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Research Article

ALTERATIONS OF HEMATOLOGICAL PARAMETERS UNDER CHLORPYRIFOS INTOXICATION IN MICE

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A B S T R A C T

Hematology is defined as the branch of biology, which deals with the morphology of blood and blood forming organs. Chlorpyrifos is one of the most widely used Organophosphates (OP) insecticides. Anemia and alteration in other hematological parameters have been recorded following repeated Chlorpyrifos exposure.In the present study Healthy adult mice of same age (100±10 days) and weight (75±10 g) 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. In the present investigation the effect of chlorpyrifos, on the blood is determined in Mice. Oral administration of chlorpyrifos produced statistically significant ($P<0.01$) decrease in RBC, Hb, PCV but WBC count shows increased level in single, double and multiple doses respectively. The Mean corpuscular volume (MCV), Mean Corpuscular Haemoglobin (MCH) and Mean Corpuscular Haemoglobin Concentration (MCHC) are dependent on the RBC count, MCV, MCH and MCHC showed statistically significant ($P<0.01$) decrease in single, double and multiple dose of chlorpyrifos intoxication in Mice.

INTRODUCTION

Blood is a specialized biological 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. The cells of the tissue of the body are in contact with body fluids which in turn are in equilibrium with the fluid portion of the blood. 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 (Baynes and Dominczak, 2005).

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Blood composed of an aqueous solution containing molecules of varying sizes and number of cellular elements. Some of the components of blood perform important role in the body's defense against external insult and in the repair of damaged tissues (Baynes and Domniczak, 2005).

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 (Calabrese *et al.,* 1975). Haematological and biochemical profiles of blood can provide important information about the internal environment of the organism (Masopust, 2000). Blood parameters are probably the more rapid and detactable variations under stress and are fuel in assessing the health condition (Hymavathi and Rao, 2000). In human medicine, investigation of haemotological parameters is necessary for clinical diagnosis of a disease and pathological condition (Hardiker and Gokhale, 2000).

Blood is the only variable tissue in occupationally exposed workers and it is a patho physiological reflector of the whole body, hence blood parameters are routinely used for diagnosing and monitoring the disease conditions in humans (Rahman and Sidiquie, 2006). For many years the study of haematological parameters has been used as diagnostic tool to investigate disease and physiological and metabolic alterations (Bansal *et al*.,1979). Haematological values are widely used to determine systemic relationship and physiological adaptations including the assessment of general health conditions (Alkinson and Judd, 1978).

Haematological parameters of different mammals and other vertebrate taxa are reported to vary with sex (Sealander, 1964; Srihari and Shakuntala Sridhara, 1986 and Haratym - maj, 2002), age (Sealander, 1964; Maity and Guru, 1998), body weight (Pandey, 1977), size (Pradhan, 1961), nutritional status (Smith, 1968) season (Natrig *et al*., 1963; Sealander, 1964), habitat and altitude (Kalabuchov, 1937; Murray *et al.,*2007). Apart from these factors, pesticides, drugs, metals (Jadwiga Chmielnicka *et al.,* 1994; Hymavathi and Rao, 2000; Suricuchi *et al*., 2000;Vasantha Sena, 2002; Mughani *et al.,* 2003; Othman 2004; Sharma *et al.,* 2006), dyes (Mathur *et al.,* 2003; Ramulu *et al*., 2006), industrial effluents (Meenakala, 1978), chemical - dimethyl formanide (Lynch *et al.,* 2003) and 'parasites infection (Arti Saxena and ShakuntalaShukla, 2006; Krishan, 2006) also cause alternation in the haematological parameters.

