ISSN 0975-6299 Vol.1/Issue-3/Jul-Sep.2010

International Journal of Pharma and Bio Sciences

CHANGES IN HEMATOLOGICAL PROFILES OF ALBINO RATS UNDER CHLORPYRIFOS TOXICITY

Y. SAVITHRI, P. RAVI SEKHAR AND P. JACOB DOSS

Department of Zoology, Sri Venkateswara Univeristy, Tirupati 517 502, AP, INDIA.

**** Corresponding Author*** jacobdoss@rediffmail.com ; drysavithri@gmail.com

ABSTRACT

Hematology is defined as the branch of biology, which deals with the morphology of blood and blood forming organs. Haematological profiles of blood can provide important information about the internal environment of the organism. In the present investigation the effect of chlorpyrifos, an organophosphate compound on the blood components is determined in albino rats. The present investigation was aimed to examine the haematological changes of of albino rats with oral sub lethal (20 mg/kg) administration of chlorpyrifos as single, double and multiple doses with 48 hr intervals. The decrease in RBC count, Hb and PCV observed in the present investigation could be described are retarded haemopoisis, destruction and shrinkage of RBC. MCV, MCH and MCHC showed significant decrease in all doses in the present investigation, due to destruction of RBC (size and shape) and decrease in Hb synthesis and hemoglobin content. These symptoms imply the microcytic- hypo chromic anemia. A decreased percentage of neutrophils in peripheral blood observed in animals poisoned with chlorpyrifos may suggest, neutrophils involves in phagocytosis during xenobiotic intoxication, during which some of the neutrophils might have ruptured.

KEY WORDS: Haematology, Chlorpyrifos, albino rat.

INTRODUCTION

A large variety of potential pesticides are now being synthesized and used by different nations replacing the lower toxic and low potency chemicals for the pest control. Chlorpyrifos is an organophosphate compounds used to kill a wide variety of insects and a broad spectrum insecticide. Chlorpyrifos caused decrease in RBC, Hb and Hct, which might be due to the effect of pesticide on blood-forming organs suggesting the anaemic condition of the treated animals.[1] The anemia may be due to the inhibition of erythropoiesis and hemosynthesis and to an increase in the rate of erythrocytes destruction in hemopoietic organs. Sushila Patel

et al[2] have reported an induction of DNA damage in hematopoietic system, viz., spleen, bone narrow and lymphocytes, showing that chlorpyrifos induce chromosomal aberrations and micronucleus formation in mouse bone narrow.

Blood is a specialized bodily fluid that delivers necessary substances to the body's cells such as nutrients, oxygen and transports of waste products away from those of same cells. Blood is the most important body fluid that governs vital functions of the body like respiration, circulation, excretion, osmotic balance and the transport of metabolic substance. Circulation of the blood within the cardiovascular system is essential for transportation of gases, nutrients, minerals, metabolic products and hormones between different organs.[3]

Blood parameters are probably the more rapid and detectable variations under stress and are fuel in assessing the health condition.[4] The importance of haematological parameters in clinical biochemistry, population genetics and medical anthropology is well established. Recent speculations have proved that they may be used as valuable indicators of disease or stress in animals.[5]

The present investigation was aimed to study the effect of chlorpyrifos, on the blood is determined in albino rats.

MATERIAL AND METHODS

Test chemical

Chlorpyrifos Technical (95.30%) was obtained from Nagarjuna Agri. Chem Limited, Ravulapalem Mandal, East Godavari District, A.P., India.

Animal model

Healthy adult albino rats of same age group (100±10 days) and weight (200 ± 10g) were obtained from the Indian institute of sciences (IISc) Banglore, India, and maintained conditions (25±2^{o}C and with 12hr light , 12hr darkness) food and water were allowed *ad libitum.*

Experimental design

Toxicity of Chlorpyrifos was evaluated by probit method of Finney[6] and the LD_{50} of chlorpyrifos to albino rats was found to be 200mg/kg bw. 1/10 of LD_{50} value (20mg/kg bw) was selected as sub lethal dose. The animals were divided into four groups having ten animals each. The second, third and fourth groups of animals were termed as experimental animals. To the animals of second group single dose of pesticide (i.e. on 1st day) was administered orally by gavage method. To the third group of animals double doses were given i.e. on 1st and 3rd day. Similarly multiple doses i.e., 1st, 3rd, 5th and 7th day were given to the fourth group of animals. The first group of animals was considered as controls. After stipulated time the animals were sacrificed and the blood was collected for haematological studies.

