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ALTERATIONS IN THE PROFILE OF BLOOD CELLS OF WISTAR RATS INDUCED BY LONG-TERM INGESTION OF CHLORPYRIFOS

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ABSTRACT

Male Wistar rats were divided into three groups -- A, B and C. Two groups (B and C) of animals were daily administered orally chlorpyrifos at a dose of 5 and 10 mg/kg b wt., respectively. One group (A) was employed as control. Rats were sacrificed 24 h after last dose on 1st, 2nd, 4th, 6th, and 8th week after initiation of the experiment. Morphological alterations in blood cell profiles induced by chlorpyrifos treatment were investigated on hematological preparations made by routine methods. Results of the present study indicate that chlorpyrifos provoked an alteration of rat erythrocyte morphology from the normal discoid shape to other forms such as echinocytes, dacrocytes, schistocytes etc. Following chlorpyrifos treatment, few faded red blood cells were encountered. Many cells were degenerated thus at places cell debris were present. Few nucleated red cells were also seen.

KEY WORDS

Chlorpyrifos, Blood cells, Organophosphate, Rat

INTRODUCTION

Pesticides, a unique group of compounds, are used to prevent, control or eliminate pests which are a major cause of crop losses in the field as well as in storage. These pests have always been a nuisance and create multidimensional problems for human beings. The discovery of organic pesticides provided man with a new and powerful weapon for his incessant war against pests. Pesticides have benefited human beings by controlling insects, disease transmitting rodents, noxious arthropods and pests of plants including crops and forests. But pesticides may have dual actions – they are important in controlling injurious pests but they may also present a hazard to species not considered to be pests in the environment. This ill-effect becomes particularly significant when pesticides directly affect populations of economically important organisms or poison organisms which are eaten by economically important animals and human beings.

The effects of toxicants may be lethal or sublethal. Lethal effects may result in death of the organism (mostly in acute effects). A typical lethal effect is failure of the chemically exposed organisms to produce viable offspring's. The most common sublethal effects are behavioural changes (e.g. swimming, attraction avoidance

and prey-predator relationships), physiological changes (e.g. growth, reproduction and development), biochemical changes (e.g. blood enzyme and ion levels) and histological changes. Together, behavioural, biochemical, physiological and histopathological tests are useful for evaluating the environmental hazard of a chemical, and they may provide important information on its mode of action (Rand and Petrocelli 1985).

The organophosphate insecticides have superseded the organochlorines owing to their rapid biodegradability and shorter persistence in the environment. But as a consequence of their indiscriminate and widespread use in agriculture and public health, these insecticides ultimately reach the environment and affect the life therein. Organophosphate compounds have neuroparalytic and enzymatic actions. The toxicity of these compounds lies in the capacity of their selective effect on enzymes of nerve tissue – cholinesterase (ChE), which lead to excessive accumulation of acetylcholine in the organisms (Pan and Dutta1998; Quistad and Casida 2000).

Chlorpyrifos [O,O-diethyl-O-(3,5,6-trichloro -2- pyridil) phosphoro-thioate], is a member of organophosphate class of insecticides that displays broad-spectrum insecticidal activity against a number of important arthropod pests (Racke 1993). It is recommended as a most effective insecticide for the control of mustard aphid, *Liapaphis erysimi* (Srivastava and Srivastava 1986). The wide-spread use of chlorpyrifos is probably due to its relatively low mammalian toxicity. Chlorpyrifos has been reported to induce immune alterations associated with lymphocyte subpopulations in rats (Blakley et al. 1999).

Wang et al. (2009) have stated chlorpyrifos is generally toxic to mammals, however, the toxicity of this pesticide to other organs and their potential interactive effects still remain unclear. Blood is a pathological indicator of the whole body, and hence hematological parameters are important for analysis of the functional status of an exposed animal to suspected toxicant (Omitoyin 2006). Moreover, blood cell profile has been considered as an important indicator of diseases and other toxicants. As pathomorphological changes are indicative of numerous diseases (Yawata 2003) there is little doubt of the importance of elucidating the mechanism governing erythrocyte shape (Pawlowski et al. 2006). To our knowledge there exists no report regarding the effect of chlorpyrifos exposure to mammals. Keeping above facts in view, the present study was designed to evaluate the effect of orally administered chlorpyrifos at different doses for two months on the blood cell profile of rat. Oral administration seems to be the most appropriate in long-term exposure of chlorpyrifos as it enters the animal or human body through the residues in food. Also, oral route for exposing chlorpyrifos in rats rather than injecting the chemical would better reflect the dietary exposure that most human population experiences. Hence, the effects due to repeated dose administration by gavage have been investigated in this study.