The haemotological parameters are studied in different pesticide poisoned in Mice with vepacide (Rahman *et al.,* 1996), thiodan (Solanke and Singh, 2000), dimethoate(Reena *et al.,* 1989),cypermethrin (Nagarjuna, 2007), primiphos methyl (Gupta *et al.,* 1982; Rajini *et al.,* 1987), DDT (Baronia *et al.,* 1992), Phosalone (Janardhana Reddy, 1988), chlordane (Vani, 1991), *in vitro* and *in vivo* fenvalerate(Lakshmi Rajyam, 1992), carbaryl (Baronia and Sahai, 1993), aldrin(Anil Kumar *et al.,* 1996), fluvalinate residual pesticide (Patel *et al.,* 1998), chloropropham (Fujitani *et al.,* 2001), acephate(Thapar *et al.,*2002, Rajeswari, 2008), endosulfan (Choudhary and Joshi, 2002), deltamethrin(Manna *et al.,*2005), novel phosphorothionate (Rahman and Siddiqui, 2006), lindane and endosulfan (AzharBaig, 2007) have reported earlier. SubhashPatil *et al.,* (2000) studied the effects of phenyl hydrazine induced hemolytic anemia and anti anaemic properties of ayurvedic drugs.

Chlorpyrifosis one of the most widely used Organophosphates (OP) insecticides. Anemia and alteration in other hematological parameters have been recorded following repeated Chlorpyrifos exposure (Goel *et al.,* 2006b; Ambali *et al.,* 2010a). Chlorpyrifos is used around the world to control insects in agricultural, residential and commercial settings. Its use in residential applications is restricted in multiple countries. According to Dow, chlorpyrifos is registered for use in nearly 100 countries and is annually applied to approximately 8.5 million crop acres. The crops with the most use are cotton, corn, almonds and fruit trees including oranges, bananas and apples.Chlorpyrifos was first registered for use in the United States in 1965 for control of foliage and soil-born insects. (U.S. EPA 2002). The chemical became widely used in residential settings, on golf course turf, as a structural termite control agent, and in agricultural use. Most residential use has been phased out in the United States; however it remains a common agricultural insecticide. (U.S. EPA 2002).

Acute Toxicity of Chlorpyrifosmay affect the central nervous system, the cardiovascular system, and the respiratory system and also skin and eye irritant (Occupational Health Services, Inc. 1986). While some organophosphates are readily absorbed through the skin, studies in humans suggest that skin absorption of chlorpyrifos is more limited (Hayes and Laws, 1990). Skin which has come in contact with this material should be washed immediately with soap and water and all contaminated clothing should be removed. The acute dermal LD_{50} for chlorpyrifos in male and female rats is greater than 2,000 mg/kg (The Dow Chemical Company, 1986).Inhalation of chlorpyrifos may cause absorption of the insecticide through the mucous membranes, resulting in systemic intoxication (Occupational Health Services, Inc. 1986). Plasma cholinesterase levels activity has been shown to be inhibited when chlorpyrifos particles are inhaled (American Conference of Governmental Industrial Hygienists, Inc. 1986).

The amount (dose) of a material that causes death in one-half (50%) of the test population, when it is given on a short-term basis by mouth is referred to as its oral lethal dose (LD_{50}). The oral LD_{50} for chlorpyrifos in rats is 82 to 270 milligrams per kilogram (mg/kg) (Leo, 1978; U.S. Environmental Protection Agency, Sept, 1984; Berg, 1986). This indicates that it takes 82 to 270 mg ofchlorpyrifos for each kg of body weight to kill 50% of the experimental animals tested (Gosselin, 1984; Occupational Health Services, Inc. 1991). The lethal concentration fifty, or LC_{50}, is that concentration of a chemical in air or water that kills half of the experimental animals exposed to it for a set time period. The 4-hour inhalation LC_{50} for chlorpyrifos in rats is greater than 200 mg/m^3 (Dow Elanco, 1992).