HAEMATOLOGICAL STUDIES

Red blood corpuscle (RBC) count

RBC count was made with a Neubauer crystalline counting chamber as described by Davidson and Henry [7]. The blood was collected in a vial containing 2% ethylene diamine tetra acetic acid (EDTA) as an anticoagulant (the dilution is 1:200). The solution was mixed well by shaking gently. It was allowed to stand for 2 or 3 minutes. The counting chamber and cover glass were cleansed and the cover glass was placed over the ruled area. Again the solution was mixed gently and stemful of solution was expelled and a drop of fluid was allowed to flow under the cover slip holding the pipette at an angle of 40^{0}, it was allowed to stand for 2 to 3 minutes to allow RBC to settle. Afterwards the ruled area of the counting chamber was focused under the microscope and the number of RBC's were counted in five small squares of the RBC

column under high power and the number of RBC per cumm were calculated accordingly.

$$\frac{\text{Number of cells X dilution factor X depth factor}}{\text{Area counted}}$$

Estimation of haemoglobin concentration (Hb)

The hemoglobin concentration was estimated by Acid - haematin method (Sahli[8]). N/10 hydrochloric acid was taken up to 20 marks in a graduated tube. Blood was collected directly from the eyeball up to 20 cu mm in the Hb pipette and the outer side was wiped out and this was transferred into the graduated tube containing N/10 hydrochloric acid.

It was allowed to stand for 10 to 20 minutes after thorough mixing. Then N/10 HCl was added drop by drop, mixing between each addition until the blood color matched with the standard color. And then the results were read from the scale on the graduated tube and the Hb concentration was expressed in grams percent.

Estimation of packed cell volume (PCV)

PCV estimated by micro hematocrit method (Schalm *et al.,* [9]).

The blood was drawn into capillary tubes containing the anticoagulant, by capillary action to 2/3 of their length. The tubes were tapped to permit blood to flow towards end and to provide sufficient space to prevent outflow when the opposite ends were sealed. The outside of the capillary tubes were wiped free of blood and the index finger was placed over the moist ends to hold the column of the blood in place as the opposite dry ends were forced into the sealing material to form a tight plug. The capillary tubes were placed in the centrifuge with the sealed ends pointing outward and centrifuged at 12,000 rpm for 5 minutes. PCV was determined by rolling the capillary tubes a reader card until the top of the plasma column was aligned with 100% line and the bottom of the packed erythrocytes was on the zero line. The line that crossed the top of the packed erythrocyte column represented the PVC in percent.

White blood corpuscles (WBC) count

Blood is drawn from the vial into WBC pipette up to 0.5 marks and immediately the diluting fluid is drawn up to 11 marks. The solution is mixed thoroughly by shaking gently. The rest of the procedure is the same as described by Davidson and Henry [7] for RBC count. In case of WBC, count was made in bigger squares of the chamber. The WBC count was expressed in cu mm.

Differential leukocyte count

A drop of blood was placed on a clean glass slide about 1-2 cm from one end with the help of a spreading slide placed at an angle of 45º approximately. The drop of blood was spread out quickly along the line of control of the spreader with the slide. The slide was placed flat on two glass rods over a sink and was covered with Leishman stain. The stain was diluted by the drop by drop addition of buffered water and stained for a period of 5-7 minutes. The stain was drained and washed with water and air dried and observed under microscope.

Counting was started under high power oil immersion objective from the edge of the smear moving the smear towards center. Leucocytes were identified and the movement was repeated till a total 100 cells were counted. The values of different morphological types were expressed as the percentage.

