ± 0.09^{bA}
	4th	1.87 ± 0.12^{aB}	1.31 ± 0.02^{dA}	1.50 ± 0.09^{cB}	1.59 ± 0.06^{bB}
	6th	2.00 ± 0.06^{aC}	1.37 ± 0.04^{dC}	1.53 ± 0.01^{cB}	1.46 ± 0.04^{bA}
A:G	2nd	0.81 ± 0.01^{aA}	0.69 ± 0.01^{dA}	0.75 ± 0.00^{cA}	0.77 ± 0.01^{bA}
	4th	0.87 ± 0.00^{aB}	0.73 ± 0.00^{dB}	0.79 ± 0.01^{cB}	0.81 ± 0.00^{bB}
	6th	0.92 ± 0.00^{aC}	0.77 ± 0.01^{dC}	0.81 ± 0.01^{cC}	0.94 ± 0.00^{bC}
AST (IU L^{-1})	2nd	208.1 ± 1.15^{dA}	227.3 ± 0.70^{aA}	220.7 ± 0.36^{bA}	214.9 ± 0.97^{cA}
	4th	217.1 ± 0.47^{dB}	255.2 ± 0.93^{aB}	234.8 ± 1.19^{bB}	223.5 ± 1.77^{cB}
	6th	224.1 ± 1.17^{dC}	278.2 ± 0.98^{aC}	251.5 ± 1.42^{bC}	232.1 ± 1.30^{cC}
ALT (IU L^{-1})	2nd	34.6 ± 0.32^{dA}	50.5 ± 0.77^{aA}	42.1 ± 0.52^{bA}	38.2 ± 0.33^{cA}
	4th	36.5 ± 0.61^{dB}	58.8 ± 0.87^{aB}	48.5 ± 0.56^{bB}	40.4 ± 0.26^{cB}
	6th	38.9 ± 0.43^{dC}	63.6 ± 0.71^{aC}	52.0 ± 0.42^{bC}	42.5 ± 0.51^{cC}
RBC Acetyl-cholineesterase (U L^{-1})	2nd	11552 ± 35.17^{dA}	10044 ± 3.94^{aA}	10321 ± 6.56^{bA}	10838 ± 27.1^{cA}
	4th	11587 ± 39.69^{dB}	9785 ± 68.70^{aB}	10113 ± 0.81^{bB}	11441 ± 36.7^{cA}
	6th	12097 ± 80.71^{dC}	7217 ± 1.53^{aC}	8857 ± 52.50^{bC}	9665 ± 55.7^{cC}
Pseudocholine-esterase (U L^{-1})	2nd	1158.2 ± 4.01^{dA}	1003 ± 1.92^{aA}	1051.06 ± 2.69^{bA}	1103.40 ± 4.53^{cA}
	4th	1206.14 ± 3.14^{dB}	960.58 ± 4.44^{aB}	980.05 ± 4.53^{bB}	1088.05 ± 0.60^{cB}
	6th	1186.61 ± 0.52^{dC}	888.76 ± 3.64^{aC}	899.47 ± 3.33^{bC}	1072.40 ± 1.69^{cC}

Mean±SE bearing at least one common lower case superscript across columns and upper case superscript across rows do not differ significantly between groups and weeks ($P<0.05$), respectively.

*Group I = control; Group II, III and IV birds treated with chlorpyrifos @ 3.2, 1.6 and 0.64 mg kg^{-1} body weight, respectively.

REFERENCES

Al-Haj, M., Nasser, A. and Anis, A. 2005. Survey of pesticides used in Qat cultivation in Dhale and Yafe and their adverse effects. *Journal of Natural and Applied Sciences,* **9**: 103-110.

Ambali, S.F. 2009. *Ameliorative Effects of Vitamins C and E on Neurotoxicological, Haematological and Biochemical Changes induced by Chronic Chlorpyrifos in Wistar Rats.* Ph.D. dissertation, Ahmadu Bello University, Zaria, Nigeria.

Ambali, S.F., Akanbi, D.O., Shittu, M. Giwa, A. Oladipo, O.O. and Ayo, J.O. 2010. Chlorpyrifos-induced clinical, hematological and biochemical changes in Swiss Albino mice - Mitigating effect by co-administration of vitamins C and E. *Life Science Journal,* **7**(3): 37-44.

Attia, A.M. and Nasir, H.M. 2009. Dimethoate-induced changes in biochemical parameters of experimental rat serum and its neutralization by black seed (*Nigella sativa*) oil. *Slovak Journal of Animal Science,* **42**: 87-94.

Burtis, C.A. and Ashwood, E.R. 1994. *Tietz Textbook of Clinical Chemistry* (2nd edn.). Elseviers Academic Press, New York, USA.

Casida, J.E. and Quinstad, G.B. 2004. Organophosphate toxicology: Safety aspects of non-acetylcholine cholinesterase secondary targets. *Chemical Research Toxicology*, **17**: 983-998.

Ceriotti, F ., Henny, J., Queraltó, J., Ziyu, S., Özarda, Y., Chen, B., Boyd, J.C. and Panteghini, M. 2010. IFCC committee on reference intervals and decision limits (C-RIDL), Committee on reference systems for enzymes (C-RSE): Common reference intervals for aspartate aminotransferase (AST), alanine aminotransferase (ALT) and γ-glutamyl transferase (GGT) in serum: Results from an IFCC multicenter study. *Clinical Chemistry and Laboratory Medicine,* **48:** 1593-1601.