Repeated or prolonged exposure to organophosphates may result in the same effects as acute exposure including the delayed symptoms.When technical chlorpyrifos was fed to dogs at doses 0.01, 0.03, 0.1, 1 and 3mg/kg/day for 2 years, increased liver weight occurred at 3.0mg/kg. Signs of cholinesterase inhibition occurred at 1 mg/kg. Rats and mice given technical chlorpyrifos in the diet for 104 weeks showed no adverse effects other than cholinesterase inhibition (U.S. Environmental Protection Agency, 1989). An occupational study on 22 pest control operators exposed to an 8 hour level of 27.6 microgram per cubic meter (ug/m^3) of Dursban showed inhibition of plasma cholinesterase when compared to a control group of the same age and sex (Toxnet, 1975-1986). A measurable change in plasma and red blood cell cholinesterase levels was seen in spray workers exposed to 0.5% chlorpyrifos emulsion in field trials for malaria control. Human volunteers who ingested 0.1 mg/kg of chlorpyrifos daily for four weeks showed significant plasma cholinesterase inhibition (American Conference of Governmental Industrial Hygienists, Inc. 1986). A low blood cholinesterase level can sometimes persist from two to six weeks with long-term exposure to chlorpyrifos (Occupational Health Services, Inc. 1986). Thus the many biochemical experiments Mice is the widely accepted model. Circulatory system serves as the primary target of any pesticide action and this study may help to take precautionary measures among workers employed in manufacture and application of these chemicals (Kennedy *et al.,* 1967).

MATERIALS AND METHODS

Species: Mice

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

Concentration selected: Tenth fold ($1/10^{th}$) lower concentration of LD_{50} was selected for sublethal treatment to the experimental mice.

Course of study: Single, double and multiple doses with 48 hours interval.

Route of administration: Oral

Tissues selected: Blood.

Pesticide stock solution: Stock solution of chlorpyrifos was prepared in acetone. Working pesticide test solutions were prepared by diluting the stock solution with distilled water.

Selection of sub lethal treatment to the experimental model

As the acute oral LD_{50} value of chlorpyrifos was determined, tenth fold lower ($1/10^{th}$) concentration was selected as sublethal to study the effect of chlorpyrifos. Healthy adult mice of same age (100±10 days) and weight (75±10 g) 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 1^{st} day) was administered orally by gavage method. To the third group of animals double doses were given i.e. on 1^{st} and 3^{rd} day. Similarly multiple doses i.e., 1^{st}, 3^{rd}, 5^{th} and 7^{th} day were given to the fourth group of animals. The first group of animals was considered as controls.

Isolation of tissues: The control and experimental animals after the stipulated period (i.e. on 9^{th} day) were sacrificed and the tissues were isolated, cleaned in physiological saline and processed immediately for microscopic analysis. The tissues were also quickly isolated under ice cold conditions and stored in deep freezer at -80ºC for biochemical analysis.

Procurement of experimental animals

Healthy wistar strain mice of the same age group 100±10 days and weight 75±10 grams were selected as experimental animals for the present study. The mice were collected from Indian Institute of Science (I.I.Sc.), Bangalore. Prior to experimentation the animals were acclimatized according to the instructions given by Behringer (1973).

Maintenance of animals

The mice were maintained at laboratory conditions in the animal house at 25±2°C with a photoperiod of 12hrs light and 12hrs darkness throughout the course of the present study. The mice were fed with standard pellet diet supplied by Sai Durga feeds and foods, Bangalore and water *ad libitum*.

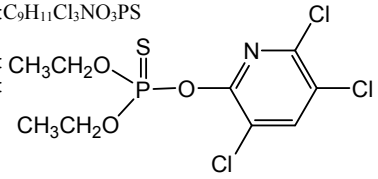
Pesticide selection

Chlorpyrifos, an organophosphate insecticide was selected for the present investigation.Chlorpyrifos O,O-diethyl O-(3,5,6-trichloro-2-pyridyl) phosphorothioate with 95.30% purity was used as the test chemical for the present study. Technical grade chlorpyrifos was obtained from Nagarjuna Agri. Chem Limited, RavulapalemMandal, East Godavari District, A.P., India. Chlorpyrifos has a wide applicability and safety compared to other compounds of its class. Hence this pesticide was selected for the present study.