RESULTS

In the present investigation marked variations in the haematological parameters were observed under chlorpyrifos toxic stress, significant decrease in total number of RBC haemoglobin, haematocrit, MCH, MCHV and neutrophils, where as significant increase in amount of white blood cells (WBC), lymphocytes, monocytes, eosinophils and basophils were represented in table – 1.

The toxic effect of chlorpyrifos on the haemogram is determined in albino rats, treated with chlorpyrifos became anaemic. It is proved in the experiment by significant decrease in RBC count, Hb and PCV levels in comparison to control animals.

DISCUSSION

Chlorpyrifos caused decrease in RBC, Hb and Hct, which might be due to the effect of pesticide on blood-forming organs suggesting the anaemic condition of the treated animals (Rahman *et al* [1]). The anemia may be due to the inhibition of erythropoiesis and hemosynthesis and to an increase in the rate of erythrocytes destruction in hemopoietic organs.

Sushila Patel *et al.,*[2] have reported an induction of DNA damage in hematopoietic system, viz., spleen, bone marrow and lymphocytes, showing that chlorpyrifos induce chromosomal aberrations and micronucleus formation in mouse bone marrow. The reduction in size and number of RBC, Hb and PCV may be a consequence of severe hemorrhage which results in the dilution of blood caused by the influx of cells and fluids from body stores .[10]

Table .1

Haemogram of control and chlorpyrifos treated albino rats

Parameters	Control	Single Dose	Double Dose	Multiple Dose
RBC (cu. mm)	8.696 ±0.018	6.710 ±0.042 (-22.838)	4.973 ±0.079 (-42.821)	4.289 ±0.107 (-50.676)
Hb/(g/100ml)	16.391 ±0.042	11.680 ±0.044 (-28.739)	7.940 ±0.049 (-51.554)	6.872 ±0.103 (-58.108)
PCV(Percent)	44.218 ±0.067	32.341 ±0.069 (26.860)	23.342 ±0.067 (47.210)	22.480 ±0.093 (49.160)
MCV(µg)	19.390 ±0.713	17.803 ±1.090 (-8.18)	17.016 ±0.632 (-12.23)	14.761 ±0.803 (-23.86)
MCH(pg)	18.693 ±0.033	16.869 ±0.045 (-9.756)	15.705 ±0.041 (-15.985)	44.961 ±2.758 (-19.307)
MCHC(Percent)	37.452 ±0.042	35.070 ±0.086 (-6.360)	34.083 ±0.097 (-8.921)	32.125 ±0.710 (-10.951)

WBC(cu. mm)	11156.635 ±94.546	12411.700 ±31.229 (11.249)	13823.745 ±77.898 (23.906)	14851.271 ±93.073 (33.116)
Neutrophils	20.114 ±1.414	17.097 ±0.091 (-15.001)	13.144 ±0.066 (-34.649)	8.7523 ±0.072 (-56.487)
Lymphocytes	74.723 ±0.057	77.409 ±0.073 (3.594)	80.377 ±0.121 (7.624)	84.148 ±0.088 (12.612)
Monocytes	1.995 ±0.007	2.304 ±0.062 (15.441)	2.844 ±0.061 (45.512)	3.521 ±0.039 (76.491)
Eosinophils	1 ±0.632	1 ±0.632 (0)	1.166 ±0.408 (16.66)	1.333 ±0.516 (33.33)
Basophils	1 ±0.632	1 ±0.632 (0)	1 ±0.632 (0)	1.166 ±0.408 (16.66)

All the values are mean ± SD of six individual observations.
SD – Standard Deviation.PC – Percent change over control.
All the values are significant at P<0.05 and P<0.01

In general anemia, reduction in the number of red blood cells or of haemoglobin in the blood can reflect impaired synthesis of haemoglobin (eg. in iron deficiency) or impaired production of erythrocytes (eg. in folic acid or Vitamin B_{12} deficiency.[11] Anemia, defined clinically as a decrease in hematocrit or Hb concentration, may be caused by blood less, excessive haemolysis, or deficient erythropoiesis.[3] Jung-Hoon Jea *et al.,*[12] studied the decline in RBC count, hemoglobin concentration and Hct presumably reflects erythrocyte hemolysis and due to either an increase in the rate at which haemoglobin concentration may be destroyed or a decrease in the haemoglobin synthesis. Decrease in hematocrit is attributable to the reduction in RBC count caused either destruction or reduction in size. Rahmen and Siddiqui[13] also observed decrease in hematocrit and mean value of hemoglobin.