MATERIALS AND METHODS

Wistar rats (males; b wt 145-165 g) were procured from the Indian Institute of Toxicology Research, Lucknow, India. The animals were acclimatized for at least 2 weeks prior to chlorpyrifos treatments. The rats were maintained on the standard laboratory feed and water *ad libitum* throughout the experimental period.

After acclimation rats were separated into three groups of 25 each. Animals in group A (GA) served as control. Rats from group B (GB) were daily administered orally chlorpyrifos (Anu Products Ltd., India) at a dose of 5 mg/kg b wt. Animals in group C (GC) received daily an oral administration of chlorpyrifos at a dose of 10 mg/kg b wt. Fresh dosing solutions of chlorpyrifos were prepared each day. Chlorpyrifos treatments were given at 08:00 each day throughout the experiment as it has been reported that efficacy of chlorpyrifos is altered depending on whether it is

administered in the morning or afternoon (Kuperberg et al., 2000).

Five rats (from all the three groups) were sacrificed 24 h after last dose on 1st, 2nd, 4th, 6th, and 8th week after initiation of the experiment under light ether anesthesia. The animals were fasted overnight before sacrifice. Fresh blood was collected (from each rat from all three groups) on each interval from jugular vein. Blood smears were prepared, dried in air and after fixing for 10 minutes in methyl alcohol stained with giemsa for 20 minutes. Stained preparations were examined with microscope (Olympus CH 20i) for morphological changes in blood cells. Photomicrographs were taken with the aid of Olympus E 420 camera.

Observations

Group A:In control rats the erythrocytes (red cells) were noticed as round, non-nucleated biconcave discs having a central pallor which covers about one third of the cell (Fig. 1).

Group B:Up to 2 weeks treatment with 5 mg/kg b wt of chlorpyrifos there was no change in the profile of the blood cells. After 4 week chlorpyrifos exposure the red blood cells were noticed to be increased in size (Fig. 2). Few red blood cells with irregularly spaced projections (acanthocytes) were noticed (Fig. 3). Teardrop shaped red blood cells (dacrocytes) were also found (Fig. 3). After 6 week chlorpyrifos treatment, red blood cells almost spherical in shape, were recorded possessing no area of central pallor like a normal red blood cell (spherocytes) (Fig. 4). Few fragmented red blood cells (schistocytes) were also noticed (Fig. 5). Following 8 week chlorpyrifos treatment, few faded red blood cells were encountered (Fig. 6). Many cells were degenerated thus at places cell debris were present (Fig. 7).

Group C: There was no change in the profile of the blood cells after 1 week treatment with 10 mg/kg b wt of chlorpyrifos. After 2 week exposure few red blood cells increased in size. Also, few red blood cells with irregularly spaced projections (acanthocytes) and echinocytes were noticed (Fig. 8). Dacrocytes also mark their presence (Fig. 9). Following 4 week treatment, spherocytes, schistocytes and many echinocytes were noticed (Fig. 10). Few degenerating red blood cells were also observed (Fig. 11). Number of lymphocytes and neutrophils increased. In 6 week chlorpyrifos treated rats red blood cells showing blue pigments (polychromatophilic) were recorded (Fig. 12). Several spherocytes (Fig. 13) were present in addition to schistocytes, dacrocytes and acanthocytes (Fig. 14). Many degenerating blood cells (red blood cells and white blood cells) were also recorded (Fig. 15). After 8 week chlorpyrifos exposure there were present several degenerating cells which clustered to form debris (Fig. 16). Also several red blood cells with an oval or rectangular area of central pallor (stomatocytes) and with a central color spot in the area of pallor (target cells) (Fig.17) were noticed. Few nucleated red cells were also seen (Fig. 18).