The following are the specifications of chlorpyrifos used in the present study.

Generic name :Chlorpyrifos
Chemical name :O,O-diethyl O-(3,5,6-trichloro-2-pyridyl)

Phosphorothioate
Synonym(s) :Phosphorothioic acid O,O-diethyl
O-(3,5,6-trichloro-2-pyridinyl) ester; chlorpyrifos-ethyl; chlorpyrifos
Registered trade name (s) :Dowco 179; ENT 27311; Dursban;
Lorsban; Pyrinex; DMS-0971
Chemical formula :$C_9H_{11}Cl_3NO_3PS$
Chemical structure :
Identification numbers :



CAS Registry :2921-88-2
NIOSH RTECS :TF6300000
EPA Hazardous Waste :059101
OHM / TADS :7800025
DOT/UN/NA/IMCO :NA 2783 Chlorpyrifos
HSDB :389
Molecular Weight :350.57
Color :White granular crystals

White to tan
Amber solid cake with amber oil
Colorless crystals
Physical state :Crystalline solid
Melting point :41-42°C
Boiling point :Decomposes at approximately 160°C
Density at 43.5°C :1.398 g/cm^3
Odor :Mild mercaptan

Solubility :
Water at 20°C :0.7 mg/L
Water at 25°C :2 mg/L
Organic solvent (s) :79% w/w in iso octane
43% w/w in methanol
Readily soluble in other organic solvents
Partition coefficients :
Log K_{ow} : 4.82
Log K_{oc} : 3.73
Vapor pressure at 20°C : 1.87 x 10^{-5} mm Hg
Vapor pressure at 25°C : 1.87 x 10^{-5} mm Hg
Henry's law constant at 25°C : 1.23 x 10^{-5} atm-m^3 / mol
Conversion factors (25°C) : 1 ppm = 14.3 mg/m^3
1 mg/m^3 = 0.070 ppm
Flammability limits at 25°C: No data

(CAS = Chemical Abstracts Services; DOT/UN/NA/IMCO = Department of Transportation/United Nations/North America / International Maritime Dangerous Goods Code; EPA = Environmental Protection Agency; HSDB = Hazardous Substance Data Bank; NIOSH = National Institute for Occupational Safety and Health; OHM/TADS = Oil and Hazardous Materials / Technical Assistance Data System; RTECS = Registry of Toxic Effects of Chemical Substances).

Haematology

Red blood corpuscle (RBC) count

RBC count was made with a Neubauer crystalline counting chamber as described by Davidson and Henry (1969). The blood was collected in a vial containing 2% ethylene diamine tetra acetic acid (EDTA) as an anticoagulant. The blood was drawn up to 0.5 marks in RBC pipette and immediately the diluting fluid was drawn up to the mark 101 (thus 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 stem ful 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, 1962). 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.

Pipette was rinsed two or three times with dilute 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*., 1975).

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.

Mean corpuscular volume (MCV)

MCV expresses the average volume of the red blood cells. For obtaining the mean corpuscular volume, the packed cell volume is divided by red blood cell count and the result is multiplied by 10. MCV is expressed in cubic microns (cuμ).

Mean corpuscular hemoglobin (MCH)

MCH represents the average weight of hemoglobin contained in each cell. MCH is influenced by the size of the cell and concentration of hemoglobin. For getting MCH the Hb concentration is usually divided by red blood cell count and the result is multiplied by 10 and is expressed as pictograms (pg).

Mean corpuscular hemoglobin concentration (MCHC)

MCHC refers to the average concentration of the Hb in the red blood cells. In contrast to MCH, MCHC is not influenced by the size of the cell. For getting MCHC the hemoglobin is divided by packed cell volume and the result is multiplied by 100. The MCHC value is expressed in terms of percentage.

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 (1969) 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.