David, M., Mushigeri S.B., Shivakumar, R. and Philip, G.H. 2004. Response of *Cyprinus carpio* (Linn) to sub-lethal concentration of cypermethrin: Alterations in protein metabolism profiles. *Chemosphere*, **56**: 347-352.

Doumas, B.T. and Peters, T.J. 2009. Origins of dye-binding methods for measuring serum albumin. *Clinical Chemistry*, **55**: 583-584.

Eaton, D.L., Daroff, R.B., Autrup, H., Bridge, J., Buffler, P., Costa, L.G., Coyle, J., McKhann, G., Mobley, W. C., Nadel, L., Neubert, D., Schutte-Herman, R. and Spencer, P.S. 2008. Review of the toxicology of chlorpyrifos with an emphasis on human exposure and neurodevelopment. *Critical Reviews in Toxicology*, **2**: 1-125.

Eisler, R. 2000. Chlorpyrifos. pp. 883-885. **In**: *Handbook of Chemical Risk Assessment*. CRC Press, LLC Printing, Florida, USA.

Essex, D.W. 2009. Redox control of platelet function. *Antioxidants and Redox Signaling*, **11**: 1191-1225.

Fenk, C.J., Kaufman, N. and Gerbig, D.G. 2007. A new colorimetric assay of tabletop sweeteners using a modified Biuret reagent. *Journal of Chemical Education*, **84**: 1676-1678.

Gayal, L., Singh, S.P., Ahmad, A.H. and Ahmad, W. 2012. Haemato-biochemical profile following multiple oral dose administration of chlorpyrifos in poultry. *Journal of Veterinary Pharmacology and Toxicology*, **11**: 88-90.

Goel, A., Dani, V. and Dhawan, D.K. 2006. Role of zinc in mitigating the toxic effects of chlorpyrifos on haematological alterations and electron microscopic observations in rat blood. *Biometals*, **19**: 483-492**.**

Gomes, J., Dawodu, A.H., Lioyd, O., Revitt, D.M. and Anilal, S.V. 1999. Hepatic injury and disturbed amino acids metabolism in mice following to prolonged exposure to organophosphorus pesticides. *Human and Experimental Toxicology*, **18**: 33-37.

Jain, N.C. 1986. *Schalm's Veterinary Haematology* (4th edn.). Lea Febiger, Philadelphia, USA.

Kaneko, J.J., Harvey, J.W. and Bruss, M.L. 2008. *Clinical Biochemistry of Domestic Animals* (6th edn.). Elsevier Academic Press, New York, USA.

Karanth, S. and Pope, C. 2000. Carboxylesterase and A-esterase activities during maturation and aging: Relationship to the toxicity of chlorpyrifos and parathion in birds. *Toxicological Sciences*, **58:** 282-289.

Kobayashi, S.D., Voyich, J.M. and DeLeo, F.R. 2003. Regulation of the neutrophil mediated inflammatory response to infection. *Microbes and Infection*, **5**: 1337-1344.

Kossmann, S., Magner-Krezel, Z., Sobieraj, R. and Szwed, Z. 1997. The assessment of nephrotoxic effect based on the determination of the activity of some selected enzymes in urine. *Przegad Lekarski*, **54**: 707-711.

Krotz, F., Sohn, H.Y. and Pohl, U. 2004. Reactive oxygen species: Players in the platelet game. *Arteriosclerosis, Thrombosis and Vascular Biology*, **24**: 1988-1996.

Lemus, R. and Abdelghani, A. 2000. Chlorpyrifos: An unwelcome pesticide in our homes. *Reviews on Environmental Health*, **15**: 421-433.

Lengyl, Z., Fazakas, Z. and Nagymajteny, L. 2005. Change in the central nervous activity of birds treated with dimethoate in combination with other neurotoxicants in different phases of ontogenesis. *Archives of Industrial Hygiene and Toxicology*, **56**: 257-264.

Mansour, S.A. and Mossa, A.H. 2009. Lipid peroxidation and oxidative stress in rat erythrocytes induced by chlorpyrifos and protective role of zinc. *Pesticide Biochemistry and Physiology*, **93**: 34-39.

Rawat, D.S., Singh, S.P., Sharma, L.D., Ahmad, A.H. and Mehta, G. 2003. Residue analysis of some pesticides in poultry egg and mean samples in Garhwal region of Uttranchal. pp. 23-24. **In:** *22nd Annual Conference of Society for Toxicology*, 20-22 November, Madras Veterinary College, Chennai, India.

Salih, M.A. 2010. Toxic effect of dimethoate and diazinon on the biochemical and hematological parameters in male rabbits. *Jordan Journal of Biological Sciences*, **3**: 77-82.

Savithri, Y., Sekhar, P.R. and Doss, P.J. 2010. Changes in hematological profiles of albino birds under chlorpyrifos toxicity. *International Journal of Pharmacy and Biological Sciences*, **1**: 1-7 (*www.ijpbs.net zoology*)

Snedecor, G.W. and Cochran, W.G. 1994. *Statistical Methods* (8th edn.). Iowa State University Press, Ames, Iowa, USA.