DISCUSSION

Results of the present study indicate that chlorpyrifos provoked an alteration of rat erythrocyte morphology from the normal discoid shape to other forms such as echinocytes, dacrocytes, schistocytes etc. Echinocytes transformation plays an antihemolytic role – the expansion of the plasma membrane increases the cell-volume ratio/membrane-area, thus allowing swelling of the cells before lysing (Isomaa et al. 1986). Zeni et al. (2002) noticed occurrence of echinocytes in *Ictalurus melas* following exposure to an anionic detergent (sodium dodecyl benzene sulphonate) and suggested that echinocytes may result from cellular adaptation of physiological parameters involved in shape maintenance. Suwalsky et al. (2008) have reported that human erythrocytes when incubated with aqueous extract of *Aristotelia chilensis* showed an alteration in erythrocyte morphology from the normal discoid shape to an echinocytic form. They

have postulated that cell membrane lipid bilayers are in general potential targets for *Aristotelia chilensis* polyphenols. The presence of echinocytes (crenated cells) might influence the blood flow by increasing viscosity, resulting from the intermeshing of the spicules (Reinhart and Chien 1986) thus obstructing the microcirculation.

In the present study fragmented red blood cells (schistocytes) were noticed after chlorpyrifos treatment to rats. This derives support from the previous findings of several investigators who have reported the occurrence of schistocytes after treatment with aluminium (rats – Vittori et al. 1999) and phenylhydrazine (calf – Sharma et al. 1991). Structural defects and changes in surface shapes of erythrocytes have been reported by Koc et al. (2008) from endosulfan and malathion exposed rats. Changes in the erythrocyte profile were also noticed in fishes by Benarji and Rajendranath (1990 – dichlorvos), Tavares et al. (1999 – trichlorphon), Khattak and Hafeez (1996 – malathion), Singh and Srivastava (1994 – formothion) and Sampath et al. (1993 – Ekolux organophosphorus preparation). Cox et al. (http://www.vet.uga.edu/vpp/clerk/cox/index.php) have stated that schistocytes are one of the poikilocytes used to monitor toxicity. Also they have suggested glomerulonephritis as one of the factors for the formation of schistocytes. We also supports that kidney and liver lesions (our unpublished results) observed after chlorpyrifos treatment to rats may be the reason for the presence of schistocytes. This can also be considered as supportive data for the other cell deformities (dacrocytes, acanthocytes) noticed in the present study. Also, it can be speculated that chlorpyrifos caused alterations in the cytoskeleton (membrane proteins and/or lipids) of red blood cells thus affecting the surface area of the cell. Comelekoglu et al. (2000) stated that some pesticides may provoke the alterations in size and surface shapes of erythrocytes. Nikimma (1992) suggested that toxic materials directly or indirectly damage the membrane structure, ion permeability and cell metabolism of erythrocytes thus may cause morphologically damaged erythrocyte formation. Akahane et al. (1987) have reported an increased cholesterol and phospholipids in red cell membrane after malotilate (a hepatotropic agent) treatment to rats and suggested that malotilate causes an increase in the surface area of the erythrocytes by accelerating the incorporation of cholesterol into their membrane and such erythrocyte might be rheologically impaired and captured more easily by the spleen.

In chlorpyrifos exposed rats polychromatophilic cells have been observed. These cells have been considered to be immature red blood cells and represent accelerated red cell production due to stressed bone marrow. Occurrence of nucleated red blood cells in the present study indicates an active marrow response releasing premature release of normoblasts into circulation.

An increase in the cellular size of the red blood cells has been recorded in rats post-exposure to chlorpyrifos. This could be due to the membrane alterations. Chlorpyrifos may have impact on the cell flexibility and permeability by mechanisms not yet defined. The increased cellular size observed in this study may derive support from the suggestions of Vives et al. (1999) who explained that the expansion of membrane increases the area/volume proportion and could allow the swelling of the cell, thus, reaching the largest volume before the lysis. Swelling of red blood cells as reflected by the increased mean corpuscular volume (MCV) has been attributed to the increase in the activity (Soivio et al. 1974). An increase of erythrocyte size (MCV) has been associated with several factors but it is generally considered as a response to stress (Weber 1982).