Statistical treatment of the data

The mean, standard deviation (SD), percent change and one – way analysis of variance (ANOVA) (Steel and Torrle, 1960) were performed using the SPSS package programming techniques on "Intel Core 2Duo Processor" personnel computer. Probability values less than 0.05 were considered significant (Snedecor and Cochran, 1968).

RESULTS AND DISCUSSION

In the present investigation the effect of chlorpyrifos, anorgano-phosphate compound on the blood is determined in Mice. The changes in haemotology of Mice as a result of chlorpyrifos after single, double and multiple doses are indicated in Table 1, Fig.1 and Table, 2.Fig.2.

Oral administration of chlorpyrifos produced statistically significant ($P<0.01$) decrease in RBC, Hb, PCV but WBC count shows increased level in single, double and multiple doses respectively. The decrease in RBC count from single dose to double dose was four fold and it was four and half fold from single dose to multiple dose. In case of Hb, five fold and five and half fold decrease was noticed from single dose to double and multiple dose. The decrease in packed cell volume was four and half from single dose to double dose and five fold from single to multiple dose.

Table 1 Haemogram of control and chlorpyrifos treated mice

Parameters	Control	Single Dose	Double Dose	Multiple Dose
RBC (cu. mm)				
Mean	6.5956 ±0.32424	4.5346 ±1.1673 (-31.2481)*	3.6496 ±1.1612 (-48.7288)*	2.813 ±1.1313 (-36.0465)*
SD				
PC				
Hb/(g/100ml)				
Mean	13.656 ±0.00187	9.5735 ±1.3127 (-29.8952)*	6.6498 ±1.2979 (-51.3049)*	5.5753 ±1.2940 (-59.1732)
SD				
PC				
PCV(Percent)				
Mean	40.338 ±1.3559	30.5625 ±1.3449 (-24.2339)*	20.4781 ±1.3877 (-49.2337)*	16.4836 ±1.2853 (-59.1362)*
SD				
PC				
MCV(µg)				
Mean	15.3143 ±1.3735	13.6458 ±1.2755 (-10.89) ***	13.36 ±1.3795 (-14.51) ***	12.6761 ±1.4087 (-17.22) **
SD				
PC				
MCH(pg)				
Mean	15.5826 ±1.25052	14.6378 ±1.3034 (-6.0631) ***	12.5593 ±1.3224 (-19.4017) **	13.7158 ±1.2991 (-11.9800)
SD				
PC				
MCHC(Percent)				
Mean	32.7061 ±1.3344	30.2483 ±1.4879 (-7.5148)	28.41 ±1.7045 (-13.1354)	27.6922 ±1.3098 (-15.3301)
SD				
PC				

All the values are mean ± SD of six individual observations.
SD – Standard Deviation.
PC – Percent change over control.
* Significant P<0.001
** Significant P<0.05
*** Significant P<0.01

Table 2 Haemogram of control and chlorpyrifos treated mice

Parameters	Control	Single Dose	Double Dose	Multiple Dose
WBC(cu. mm)				
Mean	10130.48 ±14.2065	11202.22 ±138.6469 (-10.5793) ***	12308.88 ±134.5183 (-21.5034)*	13517.47 ±147.2887 (-33.4336)*
SD				
PC				
Neutrophils				
Mean	15.1503 ±0.12031	13.2273 ±0.2178 (12.6928)	11.1368 ±0.0689 (26.4912)*	9.7405 ±0.1381 (35.7075)*
SD				
PC				
Lymphocytes				
Mean	68.7445 ±0.1500	75.4401 ±0.1414 (-9.7398) ***	77.3491 ±0.1482 (-12.5167) ***	78.2403 ±0.1481 (-13.8131) ***
SD				
PC				
Monocytes				
Mean	0.981 ±0.1837	1.9976 ±0.1709 (-103.628) *	2.1751 ±0.1324 (-121.722) *	2.9743 ±0.1855 (-203.190) *
SD				
PC				
Eosinophils				
Mean	0.9771 ±0.0177	0.9771 ±0.0177 (-26.2409)*	1.2335 ±0.2045 (-14.6863) **	1.1356 ±0.0137 (-16.2214) **
SD				
PC				
Basophils				
Mean	0.7951 ±0.3567	0.9753 ±0.1480 (-22.6638)*	0.9836 ±0.1559 (-23.7077)*	1.1576 ±0.1147 (-45.5917)*
SD				
PC				