Decreased RBC count, Hb and PCV levels was observed in rats treated with thiodan 35 E.C[14], chloropharm[15], endosulfan[16], and lindane and endosulfan[17], deltamethrin.[18]

MCV, MCH and MCHC showed significant decrease in all doses in the present investigation. due to destruction of RBC (size and shape) and decrease in Hb synthesis and hemoglobin content. These symptoms imply the microcytic hypo chromic anemia.

Decrease in MCV, MCH and MCHC was observed in rats treated with various insecticides such as endosulfan, malathion, methyl parathion, phosphomidon, monocrotophos and fenvalerate (Dhembare and Pandhe,). [19]

Increase in total leukocytes count has been suggested to be due to stimulated lymphopoiesis and/or enhanced release of lymphocytes from lymph myeloid tissue.[20] Such lymphocyte response might be due to the presence of toxic substances may be associated with the pollutant induced tissue damage and severe disturbance of the non-specific immune

system leading to increased production of leukocytes.

Several authors have noticed an increase in WBC in animals repeatedly treated with sublethal doses of insecticides. Increased WBC was observed in rats treated with diodine[21], aldrin[22], novel phosphorothionate[13] and lindane and endosulfan[17] .

A decreased percentage of neutrophils in peripheral blood observed in animals poisoned with chlorpyrifos may suggest, neutrophils involves in phagocytosis during xenobiotic intoxication, during which some of the neutrophils might have ruptured. Therefore the neutrophils count consistently decreased during different doses of chlorpyrifos intoxication in rats in the present investigation. Based on the studies on humans (males and females) participating in the production of liquid pesticides, a significant decrease was noted in the number of neutrophils.[23] Haratym-Maj [24] reported the decrease in the number of neutrophils in mice intoxication with higher deltamethrin dose.

Thus the haematological parameters in the present study showed a significant alteration under chlorpyrifos exposure. But these changes are highly significant in multiple dose treated animals than the single and double dose chlorpyrifos administered rats (Table -1) which could be an adaptive mechanism prevailed in the animal under toxic stress.

REFERENCES

1. Rahman, M.F., M.K.J. Siddiqui, M. Mahaboob and M.J. Mustafa (1990). Hematological and hepatotoxic effects of isoprocrab in chicken. *Journal of Applied Toxicology,* 10(3): 187-92.
2. Sushila Patel., Alok K. Pandey, Mahima Bajpayee, Devendra Parmar and Alok Dhawan (2006). Cypermethrin – induced DNA damage in organs and tissues of the mouse: Evidence from the comet assay. *Mutation Research,* 607:176-183.
3. Baynes, W. John and H. Marek Dominiczak (2005). In: Medical Biochemistry, Second Edition, Elsevier Mosby Ltd., Philadelphia.
4. Hymavathi, V and L.M. Rao (2000). Effect of sublethal concentrations of lead on the haematology and biochemical constituents of *Channa punctatus, Bulletin of Pure and Applied Sciences*, 19A (1): 1-5.
5. Calabrese, A.L., F.P.Thurberg, M.A.Dawson and D.R. Wenzl off (1975). Sublethal physiological stress induced by cadmium and mercury in winter flounder *Pseudopleuronectes americanus*. In sublethal effect of toxic chemicals (eds.), *J. H. Koeman* J.J.T.W.A. Strik. Elsevier, Scientific Co. Amsterdam, 15-21.
6. Finney, D.J (1971). Probit analysis, III Edition, Cambridge Univ. press, London, p.20.
7. Davidson, J., and Henry (1969). Laboratory methods 14 Ed: 139-143.
8. Sahli, T. (1962). Text Book of clinical pathology.
9. Schalm, O.W., N.C. Jain and E.J. Caroll (1975). Veterinary haematology. 3(ed.) Lea and Fibiger. Philadelphia, USA. pp.45-46.
10. Sharma, R.K and Y. Saxena (1983). Haematological study of Indian desert gerbil, *Meriones hurrianae* (Jerdon) after brodifacoum poisoning. *Indian Journal of Comparative Animal Physiology,* 1(2): 23-28.
11. Murray, Robert K., Daryl K. Granner, Peter A. Mayes and Victor W. Rodwell (2007). In: Harper's Illustrated Biochemistry. International 26th Edition, The McGraw-Hill Companies, Inc. pp 46, 47.
12. Jung – Hoon Jee, Fatima Masroor and Ju-Chan Kang (2005). Responses of cypermethrin – induced stress in haematological parameters of Korean rockfish, *Sebastes schlegeli* (Hilgendorf). *Aquaculture Research,* 36:898-905.
13. Rahman, M.F and M.K.J. Siddiqui (2006). Hematological and clinical chemistry