In the present study, stomatocytes and target cells have been observed in chlorpyrifos exposed rats. Similar cells have also been reported from rats treated with aluminium (Vittori et al. 1999; Bazzoni et al. 2005) and malotilate (Akahane et al. 1987). Vittori et al. (1999) stated that presence of atypical cells (target and crenated cells) induced by aluminium ingestion to rats strongly suggests

membrane alteration. Bazzoni et al. (2005) have attributed the presence of stomatocytes in aluminium treated rats, to an expansion of the inner monolayer area, or to a shrinkage of the external one. They further stated that the cavities which give place to the stomatocytic shapes indicate, generally, a stress of the membrane.

Chlorpyrifos treated rats showed the degeneration of white blood cells. Svoboda et al. (2001) observed decrease of non-specific immunity after diazinon (organophosphorus) exposure to fish *Cyprinus carpio* and attributed this to decreased leucocyte count. Decreased white blood cell count has also been reported from teleosts exposed to diazinon (Koprucu et al. 2006; Adedeji et al. 2009), methylparathion (Nath and Banerjee 1996) and cadmium (Kori-Siakpere et al. 2006). Reduced number of white blood cells has been noticed in chlorpyrifos exposed bird (Obaineh and Matthew 2009). These reports regarding the decreased white blood cell count provide supportive evidence to the present study in which degenerating white blood cells have been noticed after toxicant treatment to rats.

In this study, cellular debris formed by degeneration of red blood cells has been noticed in rats following post-exposure to chlorpyrifos. Several studies in past have reported decreased erythrocyte count and hemoglobin content after toxicant exposure to fish (Adhikari et al. 2004; Koprucu et al. 2006; Kori-Siakpere et al. 2006; Patnaik and Patra 2006) and rat (Koc et al. 2008). In these reports the decreased red blood cell number/hemoglobin content may be accounted for by the destruction of red blood cells/hemolysis.

Under the light of this study, it is concluded that chlorpyrifos is extremely effective in causing alterations in red blood cells and white blood cells. These changes may be potentially harmful for the survival of the organism if exposed for long-term to the toxicant. Even though limitations exist to extrapolate experimental results in animals to humans, this study might be useful in carrying *in vitro* investigations with human erythrocytes to fully understand the possible deleterious effects of the pesticide on structural alterations and survival of these cells.

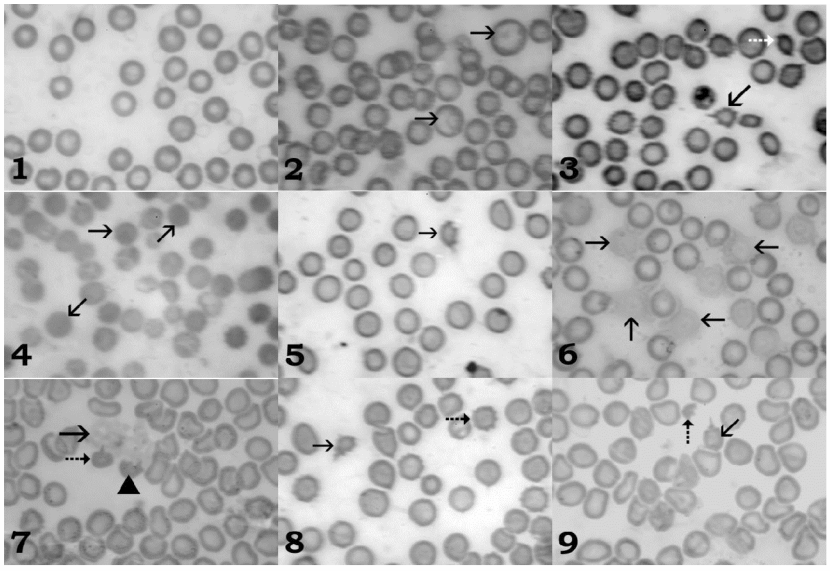

Fig. 1 ***Normal red blood cells with central pallor of control rat. x 500.***
Fig. 2 ***Red blood cells of chlorpyrifos (5 mg/kg b wt) treated rat. Note increased size of the cells (arrows). x 500.***
Fig. 3 ***Presence of acanthocytes (broken arrow) and dacrocytes (arrow) in the blood of chlorpyrifos (5 mg/kg b wt) treated rat. x 500.***
Fig. 4 ***Spherocytes (arrows) in the blood of rat treated with chlorpyrifos (5 mg/kg b wt). Note the absence of central pallor in these cells. x 500.***
Fig. 5 ***Fragmented red blood cell (schistocyte) in the blood of chlorpyrifos (5 mg/kg b wt) treated rat. x 500.***