All the values are mean ± SD of six individual observations.
SD – Standard Deviation.
PC – Percent change over control.
* Significant P<0.001
** Significant P<0.05
*** Significant P<0.01

The red cell indicators like Mean corpuscular volume (MCV), Mean Corpuscular Haemoglobin (MCH) and Mean Corpuscular Haemoglobin Concentration (MCHC) are dependent on the RBC count, Hb concentration and PCV values. MCV, MCH and MCHC showed statistically significant (P<0.01) decrease in single, double and multiple dose of chlorpyrifos intoxication in Mice. WBC count shows statistically significant (P<0.01) increased in animals of experimental group compared to the control group. The increase in WBC count from single dose to double dose was two fold and it was three fold from single dose to multiple doses.

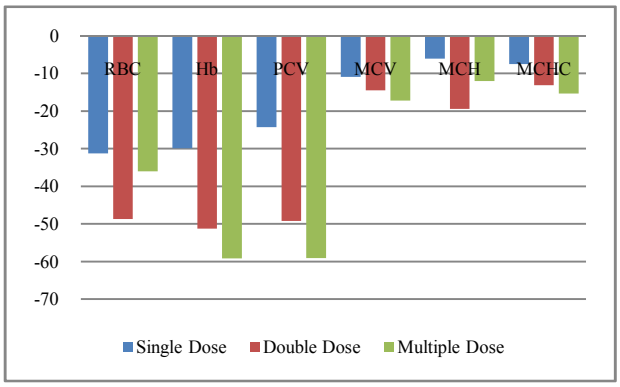


Fig 1 Changes in Haematological parameters in mice exposed to chlorpyrifos

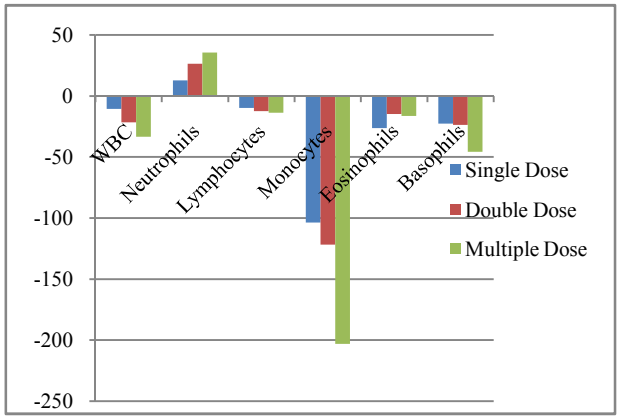


Fig 2 Changes in Haematological parameters in mice exposed to chlorpyrifos.

The differential count regarding lymphocytes and monocytes showed statistically significant (P<0.001 and P<0.05) increase in all doses of animals compared to the control group. The neutrophils showed a statistically significant (P<0.01) decrease in all dosed animals. The slightly elevated eosinophils in animals of experimental group did not differ statistically from that observed in the control group. In the case of basophils no change is observed in single, double and multiple dose of chlorpyrifos treated Mice.

In the present investigation the toxic effect of chlorpyrifos on the haemogram is determined in Mice. Mice 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.

The results in the present investigation are in line with those found by Morgan *et al.,* (1980) in bone narrow. 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*., 1990). 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.,* (2006) 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. Carbosulfan has been reported to increase chromosomal aberrations in human peripheral blood lymphocytes and bone marrow cells of Mice (Topaktas and Rencuzogullavi, 1993; Topktas *et al.,* 1996). 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 (Sharma and Saxena, 1983). It also indicates that the red blood cells are fully permeable to chlorinated hydrocarbon insecticides. Interaction of endosulfan, an OC compound with erythrocyte membrane in cat was observed by Mishra *et al*., (1982). Millar and Buhler (1974) reported HCP, an organochlorine compound binds to RBC *in vitro* and induces osmotic swelling of these cells by directly altering the permeability of membrane of Na^+ and K^+. The erythrocytes may be susceptible to oxidative damage due to the presence of poly unsaturated fatty acids (PUFA) heme iron and oxygen which may produce oxidative changes in red cells.