changes induced by subchronic dosing of a novel phosphorothionate (RPR-V) in wistar male and female rats. *Drug and Chemical Toxicology,* 29:95-110.
14. Solanke, A.K and V.H. Singh (2000). Haematological changes in rat, *Rattus rattus* after repeated exposure to thiodan 35 *EC. Environmental and Ecology,* 18(2): 529-31.
15. Fujitani, T., Y. Tada, A.T. Noguchi and M. Yoneyama (2001). Effect of chlorpropham (CIPC) on the hemopoietic system of rats. *Food Chem. Toxicology,* 39:253-259.
16. Choudhary, N and S.C. Joshi (2002). Effect of short term endosulfan on hematology and serum analysis of male rat. *Indian Journal of Toxicology,* 9(2):83-87.
17. Azhar Baig, Md (2007). Pesticidal residue analysis of organochlorine residues in different milk samples from Chittoor district in Andhra Pradesh, India. Final report of UGC Minor Research Project during the period from 2004-2006.
18. Yekeen, T.A., T.G. Okorie and A.A. Bakare (2007). Acute and chronic toxicity of the synthetic pyrethroid deltamethrin in mice. *Asian Journal of Microbiology, Biotechnology and Environmental Sciences,* 9(1):11-17.
19. Dhembare, A.J and G.M. Pondhe (2000). Haematological changes in fish, *Punctivs sophore* exposed to some insecticides. *Journal of Exp. Zoology India*, 3(1):41-44.
20. Das, B.K and S.C. Mukherjee (2003). Toxicity of cypermethrin in *Labeo rohita* fingerlings: biochemical, enzymatic and hematological consequences. *Comparative Biochemistry and Physiology* Part C, 134:109-121.
21. Jabeen Massod, M.F., J. Rahaman, M. Narasaiah and Mustafa (1991). Heamatological and Biochemical alterations in rats subjected to Dermal application of diodine. *Indian Journal of Comparative Animal Physiology,* 9 (1): 27-31.
22. Anil Kumar, D.M.H.S., N. John Sushma, D.J. Sudheer Kumar and K. Jayantha Rao (1996). Haematological changes in albino rats under aldrin intoxication. *Indian Journal of Comparative Animal Physiology*, 14(1):63-66.
23. Klucinski, P., A. Hrycek, H. Stasiura – Zielinska, S. Kossmann, J. Tustanowski, D. Friedek and B. Kaminska-Kolodziej (1996). Humoral and cellular immunity rates in chemical plant workers employed in the production of liquid pesticides. *Int J Occup Med Environ Health,* 9:103-110.
24. Haratym Mag (2002). Heamotological alteranations after pyrethroid poisoning in mince. Ann Agric Environ Med, (9): 199-206.