Fig. 6 *Faded red blood cells (arrows) in the rat exposed to chlorpyrifos (5 mg/kg b wt). x 500.*
Fig. 7 *Cell debris (arrow) formed by degeneration of cells in the chlorpyrifos (5 mg/kg b wt) treated rat. Mark also the presence of dacrocyte (broken arrow) and schistocyte (arrow head). x 500.*
Fig. 8 *Acanthocyte (arrow) and echinocyte (broken arrow) in the blood of chlorpyrifos (10 mg/kg b wt) treated rat. x 500.*
Fig. 9 *Schistocyte (broken arrow) and dacrocyte (arrow) in the rat exposed to chlorpyrifos (10 mg/kg b wt). x 500.*

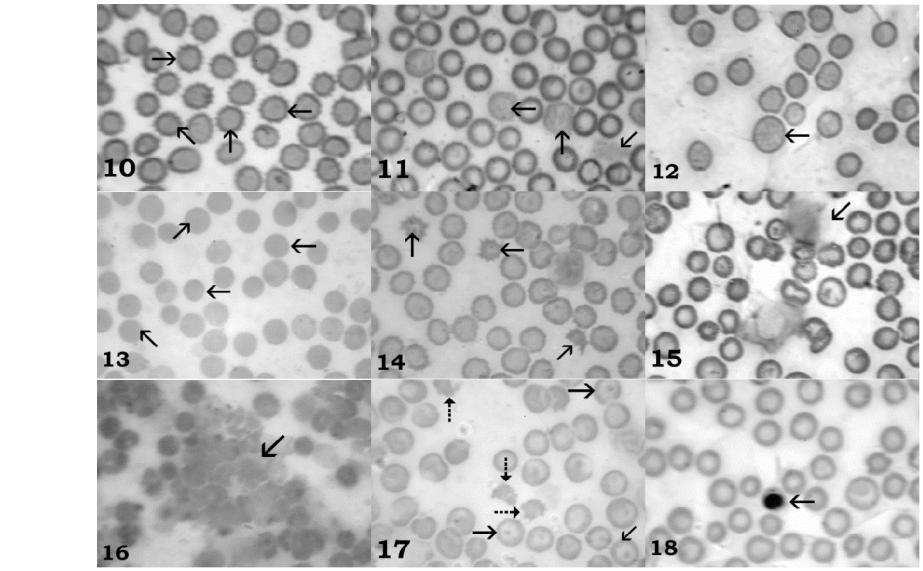

Fig. 10 *Echinocytes (arrows) in the blood of chlorpyrifos (10 mg/kg b wt) treated rat. x 500.*
Fig. 11 *Degenerating red blood cells (arrows) in the blood of chlorpyrifos (10 mg/kg b wt) treated rat. x 500.*
Fig. 12 *Polychromatophilic cell (arrow) in chlorpyrifos (10 mg/kg b wt) exposed rat. x 500.*
Fig. 13 *Spherocytes (arrows) in the blood of rat treated with chlorpyrifos (10 mg/kg b wt). Note the absence of central pallor in these cells. x 500.*
Fig. 14 *Acanthocytes (arrows) in the blood of chlorpyrifos (10 mg/kg b wt) treated rat. x 500.*
Fig. 15 *Degenerating white blood cells (arrow) in the blood of chlorpyrifos (10 mg/kg b wt) treated rat. x 500.*
Fig. 16 *Cell debris (arrow) formed by degeneration of cells in the chlorpyrifos (10 mg/kg b wt) treated rat. x 500.*
Fig. 17 *Target cells (arrows) and schistocytes (broken arrows) in the blood of chlorpyrifos (10 mg/kg b wt) treated rat. x 500.*
Fig. 18 *Nucleated red blood cell (arrow) in chlorpyrifos (10 mg/kg b wt) treated rat. x 500.*

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