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 (Murray *et al.,* 2007). Anemia, defined clinically as a decrease in hematocrit or Hb concentration, may be caused by blood less, excessive hemolysis, or deficient erythropoiesis (Baynes and Dominiczak, 2005).Whipple (1942) reported if the destruction of erythrocytes is more than the normal in the haemoglobin concentration would naturally be decreased. This may be possible that destruction of erythrocytes by chlorpyrifos leads to fall in hemoglobin concentration. Jung-Hoon Jea *et al.,* (2005) 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. Shakoori *et al.,* (1992) were reported that reduction in Hb content could be related to the decreased size of red blood cells or to the impaired biosynthesis of haem in bone marrow. The decreased RBC count, Hb and PCV levels in the blood of mice suggested a possible damage to red cells due to ribosomal abnormality (Madhavee Latha, 2006) and decreased protein synthesis.

Decrease in hematocrit is attributable to the reduction in RBC count caused either destruction or reduction in size. In support of this decrease in hematocrit and mean value of hemoglobin (Jung-HoonJee *et al*., 2005; Sivaiah, 2006; Rahmen and Siddiqui, 2006; Nagarjuna, 2007 and Rajeswari, 2008Savithri, 2009) were observed.

Decreased RBC count, Hb and PCV levels was observed in Mice treated with Primiphos methyl (Rajini, *et al.,* 1987*),* BHC(Baronia *et al.,* 1992), aldrin(Anil Kumar *et al.,* 1996), residual pesticide (Patel *et al*., 1998), cypermethrin (Insitories *et al*., 1999 and Nagarjuna, 2007), thiodan 35 E.C (Solanke and Singh, 2000), chloropharm (Fujitani *et al*., 2001), endosulfan (Choudary and Joshi, 2002), novel phosphorothionate (Rahman and Siddiqui, 2006) and lindane and endosulfan (AzharBaig, 2007), in mice treated with citrinin (Bilgrami *et al*., 1987), fenvalerate. (Anil Kumar and Singh, 1997), deltamethrin (Yakeen *et al.,* 2007), acephate, (Rajeswari, 2008), azadirachtin and monocrotophos (Sivaiah, 2006); carbofuran and glyphosphate (Yousef *et al*., 1999), in fishes treated with various insecticides such as endosulfan, malathion, methyl parathion, phosphomidon, monocrotophos and fenvalerate (Dhembare and Pondhe, 2000), and in sprayers of grape garden exposed to various pesticides (Patil Jyotsna *et al*., 2003). The present results are in consonance with earlier findings of different animals exposed to pesticides.

Several authors have attributed the decrease in RBC, Hb and PCV to certain altered physiological conditions apparently induced by the pesticides. The decrease in these parameters is known to effect anemic obviously caused by deficiency of iron thus retarding the synthesis of Hb (Bhai *et al*., 1971). It is well known that the glycolysis is known to retard the production of methemoglobin whose deficiency responsible for maintaining the iron of Hb in ferrous in which state only it acts as an efficient oxygen carrier. Various investigators have reported that pesticide intoxication cause an increase in the activity of LDH and cellular oxidation (Koundinya and Ramamurthi, 1980; Das and Mukherjee, 2003, Manna *et al.,* 2004; Velisek *et al.,* 2006; Jacob Doss *et al.,*2007). Thus indicating the prevalence of anaerobic metabolism following the pesticide administration. The disruptive iron synthesizing machinery due to inhibition of aerobic glycolysis could be the reason for the decrease of various haemotological parameters under stress conditions.

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, 2000).

The reduction in RBC count, Hb and PCV observed in the present investigation could be described are retarded haemopoisis, destruction and shrinkage of RBC.Subacute exposure of imidacloprid and quinalphos in 8 to 10 week old white leghorn cockerels did not affect packed cell volume and haemoglobin levels (Siddiqui, 2004). Erythrocyte and monocyte count did not show any change following feeding of broiler chicks with 20 ppm fenvalerate (Synthetic pyrethroid), 2 ppm monocrotophos (organophosphate) and 2 ppm endosulfan in broiler chicks (Garg *et al*., 2004). Deltamethrin showed no significant effect on Hb of the treated mice (Yakeen *et al*., 2007). Very few attempts have been made to study the WBC in animals treated with pesticides. Acephate administration did not influence haemoglobin, packed cell volume, total erythrocyte count and total leukocyte count in white leghorn birds.

Increase in total leukocytes count has been suggested to be due to stimulated lymphopoiesis and/or enhanced release of lymphocytes from lymph myeloid tissue (Das and Mukherjee, 2003). 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 (Jabeen Massod *et al.,* 1991*),* phorate (Qureshi *et al.,* 1994), aldrin (Anil Kumar *et al.,* 1996), chloropham (Fujitani *et al.,* 2001), endosulfan (Choudhary and Joshi, 2002), cypermethrin novel phosphorothionate (Rahman and Siddiqui, 2006) and

lindane and endosulfan (AzharBaig, 2007), in mice treated with BHC (Philips *et al.,* 1984), alpha-cypermethrin (Luty*et al.*, 2000), deltamethrin and fenvalerate (Tos-Luty *et al*., 2001), pyrethroids like alpha- cypermethrin, deltamethrin and fenvalerate (Haratym-Maj, 2002) and azadirachtin and monocrotophos (Sivaiah, 2006), in pigeon treated with fenitrothion (Mandai and Pulak, 1989), in fishes treated with endosulfan (Abidi Rehana and Srivatsava, 1988) cypermethrin (Das and Mukherjee, 2003 and Nagarjuna, 2007) and acephate (Rajeswari, 2008). Deltamethrin showed no significant effect on WBC of the treated mice (Yekeen *et al.,* 2007).

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 mice 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 (Klucinski *et al.,* 1996). In male mice poisoned with a higher deltamethrin dose was accompanied by a significant decrease in the number of neutrophils. In female mice, an insignificant decrease in the percentage of neutrophils was noted for a lower deltamethrin dose, whereas a higher dose caused an insignificant increase. In fenvalerate poisoning, as weakening of the process of myelopoiesis may occur, this was observed in female mice. In male animals poisoned with a lower dose of fenvalerate an insignificantly lower percentage of neutrophils was noted, while poisoning with a higher dose resulted in an insignificant increase (Tos-Luty *et al.*, 2001). Haratym-Maj (2002) reported the decrease in the number of neutrophils in mice intoxication with higher deltamethrin dose.

Studies in vitro by Podstawka (1994), aimed at the evaluation of the toxic dichlorvos effects on neutrophils in human peripheral blood, and confirmed that neutrophils showed an intensified phagocytosis activity. Luty *et al*., (1998) reported the dermal exposure to dichlorvos resulted in an increased activity of both parameters examined phagocytosis and bacterial function of neutrophils.

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 mice (Table. 1,Fig,1), which could be an adaptive mechanism prevailed in the animal under toxic stress.

CONCLUSION

Tremendous progress of research work on the pesticide toxicity in Mice on haematological changes under pesticide impact appears to have been marginally death with therefore the present investigation is undertaken to elucidate the toxic effect of chlorpyrifos on certain haematological parameters in Mice. The haematological parameters in the present study showed significant changes under chlorpyrifos exposure. But these changes are highly significant in multiple dose treated animals than the single and double dose chlorpyrifos administered mice.

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